EVOLUTION OF NERVOUS SYSTEMS

A COMPREHENSIVE REFERENCE

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1.01 A History of Ideas in Evolutionary Neuroscience

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Glossary

allometry	The notion that changes in the size of an object (e.g., the body or the brain) entail predictable changes in the proportional sizes of its components. In contrast, isometric scaling involves no changes in an object's proportions.
convergence	The independent evolution of similar structures or functions from non-homologous ancestral precursors.
developmental constraint	The notion that the mechanisms of development bias the production of phenotypic variants that natural selection can act on.
encephalization	Brain size relative to what one would expect in an organism of the same type (i.e., species or other taxonomic group) and body size. Synonym: relative brain size.
heterochrony	Phylogenetic changes in the relative timing of developmental events or in the relative rates of developmental processes.
homology	The relationship between two or more characters that were continuously present since their origin in a shared ancestor. For a more detailed definition, especially for neural characters, see Striedter (1999).
mosaic evolution	The notion that, as brains evolve, individual brain regions may change in size independently of one another. In contrast, concerted evolution indicates that brain regions must change their size in concert with one another.

The field of evolutionary neuroscience is more than 100 years old, and it has deep pre-evolutionary roots. Because that illustrious history has been reviewed repeatedly (Northcutt, 2001; Striedter, 2005) and is treated piecemeal in several articles of this book, I shall not review it fully. Instead, I will discuss a selection of the field's historically most important ideas and how they fit into the larger context of evolutionary theory. I also emphasize ideas that are, or were, controversial. Specifically, I present the field's central ideas in contrast pairs, such as 'common plan versus diversity' and 'natural selection versus constraints'. This approach scrambles the chronology of theoretical developments but helps to disentangle the diverse strands of thought that currently characterize evolutionary neuroscience. It also helps to clarify which future directions are likely to be most fruitful for the field.

1.01.1 Common Plan versus Diversity

One of the most famous battles of ideas in comparative biology was that between Etienne Geoffroy St. Hilaire and George Cuvier over the existence, or not, of a common plan of construction (or Bauplan) for animals (Appel, 1987). Geoffroy was of the opinion, previously developed by Buffon (1753), that all animals are built according to a single plan or archetype, but Cuvier, France's most illustrious morphologist, recognized at least four different types. Their disagreement erupted into the public sphere when Geoffroy in 1830 endorsed the view that the ventral nerve cord of invertebrates is directly comparable (today we say 'homologous') to the spinal cord of vertebrates. Cuvier responded that Geoffroy was speculating far beyond the available data, and he reasserted publicly that the major types of animals could not be linked by intermediate forms or topological transformations. This Cuvier–Geoffroy debate was followed closely by comparative biologists all across Europe, who

were already flirting with the idea of biological evolution or, as they called it, the transmutation of species. If Cuvier was right, then evolution was impossible. On the other hand, some of Geoffroy's hypotheses (e.g., his proposal that insect legs correspond to vertebrate ribs) did seem a trifle fanciful. Thus, the Cuvier–Geoffroy debate embodied much of the ambivalence surrounding evolution in the first half of the nineteenth century.

After Darwin offered a plausible mechanism for the transmutation of species, namely, natural selection (Darwin, 1859), the idea of biological evolution took hold and, by extension, Geoffroy's ideas gained currency. Innumerable homologies were sought and, frequently, revealed (Russel, 1916). Most impressive was the discovery of extensive molecular homologies between species that span the metazoan family tree (Schmidt-Rhaesa, 2003). It was striking, for example, to discover that many of the genes critical for early brain development are homologous between insects and vertebrates (Sprecher and Reichert, 2003). Indeed, the invertebrate and vertebrate genes are sometimes functionally interchangeable (Halder *et al*., 1995; deRobertis and Sasai, 1996). Those discoveries supported Geoffroy's view that all animals were built according to a common plan, which could now be understood to be a common genetic blueprint or 'program' (Gehring, 1996). Indeed, many biologists proceeded to search for molecular genetic homologies that could reveal previously unimagined morphological homologies (Janies and DeSalle, 1999). Geoffroy would have been thrilled. There are, however, problems with the view that animals are all alike.

The most serious problem, in my view, is that homologous genes may sometimes be involved in the development of adult structures that are clearly not homologous (Striedter and Northcutt, 1991). For example, insect wings and vertebrate nervous systems both depend on *hedgehog* function for normal development, but this does not make neural tubes and insect wings homologous (Baguña and Garcia-Fernandez, 2003). Instead, findings such as this suggest that evolution tends to work with highly conserved 'master genes' (Gehring, 1996) or, more accurately, tightly knit assemblies of crucial genes (Nilsson, 2004), which it occasionally reshuffles by altering their upstream regulatory elements and/or downstream targets. Evolution is a terrific tinkerer that manages to create novelty from conserved elements. This conclusion echoes Geoffroy's arguments insofar as it acknowledges that "Nature works constantly with the same materials" (Geoffroy, 1807), but it does not mesh with the view that evolution built all animals according to a single plan. What we have, then, is at least a partial rapprochement of the positions held by Cuvier and Geoffroy: adult organisms do conform to several different body plans, but they are built by shuffling repeatedly a highly conserved set of genes (Raff, 1996). Therefore, a crucial question for research is how evolutionary changes in networks of developmentally important genes influence adult structure and function.

Implicit in the preceding discussion has been the idea that adult species differences arise because of evolutionary changes in development (Garstang, 1922). This idea is commonly accepted now, but, back in the nineteenth century, Haeckel (1889) used to promote its polar opposite, namely, the notion that phylogeny creates ontogeny (see Gould, 1977). Haeckel also promoted the idea that all vertebrates pass through a highly conserved phylotypic stage of embryonic development (Slack *et al*., 1993). Studies have, however, challenged the phylotypic stage idea by showing that the major groups of vertebrates can be distinguished at all stages of embryogenesis (Richardson *et al*., 1997). An intriguing aspect of that early embryonic variability is that it consists mainly of differences in the timing of developmental processes (Richardson, 1999). Little is known about the genes that generate those changes in developmental timing (also known as heterochrony), but some of them, at least, are likely to be fairly well conserved across species (Pasquinelli and Ruvkun, 2002). More importantly, the notion that adult diversity is based on evolution changing the temporal relationships of conserved processes represents another reconciliation of Cuvier's insistence on adult diversity with Geoffroy's belief in a common plan. Thus, the field of evolutionary developmental biology (evo-devo for short) has overcome the once so prominent dichotomy between conservation and diversity. Its major challenge now is to discover the mechanistic details of how conserved genes and processes are able to produce such diverse adult animals.

Evo-devo thinking has also invaded neuroscience, but evo-devo neurobiology still emphasizes conservation over diversity. For example, we now have extensive evidence that all vertebrate brains are amazingly similar at very early stages of development (Puelles *et al*., 2000; Puelles and Rubenstein, 2003). However, we still know very little about how and why brain development diverges in the various vertebrate groups after that early, highly conserved stage or period. Looking beyond vertebrates, we find that insect brain development involves at least some genes that are homologous to genes with similar functions in vertebrates (Sprecher and Reichert, 2003). This is

remarkable but does not prove that insects and vertebrates are built according to a common plan – if by that we mean that the various parts of adult insect brains all have vertebrate homologues. For example, the finding that several conserved genes, notably *Pax6*, are critical to eye development in both invertebrates and vertebrates, does not indicate that all those eyes are built according to a common plan. The crucial question, which we are just beginning to explore, is how the conserved genes are tinkered with (reshuffled, co-opted, or redeployed) to produce very different adult eyes (Zuber *et al*., 2003; Nilsson, 2004). This, then, seems to be the future of evo-devo neurobiology: to discover how highly conserved developmental genes and processes are used to different ends in different species. As I have discussed, this research program has ancient roots, but it is just now becoming clear.

1.01.2 *Scala Naturae* versus Phylogenetic Bush

The idea of evolution proceeding along some kind of scale from simple to complex also has pre-evolutionary roots. Aristotle, for example, ordered animals according to the degree of perfection of their eggs (see Gould, 1977). Later religious thinkers then described an elaborate scale of nature, or *scala naturae*, with inanimate materials on its bottom rung and archangels and God at the other extreme. The early evolutionists, such as Lamarck, transformed this static concept of a *scala naturae* into a dynamic phylogenetic scale that organisms ascended as they evolved. Darwin himself had doubts about arranging species on a scale, but most of his followers had no such qualms (Bowler, 1988). Even today, the phylogenetic scale is taught in many schools and it persists in medicine and academia. For example, the National Institutes of Health's (NIH) guide for institutional animal care and use still recommends that researchers, whenever possible, should work with "species lower on the phylogenetic scale" (Pitts, 2002, p. 97). On the other hand, most contemporary evolutionists have pronounced as dead both the *scala naturae* and its postevolutionary cousin, the phylogenetic scale (Hodos and Campbell, 1969). What do those modern evolutionists cite as the scales' cause of death?

One fatal flaw in the idea that species evolve along a single scale is that, as we now know, evolution made at least some species simpler than their ancestors. Salamanders, for example, are much simpler, especially in brain anatomy (Roth *et al*., 1993), than one would expect from their phylogenetic position. Even more dramatically, the simplest of all animals, the placozoans, are now thought to have evolved from far more complicated ancestors (Collins, 1998). As more and more molecular data are used to reconstruct phylogenies, it is becoming apparent that such secondary simplification of entire animals has occurred far more frequently than scientists had previously believed (Jenner, 2004) – perhaps because they were so enamored of the phylogenetic scale. A second major problem with *scala naturae* thinking is that the order of species within the scale depends on which organismal features we consider. For example, many fishes would rank higher than mammals if we based our scale on skull complexity, which was reduced dramatically as early mammals evolved (Sidor, 2001). Similarly, dolphins rank high if we look only at brain size, but relatively low if we consider neocortical complexity, which was reduced as the toothed whales evolved (Morgane and Jacobs, 1972). Most people tacitly agree that 'higher animals' are warm-blooded, social, curious, and generally like us, but once we try to be more objective, the single 'chain of being' (Lovejoy, 1936) fractionates into a multitude of different chains, none of which has any special claim to being true.

This multiple-chains idea becomes self-evident once we have grasped that species phylogenies are just like human family trees; they are neither ladders, nor trees with just a single trunk, but bushes or tumbleweeds (Striedter, 2004) with branches growing in divergent directions. Within a given branch, or lineage, complexity may have increased at some points in time and decreased at others, but even if complexity increased more frequently than it decreased, the overall phylogeny would fail to yield a single scale, because complexity tends to increase divergently in different lineages. For example, bats, honeybees, and hummingbirds are all incredibly complex, compared to their last common ancestor, but they are each complex in different ways. Of course, we can pick one parameter and build a scale for that – we can, for instance, compare the ability of bats, honeybees, and hummingbirds to see ultraviolet (UV) radiation – but different parameters might well yield different scales. Simply put, changes that occurred divergently in different lineages will not, in general, produce a single overarching scale. This insight is old hat to evolutionary biologists, but news to many neuroscientists (Hodos and Campbell, 1969). In part, therefore, the persistence of *scala naturae* thinking in the neurosciences reflects a lack of proper training in contemporary evolutionary theory. In addition, I suspect that human minds possess a natural tendency for ordering disparate items linearly. Such a bias would be useful in many contexts, but it would make it

difficult to comprehend (without training) the divergent nature of phylogeny.

Although *scala naturae* thinking persists in neuroscience generally, evolutionary neuroscientists have labored to expunge its ghost. For example, a consortium of 28 comparative neurobiologists revised the nomenclature of avian brains to replace the terms *neo*striatum, *archi*striatum, and *paleo*striatum – which suggested that brains evolved by the sequential addition of new brain regions – with terms devoid of *scala naturae* overtones (Reiner *et al.*, 2004a, 2004b; Jarvis *et al.*, 2005). Some of the replacement names are terms that were already used for brain regions in other vertebrates; they reflect our current understanding of homologies. However, some of the new terms – e.g., nidipallium and arcopallium – are novel and intended to apply exclusively to birds. These novel names were coined because bird brains, particularly bird forebrains, have diverged so much from those of other vertebrates (including reptiles) that strict one-to-one homologies are difficult, if not impossible, to draw for several regions (Striedter, 1998, 1999). Thus, the revised terminology reflects a new consensus view that avian brains did not evolve by the sequential addition of new brain areas, yet also reminds us that bird brains are full of features that evolved quite independently of those that feature in mammalian phylogeny. In other words, the new terminology avoids *scala naturae* overtones and, instead, combines the notion of a common plan with that of divergent complexity.

As comparative neurobiologists reject the notion of a *scala naturae*, they stand to lose a central part of their traditional justification for working on nonhuman brains. No longer can they argue that research on other brains must be useful because nonhuman brains are always simpler, and therefore easier to comprehend, than human brains. Instead, they must admit that some nonhuman brains are stunningly complex and, more importantly, that their phylogenetic paths toward complexity diverged from the primate trajectory. That is, complex bird, fish, or insect brains are not mere steps along the path to human brains, but the outcome of divergent phylogenies (see Evolution of the Nervous System in Fishes, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?, Evolution of Color Vision and Visual Pigments in Invertebrates). Does this suggest that research on nonhuman brains should cease to be funded? I do not think so, but the justification for working on nonhuman brains ought to be tweaked.

One obvious alternative justification is that all brains are likely to share some features, especially if they come from close relatives. Another good justification for research on nonhuman brains is that, compared to human brains, the former are much more amenable to physiological and anatomical research. This line of justification assumes that the model differs from the target system only in those respects that make the model easier to study, and not in the respects that are modeled – an assumption that sometimes fails. It now appears, for example, that the auditory system of owls, which was generally regarded as an ideal model for sound localization in vertebrates, exhibits some highly specialized features (McAlpine and Grothe, 2003). This finding, at first glance, suggests that research on bird brains is wasteful, but this is a simplistic view. Research on the owl's auditory system has taught us much about how neurons compute behaviorally relevant information and it serves as an invaluable reference against which we can compare sound processing in other species, including humans. Furthermore, some differences between a model and its target can lead to surprising discoveries. Much might be gained, for example, from studying why some nonhuman brains are far more capable than primate brains of repairing themselves (Kirsche and Kirsche, 1964). Thus, model systems research can be useful even if the model is imprecise. A third, less frequently discussed, justification for examining the brains of diverse species is that comparative research can bring to light convergent similarities, which in turn might reveal some principles of brain design. For example, the discovery that olfactory systems in both vertebrates and many different invertebrates exhibit distinctive glomeruli strongly suggests that those glomeruli are needed for some critical aspects of odorant detection and analysis (Strausfeld and Hildebrand, 1999).

Therefore, research on nonhuman brains need not be justified in terms of a presumed phylogenetic scale. Instead, comparative neurobiology is valuable because (1) all brains are likely to share some features, (2) nonhuman brains are more amenable to some types of research, and (3) the study of diverse nonhuman brains can lead to the discovery of design rules for brains. Historically, only the first of these alternatives has been widely discussed, but all are logically sound, and none depend on the existence of a *scala naturae*.

1.01.3 Relative Size versus Absolute Size

The most obvious difference between species is that they differ enormously in size. Because life began with tiny organisms, evolutionary increases in body size must have outnumbered or outpaced the decreases. This is true of organisms generally, but it

also holds for several individual lineages, including mammals and, within mammals, primates (Stanley, 1973; Alroy, 1998). The most fascinating aspect of those changes in body size is that they involved much more than the isometric scaling up or down of the ancestral condition; they involved allometric changes in the proportions of body parts and physiologic processes. For example, skeletal mass increases disproportionately with increasing body size, whereas heart rate decreases. Countless studies – on both vertebrates and invertebrates – have documented these allometries and explored their functional implications (Calder, 1984; Schmidt-Nielsen, 1984).

Much less is known about the causes of allometry. Studies on allometry in insects showed that some scaling relationships are readily modifiable by natural or artificial selection (see Emlen and Nijhout, 2000; Frankino *et al*., 2005). This finding suggests that even tight scaling laws are not immutable, which would explain why many traits scale differently (e.g., with different exponents) in different taxonomic groups (Pagel and Harvey, 1989). A very different, more theoretical line of research has shown that numerous allometries, specifically those with power law exponents that are multiples of 1/4, may have evolved because the optimal means of delivering metabolic energy to cells is through an hierarchically branching, fractal network of vessels whose termini (e.g., capillaries) are body size-invariant (West *et al*., 1997; Savage *et al*., 2004; West and Brown, 2005). This theory is mathematically complex and still controversial (Kozlowski and Konarzewski, 2004; Brown *et al*., 2005; Hoppeler and Weibel, 2005), but it is elegant. Furthermore, because the theory of West *et al*. is based in part on the assumption that natural selection optimizes phenotypes, it is consistent with the aforementioned finding that allometries are modifiable by selection. However, West *et al*.'s (1997) theory cannot explain (or does not yet explain) why some organs, such as the brain, scale with exponents that are not multiples of 1/4. Nor can it easily explain taxonomic differences in scaling exponents. Thus, the causal – physiological and/or developmental – bases of allometry are coming into focus but remain, for now, mysterious.

Brain scaling, in particular, remains quite poorly understood (see Principles of Brain Scaling, Scaling the Brain and Its Connections, How to Build a Bigger Brain; Cellular Scaling Rules for Rodent Brains). The discovery that brains become proportionately smaller with increasing body size dates back to the late eighteenth century (Haller, 1762; Cuvier, 1805–1845). Since then, numerous studies have documented brain allometry in all the major groups of vertebrates (Deacon, 1990a; van Dongen, 1998) and even some invertebrates (Julian and Gronenberg, 2002; Mares *et al*., 2005). Generally speaking, those studies confirmed that in double logarithmic plots of brain size versus body size, the data points for different species within a given lineage tend to form a reasonably straight line, indicating the existence of a simple power law. The slope of those best-fit lines are almost always less than 1, which reflects the aforementioned fact that brains generally become proportionately smaller with increasing body size. The large body of work on brain–body scaling further revealed that data points for different taxonomic groups often form lines with similar slopes but different y intercepts. These differences in y intercepts are known as differences in relative brain size or encephalization. They seriously complicate efforts to draw a single allometric line for any large taxonomic group (Pagel and Harvey, 1989), but they allow us to identify evolutionary changes in relative brain size among some smaller taxonomic groups. For example, they allow us to determine that relative brain size increased with the origin of mammals, with the origin of primates, several times within primates, with the origin of the genus *Homo*, and, last but not least, with the emergence of *Homo sapiens* (see Primate Brain Evolution in Phylogenetic Context, The Hominin Fossil Record and the Emergence of the Modern Human Central Nervous System, The Evolution of Human Brain and Body Growth Patterns). Overall, such phylogenetic analyses suggest that, among vertebrates, relative brain size increased more frequently than it decreased (Striedter, 2005).

Enormous effort has gone into determining the functional significance of evolutionary changes in brain–body scaling. Darwin, for example, had argued that relative brain size is related to "higher cognitive powers" (Darwin, 1871), but defining those powers and comparing them across species has proven difficult (Macphail, 1982). Consequently, most subsequent investigators shied away from the notion of general intelligence, or 'biological intelligence' (Jerison, 1973), and focused instead on more specific forms of higher cognition. Parker and Gibson (1977), for example, proposed that a species' degree of encephalization is related to its capacity for extracting nutritious fruits and nuts from their protective shells. Several authors have stressed correlations between brain size and 'social intelligence' (Byrne and Whiten, 1988; Dunbar, 1998; Reader and Laland, 2002). Collectively, these studies reinforced the sense that relative brain size is, somehow, related to some forms of intelligence. However, relative brain size also correlates with several other attributes, such as longevity, home-range size, diet, and metabolic rate (for a review, see van Dongen, 1998). The latter correlations, with diet and metabolism, have received

particularly lavish attention (Martin, 1981; McNab, 1989; Aiello and Wheeler, 1995). Paradoxically, the discovery of so many correlations has led some evolutionary neuroscientists to despair: there are too many correlates of relative brain size, and many of them come and go, depending on which taxonomic group is being examined and which statistical methods are used for the analyses (e.g., Bennet and Harvey, 1985; Iwaniuk *et al.*, 1999; Deaner *et al.*, 2000; Beauchamp and Fernández-Juricic, 2004; Jones and MacLarnon, 2004; Martin *et al.*, 2005). Too many contested hypotheses, too little certitude.

There is not much clarity on why brains scale so predictably with body size. Early workers argued that brains generally scale against body size with a power law exponent close to 2/3 because the brain's sensory and motor functions were related to the body's surface area, which presumably scales with that same exponent (Snell, 1891; Jerison, 1973). According to this view, brain sizes in excess of that predicted by the 2/3 power law are due to increases in the brain's nonsomatic, cognitive regions. This would explain the correlations between relative brain size and some forms of intelligence. Unfortunately, there are two major problems with this view. First, brain–body scaling exponents often differ substantially from 2/3 (van Dongen, 1998; Nealen and Ricklefs, 2001). The second problem is that the brain's more cognitive regions also scale predictably with body size (Fox and Wilczynski, 1986), undermining the assumption that brains are divisible into regions that scale with body size and regions that do not. Therefore, the excess neuron hypothesis (Striedter, 2005) is dead. In searching for an alternative, some have suggested that brain–body allometry is linked to the scaling of metabolic rates. This hypothesis is based on the observation that, in at least some taxonomic groups, brain size and basal metabolic rate scale against body size with similar exponents (Martin, 1981; Mink *et al.*, 1981). However, other studies have shown that the correlation between brain size and metabolism is not tight, once the mutual correlation with body size is factored out (McNab, 1989). This correlational slack presumably arises because species differ in how much of the body's total energy supply they deliver to the brain (Aiello and Wheeler, 1995; Kaufman, 2003), but this just underscores that relative brain size is not so tightly linked to metabolic rate.

Overall, the lack of clarity on what causes brains to scale predictably with body size, and how to interpret deviations from the scaling trends, has caused interest in relative brain size to fade. Increasingly, evolutionary neuroscientists have turned away from relative brain size and asked, instead, how the size of individual brain regions correlates with various behavioral parameters (Harvey and Krebs, 1990; see Brain Size in Primates as a Function of Behavioral Innovation, Mosaic Evolution of Brain Structure in Mammals). This shift in research strategy makes sense, because, after all, the brain is functionally heterogeneous. However, even studies that focus on correlations between single brain areas and specific behaviors – some refer to them as neuroecological studies – are controversial because: (1) the behavioral parameters are difficult to quantify and/or define (Bolhuis and Macphail, 2001), (2) neuronal structure–function relationships are complex and often poorly understood, (3) it is difficult to decide *a priori* whether one should correlate behavioral parameters against a region's absolute size, its proportional size, or its size relative to expectations (Striedter, 2005), and (4) the methods for establishing statistically significant correlations in phylogenetic data remain debatable (Felsenstein, 1985; Garland *et al.*, 1992; Smith, 1994; Martin *et al.*, 2005). Brave neuroscientists are continuing to tackle those problems, but the larger problem of how to deal with relative brain size – how to find its causes and its functional significance – is fading from view. Perhaps we need a new approach to understanding relative brain size – perhaps one that is linked more directly to the physiological and geometric properties of brains (West and Brown, 2005) – but this novel direction is not yet apparent.

As interest in relative brain size waned, interest in absolute brain size waxed, mainly because many of the brain's internal structural and functional features turn out to scale predictably with absolute brain size. Best studied is the phenomenon of size-related shifts in brain region proportions (Sacher, 1970; Finlay and Darlington, 1995). In mammals, for example, the neocortex becomes disproportionately large as absolute brain size increases, whereas most other regions become disproportionately small. A second interesting scaling law is that a brain's degree of structural complexity tends to increase with absolute brain size. Within the neocortex, for example, the number of distinct areas increases predictably with neocortex size (Changizi and Shimojo, 2005). A third fascinating aspect of brain scaling is that the amount of white matter within mammalian brains scales allometrically with absolute brain size (Ringo, 1991; Zhang and Sejnowski, 2000). This connectional allometry, taken together with the fact that synapse size and density are relatively size-invariant, indicates that brains become less densely interconnected, on average, as they increase in size (Stevens, 1989; Deacon, 1990a, 1990b; Striedter, 2005; see Scaling the Brain and Its Connections). All of this signifies that brains change structurally in many ways as they vary in absolute size. Many of those changes have clear functional

implications. For example, it has been suggested that, as hominid brains increased in size, the axons interconnecting the two cerebral hemispheres became so sparse and long that the hemispheres became less capable of interacting functionally, which led to an increase in functional asymmetry (Ringo *et al*., 1994; see Cortical Commissural Connections in Primates, The Evolution of Hemispheric Specializations of the Human Brain). Considerations such as these suggest that absolute brain size is a much better predictor of brain function than relative brain size, at least among close relatives (Striedter, 2005).

In retrospect, we can say that evolutionary neuroscientists historically have overemphasized relative brain size. As Dunbar (2006) put it, comparative neurobiologists have too long been "dragooned into worrying about relativizing brain size by a very peculiar view that body size must be the default determinant of brain volume." Can we explain this undue emphasis? Partly, evolutionary neuroscientists may have worried that focusing on absolute brain size and linking it to higher cognitive powers would force us to conclude that whales and elephants, with their enormous brains, are smarter than humans (see Cetacean Brain Evolution, Evolution of the Elephant Brain: A Paradox between Brain Size and Cognitive Behavior). This is a valid concern, for few would doubt that humans are – or at least can be – the most intelligent creatures on earth. However, whales and elephants are behaviorally complex, and humans may well be special because they are unique in possessing symbolic language (Macphail, 1982). Furthermore, it seems to me that large whales, with large brains, are more intelligent (both socially and in their hunting strategies) than dolphins or small whales. This hypothesis remains to be tested, but it points to a strategy for reconciling absolute and relative brain size: among close relatives, comparisons of absolute brain size are most informative, but in comparisons of distant relatives (e.g., whales and humans), relative brain size is a more potent variable (Striedter, 2005). This view is consistent with the finding that, among primates, social group size correlates more strongly with absolute brain size than with relative brain size (Kudo and Dunbar, 2001; Striedter, 2005). It also serves as a productive counterweight to the field's traditional, almost exclusive emphasis on relative brain size.

1.01.4 Natural Selection versus Developmental Constraints

Darwin's theory of natural selection entails two main components, namely, that (1) organisms produce offspring with at least some heritable variation and (2) that organisms generally produce more offspring than their environment is able to sustain. Given those two components, some variants are bound to be fitter than others in the sense that their offspring are more likely to survive and produce offspring. This difference, in turn, will cause the heritable traits of the fitter variants to spread in the population. Given this, Darwin's most "dangerous idea" (Dennett, 1995), one can explain an organism's attributes in terms of the selective pressures that promoted their spread and, hence, their current existence. An enormous number of such adaptational explanations have been proposed. Many stress that natural selection optimized features for specific functions; others emphasize that natural selection tends to produce optimal compromises between competing functions and/or costs (Maynard Smith, 1982). Generally speaking, the explanatory power of these adaptational explanations derives solely from natural selection's second step, the sorting of offspring. Generation of the variants that are sorted is usually assumed to be random and, hence, irrelevant to explanations of the phenotype. This 'adaptationist paradigm' (Gould and Lewontin, 1979) has dominated evolutionary theory for most of its history.

In the 1970s and 1980s, however, the adaptationist paradigm was challenged by authors who stressed that the variants available to natural selection may not really be random (Gould and Lewontin, 1979; Alberch, 1982; Maynard Smith *et al*., 1985). Central to those challenges was the idea that, even if mutations are random at the genetic level, those random genetic mutations are channeled, or filtered, through mechanisms of development that favor the emergence of some phenotypes. Some structures may be impossible for embryos to develop; others are likely to emerge (Alberch, 1982). If this is true, then natural selection chooses not among a random selection of phenotypes but from a structured set that is determined, or at least biased, by the mechanisms of development. This idea is important, because it suggests that development constrains the power of natural selection to set the course of evolutionary change. It threatens natural selection's widely assumed omnipotence. Some authors carried this threat so far as to exhort biologists to halt their search for adaptive scenarios and to research, instead, the 'generative' mechanisms of development (Goodwin, 1984). Fortunately, most evolutionary biologists today seek a more balanced rapprochement of embryology and evolutionary biology (Gilbert *et al*., 1996; Wagner and Laubichler, 2004).

Specifically, evo-devo biologists today tend to accept the concept that natural selection is the most prominent determinant of who thrives and

who dies, no matter how constrained development might be. They also tend to stress that development itself is subject to descent with modification – i.e., evolution – which means that even fairly tight constraints can change. Therefore, explanations couched in terms of natural selection are not antithetical to those involving developmental constraints, but complementary (Striedter, 2005). Still, the synthesis of natural selection and developmental constraints remains uncertain in one key respect: what if the mechanisms of development were shaped by natural selection to produce variants that are much fitter than one would expect by chance? Then the distinction between the generative and selective components of natural selection (see above) would blur. The developmental production of variants would no longer be random with respect to a species' ecology. This hypothesis, which was pushed furthest by Riedl (1977), is interesting and potentially profound, but not yet supported by much evidence.

Brains were historically considered to be shaped by natural selection, unencumbered by developmental constraints. In general, the size and structure of both entire brains and individual brain regions were thought to be optimized. Jerison (1973, p. 8), made this idea explicit when he wrote that "the importance of a function in the life of each species will be reflected by the absolute amount of neural tissue of that function in each species." How development produced that fine-tuning was never specified. Presumably, the idea was that genetic mutations could vary the size and structure of individual brain regions freely, leading to steady improvements in fitness until an optimum was reached. Little thought was given to the possibility that brains might be constrained in how they could evolve. However, a few authors proposed that trophic dependencies between interconnected brain regions might cause entire circuits or systems to change size in unison rather than piecemeal (Katz and Lasek, 1978). Such 'epigenetic cascades' (Wilczynski, 1984) might channel evolution (Katz *et al*., 1981), but they would not constrain natural selection, because the cascades help to optimize functional brain systems by matching the size of interconnected neuronal populations. That is, epigenetic cascades act not against, but in conjunction with, the optimizing power of natural selection; they are not classical constraints, which may explain why they have rarely been discussed (Finlay *et al*., 1987).

The idea of brains evolving under a restrictive developmental rule was proclaimed forcefully by Finlay and Darlington (1995). Their argument was founded on the observation that the various major brain regions in mammals scale against absolute brain size with different allometric slopes (Sacher, 1970; Gould, 1975; Jerison, 1989). Although this finding was well established at the time, it had not been explained; it was a scaling rule without a cause. Finlay and Darlington's major contribution was to propose that the height of a region's allometric slope was related to the region's date of birth (i.e., the time at which the region's precursor cells cease to divide), with late-born regions tending to become disproportionately large with increasing brain size. Why does this relationship exist? Finlay and Darlington (1995) showed that their late-equals-large rule emerges naturally if neurogenetic schedules (i.e., the schedules of what regions are born when) are stretched as brains increase in size and compressed when they shrink. This insight, in turn, prompted Finlay and Darlington to hypothesize that brain evolution is constrained to stretch or compress neurogenetic schedules and cannot, in general, delay or advance the birth of individual regions. In other words, even if evolution 'wanted' to increase the size of only one brain region, it would be 'forced' to change also the size of many other brain regions. Thus, Finlay and Darlington argued that development constrains brains to evolve concertedly, rather than mosaically.

Finlay and Darlington's developmental constraint hypothesis has been challenged by various authors, who all pointed out that brains do sometimes evolve mosaically (Barton and Harvey, 2000; Clark *et al*., 2001; de Winter and Oxnard, 2001; Iwaniuk *et al*., 2004; Safi and Dechmann, 2005). In addition, Barton (2001) has argued that correlations between region size and absolute brain size are due to functional requirements, rather than developmental constraints. Specifically, Barton (2001, p. 281) reported that the sizes of interconnected brain regions in what he called a functional system exhibited "significantly correlated evolution after taking variation in a range of other structures and overall brain size into account." Finlay *et al*. (2001) countered that such system-specific evolution may indeed occur, particularly for the so-called limbic system (see also Barton *et al*., 2003), but that this does not negate the existence of developmental constraints. In a review of this debate, I concluded that most of it may be resolved by arguing that instances of mosaic (and/or system-specific) evolution occur against a background of concerted, developmentally constrained evolution (Striedter, 2005; see Mosaic Evolution of Brain Structure in Mammals). Both Finlay and Barton seem open to this kind of rapprochement (Finlay *et al*., 2001; Barton, 2006).

The debate on mosaic versus concerted evolution highlights how little we know about the evolution of

neural development or, for that matter, about the role that natural selection played in shaping brains. The developmental data used to support Finlay *et al.*'s (2001) hypothesis came from just 15 species and were collected by several different laboratories, using diverse methodologies. Moreover, the data are limited to dates of neurogenesis. We know virtually nothing about species differences (or similarities) in how large brain regions are prior to neurogenesis, how quickly the regions grow, or how much cell death they endure. Data on these other, relatively neglected aspects of brain development might reveal additional constraints, and they might clarify how regions can evolve mosaically even if neurogenetic schedules are conserved.

Similarly lacking are data on natural selection and the brain. Although several analyses have shown that the size of some brain regions (relative to absolute brain size) correlates with aspects of a species' behavior or ecology (e.g., Clark *et al.*, 2001; de Winter and Oxnard, 2001; Iwaniuk *et al.*, 2004), such correlations are only indirect evidence for natural selection. More direct data are difficult to gather, because direct demonstrations of natural selection at work require measurements of heritability and fitness functions. As it is, we know so little about how selection acts on brains that debates on its potency are bound to erupt. Clearly, more studies must be performed before we can reach firm conclusions about which aspects of brain development and evolution are tightly constrained and which are subject to specific selective pressures.

1.01.5 One Law, Many Laws, or None

Is human history explicable in terms of general principles or laws? This question has been debated extensively. Some scholars insist that history is based largely on a few major laws, playing out against a background of far less important noise. Others argue, instead, that history is so full of contingencies (or accidents) that general or universal laws are blown to bits. I am not competent to review this debate but find myself most sympathetic to the intermediate position taken by Hempel (1942) in his call for a nomological–deductive approach to history. Basically, Hempel argued that historical events can be explained only by reference to various general (deterministic or probabilistic) laws that causally link preceding events or conditions to the event being explained. For example, an account of why an automotive radiator cracked during a frost would involve both historical contingencies and general laws relating temperature to pressure (Hempel, 1942). Similarly, events in human history can be explained by "showing that the event in question was not 'a matter of chance', but was to be expected in view of certain antecedent or simultaneous conditions" (Hempel, 1942) and the operation of several, often implicitly assumed, general laws. This nomological–deductive methodology waxes and wanes in popularity (Kincaid, 1996; McIntyre, 1996), but it seems logical in principle. Naturally, one may debate whether human behavior is predictable enough to yield the kind of laws that are needed for nomological–deductive explanations (Beed and Beed, 2000).

Evolutionary biologists have likewise debated the role of general laws in explaining the past, which in their realm is phylogeny. Some have argued that natural selection is a universal law that can be used to explain the emergence of many, if not most, biological features. Others have countered that natural selection is a mathematical truth, rather than an empirically determined law (Sober, 2000). More importantly, many biologists have pointed out that the results of natural selection are not highly predictable. Gould (1989) made this argument when he declared that rewinding the tape of life on earth and playing it again would not lead to a repeat performance. Biological history is full of accidents, of happenstance. Therefore, Gould argued, evolutionary explanations must be crafted one event at a time, without recourse to general laws. On the other hand, Gould did grant that evolution is constrained by diverse physical principles, by rules of construction and good design, and by some scaling rules (Gould, 1986, 1989). In his view, "the question of questions boils down to the placement of the boundary between predictability under invariant law and the multifarious possibilities of historical contingency" (Gould, 1989, p. 290). Gould placed this boundary "so high that almost every interesting event of life's history falls into the realm of contingency" (Gould, 1989, p. 290). This appears to be an extreme position, for many other evolutionary biologists place that same boundary lower. They tend to be far more impressed than Gould by the degree of convergent evolution in the history of life (Carroll, 2001; Willmer, 2003). They look, for example, at the convergent similarities of eyes in vertebrates and octopi and conclude that some design rules for eyes exist. In sum, disagreements persist about the placement of Gould's boundary between predictability and contingency, but most biologists accept that evolutionary explanations must involve at least some causal laws (Bock, 1999).

Given this context, it is not surprising that neuroscientists are conflicted about the importance of

general laws for explaining the evolutionary history of brains. Marsh (1886) had proposed that brains consistently increase in size over evolutionary time, but later authors vehemently disagreed (see Jerison, 1973; Buchholtz and Seyfarth, 1999). Personally, I think that Marsh did have a point, for brain and body size have both increased, at least on average, in several vertebrate lineages (see Striedter, 2005). Still, Marsh's laws were merely descriptions of phylogenetic trends, not causal laws. The first explicitly causal law of brain evolution was Ariëns Kappers' (1921) law of neurobiotaxis, which states that cell groups in evolution tend to move toward their principal inputs. Unfortunately for Ariëns Kappers, later studies showed that cell groups do not move quite so predictably and called into question some of the mechanisms that supposedly produced neurobiotaxis. The next major putative law of brain evolution was Ebbesson's (1980) parcellation principle, which states that brains become more complex by the division of ancestrally uniform cell groups into daughter aggregates that selectively lose some of their ancestral connections. This principle was strenuously criticized by most comparative neuroanatomists, mainly because its empirical foundation was shaky (see Ebbesson, 1984). Although a weak version of Ebbesson's theory, stating merely that brains become less densely connected as they increase in size, is probably defensible (Deacon, 1990a; Striedter, 2005), the strong version of Ebbesson's original idea has failed the test of time: plenty of data now show that brains evolve not only by the loss of connections, but also by creating novel projections.

Confronted with this abundance of failed brain evolution laws, most evolutionary neuroscientists have emphasized only a single, undisputed regularity of brain evolution, namely, that numerous aspects of brain structure and function are highly conserved across species. Specifically, they focused, á la Geoffroy St. Hilaire, on the existence of common plans of construction and highlighted molecular homologies between invertebrates and vertebrates (see above). This has been productive. It is important to note, however, that the principle of phylogenetic conservation predicts stability and does not deal explicitly with change. Is brain phylogeny subject to just a single law, which states that brains change little over time? Or are there also laws of evolutionary change in brains? I affirmed the second possibility (Striedter, 2005), but laws of evolutionary change in brains are no doubt difficult to find. C. J. Herrick, a founding father of evolutionary neuroscience, put it well:

> Most scientific research has been directed to the discovery of the uniformities of nature and the codification of these in a system of generalizations. This must be done before the changes can be interpreted. The time has come to devote more attention to the processes and mechanisms of these changes… but it is much more difficult to find and describe the mechanisms of … [the] apparently miraculous production of novelties than it is to discover the mechanical principles of those repetitive processes that yield uniform products (Herrick, 1956, p. 43).

The last few years have seen an uptick in the number of studies that address evolutionary change and novelty in brains (Aboitiz, 1995; Catania *et al*., 1999; Rosa and Tweedale, 2005), and modern research on brain scaling and developmental constraints (see above) has advanced our understanding of the regularities that lurk within brain variability. In addition, a rapidly increasing number of studies is beginning to reveal genomic changes that are probably linked to changes in brain size and/or structure (e.g., Dorus *et al*., 2004; Mekel-Bobrov *et al*., 2005). Therefore, the time Herrick discussed, when evolutionary change becomes a focus of analysis (see also Gans, 1969), is probably at hand.

Thus, I envision a future in which most evolutionary neuroscientists will embrace many different laws, some dealing with constancy and some with change. A few philosophers of science (e.g., Beatty, 1995) might decry such a vision, because they think that any natural law deserving of its name must apply universally, in all contexts and without room for other, countervailing laws. I have no training in philosophy, but think that all scientific laws apply only in specified domains and given assumptions (Striedter, 2005). In the real world, particularly in the complex world of biological systems, most laws or principles are sometimes excepted. This does not make them useless but, instead, prompts us to ask what causes the observed exceptional cases (West and Brown, 2005). If we understand the causal basis of our laws, then the exceptions should, with further work, become explicable. In other words, I think that evolutionary neuroscientists can fruitfully avail themselves of Hempel's nomological–deductive approach to history. To some extent, they always have.

1.01.6 Conclusions and Prospects

In summary, the history of evolutionary neuroscience features some serious missteps, such as the idea that brains evolved in a phylogenetic series and Ariëns Kappers' law of neurobiotaxis, but it also reveals considerable progress. The *scala naturae* has ceased to guide the research of evolutionary neuroscientists

and the idea of neurobiotaxis has quietly disappeared. The once stagnant field of brain allometry is showing signs of revival, largely because of new statistical techniques and a new emphasis on absolute brain size. The debate about concerted versus mosaic evolution persists, but directions for rapprochement are emerging. In general, the field has flirted with a broad variety of theoretical ideas and found some of them wanting and others promising. In terms of theory, the field is still quite young, but it is poised to mature now.

Predicting directions of growth for any science is problematic, but I believe that most future developments in evolutionary neuroscience will parallel developments in other, non-neural domains of evolutionary biology. After all, the history of evolutionary neuroscience is full of ideas that originated in non-neural areas of biology. For example, the methodology of phylogenetic reconstruction or cladistics (which I did not discuss in this article but have treated elsewhere; see Striedter, 2005) was originally developed by an entomologist (Hennig, 1950; see also Northcutt, 2001). Similarly, evolutionary developmental biology was burgeoning before it turned to brains (Hall, 1999). Therefore, I think it likely that the future of evolutionary neuroscience has already begun in some non-neural field. Maybe molecular genetics, with its new emphasis on evolutionary change (Dorus *et al*., 2004), will soon take center stage. Maybe the excitement about linking physiological allometries to metabolic parameters (West and Brown, 2005) will infect some mathematically inclined evolutionary neuroscientists. Or perhaps the next big thing in evolutionary neuroscience will be microevolutionary studies that integrate across the behavioral, physiological, and molecular levels (Lim *et al*., 2004). Maybe the future lies with computational studies that model *in silico* how changes in neuronal circuitry impact behavior (e.g., Treves, 2003). It is hoped that all of these new directions – and more – will bloom. If so, the field is headed for exciting times.

On the other hand, evolutionary neuroscientists are still struggling to make their findings relevant to other neuroscientists, other biologists, and other taxpayers (see Relevance of Understanding Brain Evolution). It may be interesting to contemplate the evolution of our brains, or even the brains of other animals, but can that knowledge be applied? Does understanding how or why a brain evolved help to decipher how that same brain works or, if it does not work, how it can be repaired? Are advances in evolutionary neuroscience likely to advance some general aspects of evolutionary theory? All of these questions remain underexplored (see Bullock, 1990).

Near the end of the nineteenth century, Jackson (1958) attempted to apply evolutionary ideas to clinical neurology, but his efforts failed. It has been pointed out that some species are far more capable than others at regenerating damaged brain regions (e.g., Kirsche and Kirsche, 1964) and that nonhuman apes tend not to suffer from neurodegenerative diseases such as Alzheimer's (Erwin, 2001). Such species differences in brain vulnerability and healing capacity might well help us elucidate some disease etiologies or lead to novel therapies. Unfortunately, this research strategy has not yet succeeded. Thus far, evolutionary neuroscience's most important contribution has been the discovery that human brains differ substantially from other brains, particularly nonprimate brains, which means that cross-species extrapolations must be conducted cautiously (Preuss, 1995). This is an important message, but it can be construed as negative in tone. Hopefully, the future holds more positive discoveries.

Work on justifying evolutionary science is especially important in the United States, where anti-evolutionary sentiment is on the rise. Many conservative Christians believe that evolution is a dangerous, insidious idea because it makes life meaningless (Dennett, 1995). Add to this fear the notion that our thoughts and feelings are mere products of our brains (e.g., Dennett, 1991) and evolutionary neuroscience seems like a serious threat to God's supremacy. Although this line of argument is well entrenched, Darwin and most of his immediate followers were hardly atheists (Young, 1985). Instead, they either distinguished clearly between God's words and God's works, as Francis Bacon put it, or argued that God's creative act was limited to setting up the laws that control history. Either way, God was seen as quite compatible with evolutionary theory. Moreover, Darwin's view of life need not produce a meaningless void. Instead, it helps to clarify our relationships with other humans, other species, and our environment. Those relationships, in turn, give meaning to our lives, just as linguistic relationships give meaning to our words. Thus, Darwin knew – and we would do well to recall – that evolutionary biology can be useful even if it yields no direct medical or technological applications. Even Huxley (1863), who was a very pragmatic Darwinian and coined the word 'agnostic', knew that the uniquely human quest to comprehend our place in nature is not driven by mere curiosity or technological imperatives, but by a profound need to understand ourselves, our purpose, our existence. Within that larger and enduring enterprise, evolutionary neuroscience will continue to play a crucial role.

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1.02 Metazoan Phylogeny

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Glossary

apomorphy An evolutionarily derived character or character state, an evolutionary novelty. An autapomorphy refers to a derived character or character state unique for a single taxon; a synapomorphy refers to a shared derived character or character state possessed by at least two taxa.

body plan Set of characters primitively shared by the members of a clade, irrespective of taxonomic level. These characters both include ancestrally inherited plesiomorphies and newly evolved apomorphies for that clade.

clade Monophyletic taxon, all descendants from a most recent common ancestor.

coelom Internal body cavity lined by a mesodermally derived epithelium.

crown group A clade of extant taxa, as distinct from a stem group (see also 'stem group').

eumetazoa Metazoa minus Porifera (traditionally Eumetazoa also excluded Placozoa, but new molecular findings indicate a less basal position of Placozoa).

grade A paraphyletic group of taxa.

homoplasy Independently evolved, nonhomologous features, such as convergences and independent losses.

Hox genes Important developmental regulatory genes involved in specifying positional information along animal body axes, including appendages.

monophyletic Having a single evolutionary origin. A monophyletic taxon includes an ancestor and all its descendants. See also 'paraphyletic'.

paraphyletic Having a single evolutionary origin, but differing from a monophyletic taxon by including an ancestor, but only a subset of its descendants.

phylum Highest Linnean category for classification of animals.

plesiomorphy Ancestral character or character state.

sister taxon The closest relative of a given taxon.

stem group A clade or grade of extinct taxa, as distinct from a crown group.

synapomorphy See 'apomorphy'.

1.02.1 Introduction to Metazoan Phylogeny

This article provides an outline of our current understanding of the phylogeny of the kingdom Metazoa, which comprises all multicellular animals (most authors equate Metazoa with Animalia, but a few apply the latter name to a more inclusive group of Metazoa plus Choanoflagellata, the most likely sister taxon of animals). Metazoan phylogenetics has a long pedigree. The first generation of evolutionary biologists in the period immediately following the publication of Charles Darwin's *On the Origin of Species* enthusiastically embarked upon the challenging task of reconstructing the genealogy of life (Bowler, 1996). The influential research program of evolutionary morphology that was established by Ernst Haeckel and Carl Gegenbaur at the University of Jena in Germany

had an especially prominent place at the cradle of metazoan phylogenetics as they and their followers brought both anatomical and developmental evidence to bear on the problems of animal phylogeny.

However, after a strong start in the last decades of the nineteenth century during which phylogenetic research virtually came to define evolutionary biology, interests in reconstructing the phylogeny of life diminished concomitant with a surge to embrace a more experimental approach to biological questions (see Relevance of Understanding Brain Evolution, Evolutionary Neuroethology – A Case Study: Origins and Evolution of Novel Forms of Locomotion in Hippid Sand Crabs (Malacostraca, Decapoda, Anomala).

Yet, although metazoan phylogenetics became a much less conspicuous discipline in biology at the beginning of the twentieth century, research into the deep history of the Metazoa certainly did not disappear altogether. Now, in the twenty-first century, the study of metazoan phylogeny has reached its zenith of popularity. Several factors are responsible for this situation. First, in the late 1980s and early 1990s the first analyses of metazoan phylogeny based on molecular sequence evidence were published, and these provided a huge stimulus for other workers to start analyzing metazoan phylogeny (Bergström, 1986; Field *et al.*, 1988; Lake, 1990). Importantly, the continued collection and analysis of new molecular evidence have now established a widely accepted so-called new view of animal evolution (Halanych, 2004).

Second, the early 1990s witnessed the first computerized cladistic analyses of morphological evidence (Schram, 1991; Eernisse *et al.*, 1992). The compilation and analysis of explicit morphological data matrices made the practice of morphological phylogenetics much more transparent than heretofore. Current phylogenetic analyses of the Metazoa habitually analyze both molecular and morphological data either separately or combined into a single large data matrix.

Third, the last quarter of the twentieth century also saw the gestation and birth of evolutionary developmental biology – evo-devo in short. The origins of this increasingly mature discipline similarly extend back to the evolutionary morphology of the late nineteenth century. Understanding the large-scale pattern of animal relationships is central to evo-devo because at the core of its research agenda is the desire to understand the evolution of animal body plans in terms of changes in developmental patterns and processes (Hall, 1999).

This article will outline our current understanding of metazoan phylogeny on the basis of available molecular and morphological evidence. The reader should note, however, that no definitive picture of animal relationships can be presented at this time. Metazoan phylogenetics is currently a highly active discipline characterized by a great flux of ideas. Many questions still remain unresolved, and the hope for an overarching consensus should at this time be tempered by the realization that different data sources and methods of analysis may yield conflicting phylogenies, as will be discussed below. Therefore the picture of metazoan phylogeny presented in this article is necessarily provisional. However, one should not underestimate the significant progress that has been made in our understanding of animal relationships in many different parts of the metazoan phylogeny in the last 15 years or so.

It should be noted that the subject area covered in this article is enormous, covering a huge literature. In view of space limitations and in the interest of readability I have included only a minimum of in-text citations, principally only a small sample of important recent and comprehensive works that are expressly focused on high-level metazoan phylogenetics, and review papers. These key references should help guide the interested reader into the vast literature on animal phylogeny. I especially recommend Halanych (2004) and selected chapters in Cracraft and Donoghue (2004) as optimal starting points for a more indepth study of metazoan phylogeny.

1.02.1.1 Data

This section will briefly introduce the different kinds of evidence used to reconstruct metazoan phylogeny, and review their respective strengths and weaknesses. Until about 15 years ago all metazoan phylogenies were strictly based upon morphological evidence, including information about embryology. With the advent of molecular phylogenetics this situation has changed dramatically, and many workers now prefer to infer phylogenies solely with molecular data, or in combination with morphological evidence. In contrast, information derived from fossils plays only a minor role in metazoan phylogenetics, with the exception of several taxa for which a relatively rich and detailed fossil record is available, notably the arthropods, echinoderms, priapulids, brachiopods, and chordates.

1.02.1.1.1 Molecules Our understanding of the tree of life on all levels is increasingly based upon molecular evidence. Although many zoologists still endorse the value of morphological evidence, the molecular hegemony is increasingly gaining strength in metazoan phylogenetics as well.

The foundation for the new view of metazoan phylogeny is firmly based upon analysis of the 18S rRNA, or nuclear small ribosomal subunit (SSU), gene (Eernisse and Peterson, 2004; Halanych, 2004). The SSU gene was one of the first genes that was sequenced for species belonging to different animal phyla. For most of the traditionally recognized animal phyla the SSU gene has now been sequenced for at least one species, and for increasing numbers of phyla multiple species have been sequenced. Consequently, the SSU gene is currently the most broadly sampled gene within the Metazoa. Its relatively slow mutation rate makes it an appropriate gene to study the deep divergences of the Metazoa, but the SSU gene has not resolved all high-level phylogenetic questions.

To complement and test the results based on the SSU gene, researchers are now investigating the phylogenetic utility of different molecular loci as well, including the 28S rRNA, or nuclear large ribosomal subunit (LSU) gene, the myosin heavy-chain type II gene, various mitochondrial genes, as well as mitochondrial gene order, Hox genes, and the genes for elongation factor-1α and elongation factor-2 (Giribet, 2002, 2003; Ruiz-Trillo *et al.*, 2002; Halanych, 2004).

In addition, the sequencing of the entire genomes of several model metazoans such as the nematode *Caenorhabditis elegans*, the fly *Drosophila melanogaster*, and the primate *Homo sapiens* has allowed for a different strategy of phylogenetic analysis. Instead of sampling many taxa for the same gene, several phylogenomic studies have recently been published that have attempted to reconstruct metazoan phylogeny by comparing large numbers of homologous sequences for just the small set of animals for which genome sequence data are available (Copley *et al.*, 2004; Wolf *et al.*, 2004; Dopazo and Dopazo, 2005; Philip *et al.*, 2005; Philippe *et al.*, 2005). Interestingly, the results of several of these studies do not support the findings of phylogenetic analyses based on fewer genes, but with larger samples of species. This apparent discrepancy focuses attention on two important issues.

First, these apparent phylogenetic conflicts indicate the importance of adequate taxon sampling. In order to represent properly phylogenetically important variation in gene sequences, and to prevent being misled by homoplasy, phylogeneticists should aim at a sufficiently large representation of metazoans in their phylogenies.

Second, recent research has shown that, for the same set of taxa, independent phylogenetic analyses based on only one or a few genes can be in significant conflict with each other (Rokas *et al.*, 2003b). However, in such cases the conflict may be resolved by combining all molecular evidence into a single phylogenetic analysis, which may then yield a single, well-supported phylogeny. This shows that it is crucial to study metazoan phylogeny with multiple molecular loci, and that one should be wary of accepting phylogenies based on just one or two loci.

As an illustration of the high pace of developments in the discipline, new studies published between submission and revision of this article show that the conflict between standard molecular phylogenetic analyses and phylogenomic studies is probably only apparent (Copley *et al.*, 2004; Philippe *et al.*, 2005). It is the probable result of long-branch attraction resulting from insufficient sampling in the first phylogenomic studies. The long-diverged gene sequences of the distantly related model organisms have accumulated so many mutations that chance similarities may cause them to be grouped together in a phylogenetic analysis. Increasing taxon sampling may break up such long branches, decreasing the conflict with the much-better-sampled analyses that focus on only one or a few genes.

Finally, molecular evidence has not only added greatly to our ability to reconstruct metazoan phylogeny, it has also allowed us for the first time to estimate the approximate divergence times of the major metazoan taxa, even when the fossil record is mostly mute about most of these divergence events (Smith and Peterson, 2002). This application of molecular evidence is among the most exciting, but also the most controversial of topics in evolutionary biology. Of particular interest are the problems of apparent discrepancies of divergence time estimates based on molecular and fossil evidence observed for many groups of organisms, ranging from vascular plants to birds, and the possibility that the major metazoan lineages diverged very rapidly, making it very difficult to reconstruct and precisely date the sequence of divergence events giving rise to modern crown groups (Smith and Peterson, 2002; Graur and Martin, 2004; Reisz and Müller, 2004; Rokas *et al.*, 2005; Ho and Larson, 2006). However, through an increasing understanding of the relative strengths and weaknesses of molecular and fossil divergence times estimates, recent studies have been able to bring molecular estimates of divergence times and the metazoan fossil record increasingly in closer agreement, although a period of cryptic evolution of the major metazoan lineages is still suggested, about which the fossil record is silent.

1.02.1.1.2 Morphology Morphological characters, obtained from the study of all stages of the life cycle, from zygote to adult organism, had been the sole source of phylogenetic information until the late 1980s. Even today morphological evidence is habitually used for phylogenetic analyses of the Metazoa, either alone, or in combination with molecular evidence (Ax, 1995–2001; Zrzavy *et al.*, 1998; Giribet *et al.*, 2000; Nielsen, 2001; Peterson and Eernisse, 2001; Glenner *et al.*, 2004; Jenner and Scholtz, 2005).

Certain types of morphological character have traditionally been imbued with great phylogenetic value. The most familiar major divisions in the animal kingdom reported in textbooks reflect many of these characters. For example, the possession of bilateral symmetry, with distinct anteroposterior, dorsoventral, and left–right axes has long been regarded as the principal synapomorphy of the Bilateria. The possession of a body composed of three germ layers, ectoderm, endoderm, and mesoderm, is reflected in the name Triploblastica, a synonym of Bilateria.

Within the Bilateria the clades Protostomia and Deuterostomia are typically diagnosed on the basis of different fates of the embryonic blastopore, which characteristically is said to give rise to the adult mouth in protostomes, and the anus in deuterostomes.

Great phylogenetic value has also been attached to the possession of characteristic cleavage patterns in the early embryo. For example, the widely recognized clade Spiralia is characterized by spiral cleavage, found in such phyla as the mollusks, annelids, and nemerteans.

The nature of body cavities has been of paramount importance in metazoan phylogenetics, and the bilaterian groups Coelomata, Pseudocoelomata, and Acoelomata have often been distinguished in textbooks, with the first possessing a coelom, and the latter two lacking a coelom.

In addition to these important characters, the organization of the central nervous system (brain structure and configuration of main nerve cords) and the nature of the life cycle (indirect development with larvae, or direct development from egg to adult without an intervening free-living larva) have also played important roles in generating phylogenetic hypotheses for the Metazoa. Currently available morphological data matrices may include hundreds of characters, and the largest published morphological data set for the Metazoa included more than 16 000 data entries (Zrzavy *et al.*, 1998).

Despite this wealth of morphological information and the publication of a significant number of morphological phylogenetic analyses of the Metazoa over the 15 years, no detailed consensus view of animal relationships has yet resulted on the basis of morphology alone. This is because the selection and interpretation of phylogenetic characters are fraught with difficulties, and different decisions feeding into data matrix constructions may lead to different phylogenies (Jenner and Schram, 1999; Jenner, 2001, 2003, 2004a). Even when a data set has been properly compiled, homoplasy of characters may mislead the phylogenetic analysis. Recent research increasingly shows that key phylogenetic characters once thought to have evolved only once may in fact be evolutionarily very labile.

1.02.1.1.3 Fossils Although fossils may be thought of as providing perhaps the most direct evidence of the course of evolution, fossils have nevertheless not played a leading role in establishing high-level metazoan phylogeny. So far the fossil record remains largely silent about the details of the origin of the animal phyla, but as the fossil record continues to be mined, new and valuable insights into metazoan evolution continue to accrue (Valentine, 2004).

The most important contribution of fossils to metazoan phylogeny is in supplying the information to reconstruct the stem taxa of modern or crown group taxa (Budd and Jensen, 2000). Of special relevance are various exceptionally preserved Cambrian faunas, such as the Burgess Shale fauna of British Columbia, the Sirius Passet fauna of northern Greenland, and the Chengjiang fauna of southwest China. Fossils collected at these and other localities have greatly informed the early evolution of groups such as the arthropods, priapulids, and chordates. However, the phylogenetic significance of many fossils remains elusive, in particular the highly problematic Ediacaran (Precambrian) fossils, which may be a diverse assemblage that contains genuine members of extant phyla, and real oddballs that cannot be placed inside the Metazoa.

The inclusion of fossils into phylogenetic analyses of living taxa can have a huge impact on tree topology (Wheeler *et al.*, 2004; Lee, 2005). Consequently, ignoring available fossil evidence cannot be easily justified, even when one is only interested in the phylogenetic relationships of living taxa.

1.02.1.2 Methods

All widely used phylogenetic methods have been used to reconstruct metazoan phylogeny, including distance methods, parsimony analysis, likelihood

analysis, and, most recently, Bayesian analysis. Currently, molecular sequence data have been analyzed with all these methods, and morphological evidence has been studied with both parsimony and Bayesian analysis.

1.02.2 Overview of Major Metazoan Clades and Grades

Comprehensive molecular and morphological phylogenetic analyses have generated abundant support for the monophyly of the Metazoa. Among the morphological features shared by most metazoans are the possession of an extracellular matrix, intercellular junctions such as septate junctions, spot desmosomes, and spermatozoa. On the molecular level the evolution of at least one Hox gene seemed to have accompanied the origin of the Metazoa. The multicellularity characteristic of plants and fungi has evolved independently of metazoan multicellularity.

Among the unicellular eukaryotes the choanoflagellates are likely the closest living relatives of the animals (Lang *et al.*, 2002). Morphological and functional similarities between choanoflagellate cells and the choanocytes found in sponges (Porifera) have long been interpreted as providing support for a close relationship between animals and choanoflagellates (Maldonado, 2004). Increasing research efforts are now under way to elucidate the genomic makeup of the choanoflagellates, which could yield insights into the nature of the first metazoan genomes.

Recent phylogenetic analyses of SSU and LSU sequences have suggested another protistan candidate as the closest relative of metazoans. These studies found the mesomycetozoans (also know as ichthyosporeans) to be the closest relative of either the Metazoa or the choanoflagellates. Nevertheless, because the mesomycetozoans are mostly specialized tissue parasites, the free-living choanoflagellates may better serve as less-modified models of the metazoan ancestor.

The remainder of this section presents a brief overview of the main branches on the metazoan phylogeny (Figure 1). This is followed by a more detailed overview of the precise composition and phylogenetic relationships within the main animal clades (Figures 2–8).

1.02.2.1 Nonbilaterians and Acoelomorpha

Although the Porifera (sponges), Placozoa (*Trichoplax adhaerens*), Cnidaria (e.g., jellyfish, sea anemones), and Ctenophora (comb jellies) are

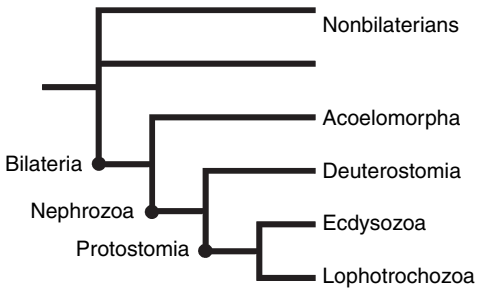

Figure 1 Concise phylogeny of the Metazoa, indicating the main grades and clades. A grade of nonbilaterians comprises Porifera, Placozoa, Cnidaria, Ctenophora, and Myxozoa. The Bilateria comprises the Acoelomorpha, which unites Acoela and Nemertodermatida. The remaining bilaterians are called Nephrozoa because of the widespread occurrence of nephridia. The three main clades are Deuterostomia, Ecdysozoa, and Lophotrochozoa. See text for further discussion.

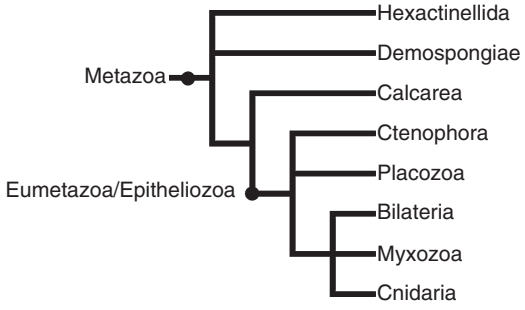

Figure 2 Basal metazoan relationships. The poriferans form a basal grade, with calcarean sponges more closely related to the other metazoans. The branching sequence of the nonbilaterians is not well understood. Cnidaria, and possibly Myxozoa, are probably most closely related to the Bilateria. See text for further discussion.

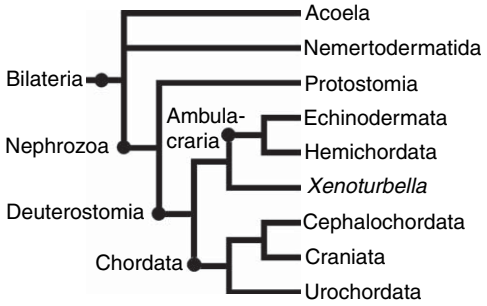

Figure 3 Overview phylogeny of the Bilateria, with an emphasis on deuterostome phylogeny. The acoels and nemertodermatids are the earliest diverging extant bilaterians. The Protostomia and Deuterostomia are likely sister taxa. Within the deuterostomes there are two sister clades. The clade Ambulacraria unites echinoderms and hemichordates, and probably *Xenoturbella*. The clade Chordata unites the urochordates, cephalochordates, and the craniates. See text for further discussion.

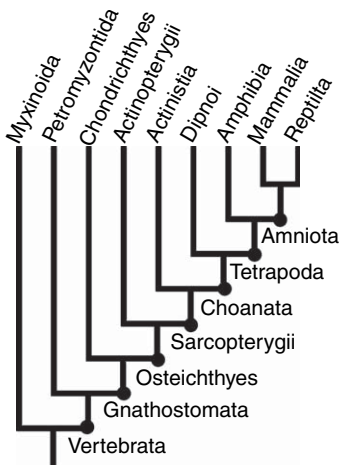

Figure 4 Craniate phylogeny. The extant agnathans (hagfish and lamprey) form two successive sister groups to the gnathostomes. Within the Gnathostomata there is a basal division between bony fishes (Osteichthyes) and cartilaginous fishes (Chondrichthyes). Within the clade of bony fishes there is a division between lobe-finned fishes (Sarcopterygii) and ray-finned fishes (Actinopterygii). Coelacanths (Actinistia) and lungfishes (Dipnoi) are successive sister taxa to the Tetrapoda, which include all terrestrial vertebrates. See text for further discussion.

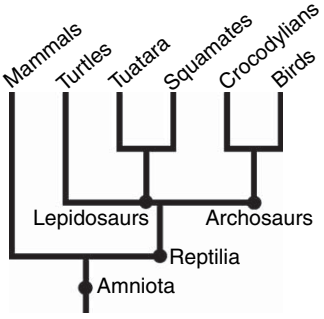

Figure 5 Phylogeny of extant amniotes, with emphasis on basic reptile relationships. Among living amniotes the mammals and reptiles are sister clades. Within the living reptiles, Lepidosauria and Archosauria are likely sister taxa. The tuataras and squamates (snakes and lizards) comprise the Lepidosauria, while birds and crocodilians comprise the Archosauria. The phylogenetic position of the turtles remains unresolved. See text for further discussion.

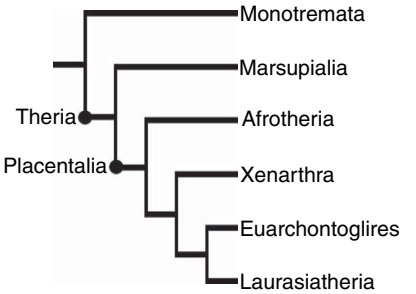

Figure 6 Phylogeny of extant mammals. The living monotremes (platypus and echidnas) and marsupials are successive sister taxa to the living placental mammals. Four major clades comprise Placentalia. The Afrotheria and Xenarthra have southern hemisphere origins, in Africa and South America, respectively. The clades Euarchontoglires and Laurasiatheria have northern hemisphere origins. Afrotheria includes elephants, manatees, golden moles, and aardvarks. Xenarthra comprises anteaters, sloths, and armadillos. Euarchontoglires includes primates and rodents. Laurasiatheria includes artiodactyls, perissodactyls, cetaceans, bats, and carnivores. The Afrotheria and Laurasiatheria comprise two independent parallel radiations of similar morphologies, ranging from mole-like forms to ant-eating forms. See text for further discussion.

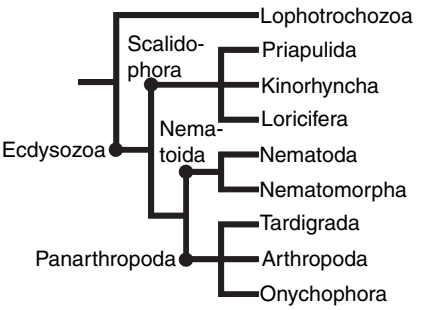

Figure 7 Protostome phylogeny with emphasis on ecdysozoan relationships. The three main ecdysozoan clades are Scalidophora, Nematoida, and Panarthropoda. The scalidophorans comprise the loriciferans, penis worms (priapulids), and mud dragons (kinorhynchs). Nematoida comprises the horsehair worms (nematomorphs), and roundworms (nematodes). Panarthropoda comprises the arthropods, water bears (tardigrades), and velvet worms (onychophorans). See text for further discussion.

often typified as diploblasts, these earliest diverging nonbilaterian metazoans (Figures 1 and 2) could hardly be said to be characterized by the possession of a common body plan, nor could they be considered as members of a monophyletic clade. I therefore prefer to refer to them simply as nonbilaterians. Although the precise evolutionary branching sequence of these groups is still contentious (Rokas *et al.*, 2003a), they likely form a grade of organization (paraphyletic group) basal to the Bilateria. The morphological disparity between these phyla spans an enormous range of body architectures, with the maximally simple placozoans at opposite extremes, with just four differentiated somatic cell types, and the cnidarians, of which some of the most complex forms, such as cubozoans, possess differentiated muscle and nervous systems, and some remarkably complex sensory organs. The elucidation of the precise sequence of divergences of these taxa is therefore vital to understanding the assembly of more complex body plans at the base of the Bilateria. However, all these groups are traditionally considered to lack bilateral symmetry.

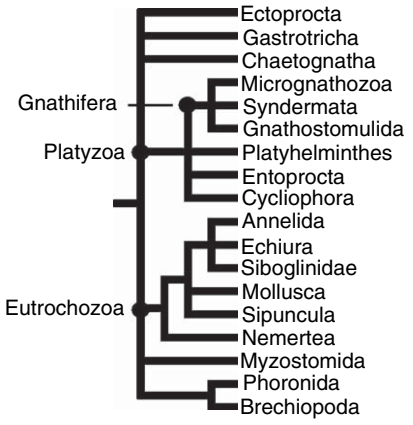

Figure 8 Phylogeny of the Lophotrochozoa. Only several clades can currently be recognized. Phoronids and brachiopods form a clade. The eutrochozoans form a clade characterized by a trochophore larva (also found in entoprocts). The Platyzoa is a putative clade of noncoelomate lophotrochozoans, and the clade Gnathifera is supported by characteristic features of their cuticularized jaws. The relationships of the remaining members of the Lophotrochozoa remain unresolved. See text for further discussion.

Surprisingly, recent investigations have added another group to the base of the Bilateria. The enigmatic, microscopical, and parasitic myxozoans were until very recently considered to be protozoans. However, recent advances in molecular phylogenetics and ultrastructural research have established their metazoan affinities, and their possible relationships to either the cnidarians or the Bilateria (Okamura and Canning, 2003; Zrzavy and Hypsa, 2003).

The earliest diverging unambiguously bilaterally symmetrical organisms appear to be the Acoelomorpha, comprising two taxa: Acoela and Nemertodermatida (Figures 1 and 3). Previously considered to be basal flatworms (Platyhelminthes), molecular data and a reinterpretation of available morphological evidence instead suggest that these relatively simple flatworm-like organisms are the most basally branching crown group bilaterians (Baguñà and Riutort, 2004). Consequently, the acoelomorphs are considered to be the sister group to the remaining bilaterians, the three main clades of which will be introduced below.

1.02.2.2 Deuterostomia

The deuterostomes are a well-circumscribed clade, comprising the Echinodermata (e.g., sea stars, sea lilies, sea urchins, sea cucumbers), Hemichordata (acorn worms and pterobranchs), and Chordata (sea squirts, lancelets, craniates plus vertebrates) (Figures 1 and 3–6). Traditionally, the membership of the Deuterostomia was considered to be wider, also including the three lophophorate phyla Brachiopoda (lamp shells), Phoronida, and Ectoprocta (moss animals), and frequently the Chaetognatha (arrow worms) as well. However, re-evaluation of morphological evidence (Lüter, 2000; Bartolomaeus, 2001; Gruhl *et al.*, 2005) is throwing serious doubt on the deuterostomian synapomorphies of brachiopods and phoronids, and accumulating molecular evidence now firmly places the lophophorates in the protostome clade Lophotrochozoa, while the phylogenetic position of the chaetognaths remains elusive. More recently, the enigmatic worm *Xenoturbella bocki* has been proposed to be a deuterostome as well.

Apart from clearly circumscribing the membership of the Deuterostomia, recent research has also reorganized the phylogenetic relationships within the deuterostomes on many levels (Blair and Hedges, 2005). In contrast to traditional ideas, the echinoderms and hemichordates are now united as sister groups in a clade Ambulacraria. Furthermore, within the chordates, the sister group relationship between the cephalochordates (lancelets) and the craniates (hagfish plus vertebrates) has been consolidated. However, the widely accepted position of the tunicates as the sister group to cephalochordates plus craniates has now come under fire from phylogenomic analyses that support instead an unexpected sister group relationship between tunicates and vertebrates, to the exclusion of the cephalochordates (Blair and Hedges, 2005; Delsuc *et al.*, 2006). Intriguingly, a recent morphological phylogenetic analysis yielded the same result (Ruppert, 2005).

A large amount of new phylogenetic research within the vertebrates on many taxonomic levels has in several instances generated an evolutionary picture that is significantly at odds with established views, for example relationships within the placental mammals (Figure 6; Murphy *et al.*, 2004; Springer *et al.*, 2004). Since invertebrates comprise the vast majority of animal diversity this article is chiefly concerned with invertebrate phylogeny. However, in view of the fact that vertebrates are disproportionately represented in research on nervous systems, a brief overview of our current understanding of vertebrate phylogeny will also be presented (see Section 1.02.3.3; Figures 4–6).

1.02.2.3 Ecdysozoa

The Ecdysozoa is the second major clade within the Bilateria (Figures 1 and 6), and it includes a subset of the animal phyla generally considered part of the

Protostomia. The key synapomorphy uniting the ecdysozoans is the possession of a cuticle that is periodically molted (a process named ecdysis). The ecdysozoan phyla are the arthropods (e.g., insects, crustaceans, myriapods, and chelicerates), onychophorans (velvet worms), tardigrades (water bears), nematodes (roundworms), nematomorphs (horsehair worms), priapulids, loriciferans, and kinorhynchs (mud dragons). Probably concomitant with the evolution of a cuticle that covers the entire body surface, the ecdysozoans lost the motile epidermal cilia that are widespread in other animal phyla. Moreover, in contrast to many other invertebrates, the life cycle of ecdysozoans does not include a free-living ciliated larval stage. They are therefore referred to as exhibiting direct development, instead of indirect development via a larval stage, which characterizes the life cycles of many marine invertebrates.

The discovery of the clade Ecdysozoa has generated a large amount of debate, principally by refuting the widely accepted Articulata hypothesis (Giribet, 2003; Jenner and Scholtz, 2005). Traditionally, the annelids and arthropods are considered to be closely related as articulatans on the basis of the shared possession of body segmentation. The striking morphological and developmental similarities between segmentation in these two groups were widely regarded as being among the most reliable homologies that could be identified between different animal phyla. By placing the arthropods in the Ecdysozoa, and relegating the annelids to the third major clade of bilaterians, the Lophotrochozoa, it had to be assumed that either segmentation evolved independently in annelids and arthropods, or if in fact homologous, segmentation must have been lost in many other animal phyla.

1.02.2.4 Lophotrochozoa

The third major clade within the Bilateria is Lophotrochozoa (Figures 1 and 8). This clade includes the largest number of traditionally recognized animal phyla, and it embodies the greatest amount of disparity among body plans found within the Metazoa. Lophotrochozoans span an enormous range of body architectures, life cycles, developmental modes, habitats, behaviors, and include both the smallest and the biggest living invertebrates, ranging from the tiny loriciferans that measure about a tenth of a millimeter, to the gigantic deep-sea squids with body lengths measuring in multiple meters. Consequently, without question the Lophotrochozoa represents the phylogenetically most challenging metazoan clade.

The name Lophotrochozoa derives from the fact that many members of this clade either possess ciliated feeding tentacles, called a lophophore, such as the brachiopods and phoronids, or they include a ciliated trochophore larva in their life cycles, such as the mollusks, annelids, echiurans (spoon worms), sipunculans (peanut worms), and the entoprocts. However, the Lophotrochozoa also includes the ectoprocts (moss animals), platyhelminths (flatworms), nemerteans (ribbon worms), gnathostomulids, rotifers, acanthocephalans, cycliophorans, micrognathozoans, and possibly the gastrotrichs and chaetognaths.

Traditionally, all nondeuterostome animals were united in a clade Protostomia, and indeed in several recent phylogenetic analyses the clades Lophotrochozoa and Ecdysozoa together form a clade Protostomia as sister group to the deuterostomes. However, molecular phylogenetic analyses have not yielded strong support for the monophyly of Protostomia. Moreover, the value of the morphological characters in support of a clade Protostomia is currently being reinterpreted, and it might turn out that the protostomes are a paraphyletic taxon, perhaps giving rise to the deuterostomes.

1.02.3 Phylogenetic Relationships Within the Major Clades and Grades

In this section a detailed description will be given of our current state of understanding of the phylogeny of the Metazoa. However, as explained in the introduction, the reader should realize that no fully resolved high-level metazoan phylogeny is yet available. As a result, the view of metazoan phylogeny presented here is a conservative estimate based on the synthesis of available molecular and morphological data. The metazoan phylogeny presented here does not represent the result of a single comprehensive phylogenetic analysis of all available evidence. Wherever it is relevant, congruence and conflict between data sources will be mentioned.

1.02.3.1 Nonbilaterians: Porifera, Placozoa, Ctenophora, Cnidaria, and Myxozoa

Porifera, Placozoa, Ctenophora, Cnidaria, and Myxozoa are the earliest diverging animal groups (Figures 1 and 2). Available evidence supports Porifera as the basal-most of these taxa. However, in contrast to traditional ideas, the sponges may not form a clade, and the interrelationships of the other basal metazoans are unclear at the present time (Rokas *et al.*, 2003a; Halanych, 2004).

Three main groups of living sponges are recognized: (1) Calcarea, or sponges with a skeleton of calcareous spicules; (2) Demospongiae (including most familiar sponges); and (3) Hexactinellida (the glass sponges, whose bodies are largely composed of syncytial tissues). Both demosponges and hexactinellids possess a skeleton of siliceous spicules.

The three groups of sponges share a unique adult body plan that is characterized by the possession of a water canal or aquiferous system, which consists of a complex of spaces inside the sponge body that are in open connection with the surrounding water. These spaces are lined by so-called collar units that are built from either single cells (choanocytes or collar cells) or syncytia (choanosome). These special cells bear a single flagellum that is encircled by a collar of microvilli. This microvillar collar assists in the capture of food particles from the water inflow created by the beating flagella. Sponges possess only a few general types of differentiated cell types, and the nerve cells, special sensory cells, and muscle cells that are characteristic of other metazoans are not developed in sponges (some hexactinellids do show the ability to conduct electrical impulses along their syncytia, and demosponges may possess contractile cells, sometimes referred to as myocytes).

Although traditionally considered to be a monophyletic group, recent molecular (SSU) phylogenetic analyses with a broad sampling of basal metazoans and nonmetazoan outgroups have instead found Porifera to be paraphyletic. Intriguingly, the calcareans were found to be the sister group to the remaining metazoans, which may be called Eumetazoa, with the demosponges and hexactinellids diverging earlier. The precise relationships of the demosponges and hexactinellids to each other and to the clade Calcarea plus Eumetazoa are not yet clear.

The paraphyly of the sponges (Figure 2) provides significant support for the hypothesis that the eumetazoan ancestor was sponge-like. Sponges have generally not been considered a fertile substratum for the evolution of eumetazoan diversity. Their morphological simplicity on the one hand (e.g., lacking true germ layers, muscles, nervous and sensory systems, and generally, epithelia with basement membranes), and their morphological and functional specialization as epibenthic, sessile, and semipermeable water filters on the other hand, have led sponges to be regarded as a specialized dead end in evolution. Now, possible sponge paraphyly forces us to consider them more seriously as a starting point from which to derive eumetazoan diversity. The recent description of the carnivorous sponge *Asbestopluma hypogea*, which has completely lost the characteristic poriferan aquiferous system with choanocytes, and thus its ability to filter-feed, exemplifies that in principle the poriferan body plan has the flexibility to transform into a fully functional macrophagous feeder, a transition that is thought to have taken place during the early evolution of the Metazoa (Vacelet and Duport, 2004).

Furthermore, molecular biological research has shown that the sponge genome contains a surprising array of genes that code for important structural and regulatory molecules present in more derived metazoans, such as the bilaterians (Müller, 2003). These molecules include a range of transmembrane receptor molecules, transcription factors, extracellular matrix proteins, and potential neurotransmitters and antibodies (neuronal-like receptors, homeobox genes, tyrosine kinases, and phosphatases, serotonin, crystallin, integrin, fibronectin, and immunoglobulin-like molecules). Extensive gene duplication is also indicated to have taken place before the divergence of the sponges and the remaining metazoans.

Only one species of placozoan has been described so far, *Trichoplax adhaerens*, and all placozoans known to date conform to the morphological description of *T. adhaerens*. However, new evidence from nuclear and mitochondrial markers in placozoans collected from different localities around the world shows a remarkable degree of genetic divergence, with genetic distances being equal to those between different families of other marine nonbilaterians (Voigt *et al.*, 2004). Placozoans are small (usually 1–2 mm long), flat, ciliated, creeping organisms, without any fixed body axis. The body is composed of just four differentiated somatic cell types, organized into a ventral and a dorsal epithelium, and an enclosed space with so-called fiber cells. The presence of true epithelia in placozoans, with cells connected by belt desmosomes and septate-like junctions has been interpreted as a synapomorphy with all animals excluding sponges, which are generally thought to lack these features. The clade of Placozoa plus all nonsponge animals is therefore sometimes named Epitheliozoa (Figure 2). However, evidence for true epithelia exists in sponges as well, although it is frequently overlooked.

However, the consensus based strictly on morphological evidence that placozoans are the sister group to the Eumetazoa is now contested by accumulating molecular evidence (SSU). Molecular evidence suggests that placozoans may be less basal metazoans than previously thought, diverging from the remaining animals after the

morphologically much more complex ctenophores or ctenophores plus cnidarians have branched off (Wallberg *et al.*, 2004). This would imply that placozoans have become morphologically extremely simplified, losing complex differentiated tissues and organs, including a nervous system, muscles, and sensory organs. Although there is some preliminary phylogenomic research that has indicated the possibility that Placozoa are in fact the sister group to all metazoans, including sponges, this finding has not yet been convincingly substantiated.

In addition, efforts are under way to sequence the whole genome of *Trichoplax*, and genetic studies as well as the first analyses of expression patterns of developmental regulatory genes have started to yield new insights into the biology of Placozoa, such as the presence of genetic signatures of the occurrence of sex in placozoans (placozoan sex has never been observed), and hints indicating that *Trichoplax* may exhibit a greater degree of histological differentiation than previously thought (Jakob *et al.*, 2004; Signorovitch *et al.*, 2005).

Living ctenophores are very delicate, transparent, mostly pelagic, biradial animals, and they exhibit a passing resemblance to cnidarian jellyfish (hence their vernacular name comb jellies). However, they can be clearly distinguished from medusae by the possession of eight rows of comb plates that run the length of the body. Comb plates consist of closely apposed cilia of several aligned cells. Ctenophores also possess a complex aboral apical organ that is the main sensory center of the animal. An additional unique feature found in many ctenophores is the colloblast, a specialized epidermal cell type located on the tentacles of tentaculate ctenophores that is used for catching prey. Ctenophores possess nerve cells organized in a nerve net at the base of the epidermis and in the mesodermal derivatives. They also possess subepidermal, nonepithelial muscle cells, which are also widespread in bilaterians, but are primitively absent from the other nonbilaterians, including cnidarians. In common with bilaterians, ctenophore muscles are derived from endomesoderm, although genuine epithelial germ layers are not formed during ctenophore development.

The possession of mesodermally derived nonepithelial muscles has been taken as the main character to indicate a sister group relationship between ctenophores and bilaterians. Several workers defend the union of ctenophores and cnidarians in a clade Coelenterata on the basis of similarities in the early embryonic development of these groups, but molecular evidence (SSU and LSU) does not support either this or the previous hypothesis. Instead, these data indicate that ctenophores are very basal metazoans, grouping either with calcarean sponges, or as a sister group to calcareans and the remaining metazoans. Yet, a recent comprehensively sampled phylogenetic analysis of SSU sequence data dismissed these results as being artifacts due to insufficient taxon sampling, and instead found strong support for a sister group relationship between ctenophores and (Cnidaria plus Bilateria) (Wallberg *et al.*, 2004).

The new basal phylogenetic position of ctenophores or their close juxtaposition to the much simpler calcarean sponges has interesting implications for our understanding of the course of character evolution if proved correct. It may imply that the morphological features shared between bilaterians, cnidarians, and ctenophores, including well-developed nervous systems, muscles, sensory organs, and digestive system, are either convergent on some level or have been lost altogether in calcarean sponges.

Cnidaria is a well-supported monophyletic group, comprising the Anthozoa (including sea anemones and corals), Hydrozoa (including the familiar laboratory animal *Hydra* and the colonial siphonophores), Scyphozoa (including most familiar jellyfishes), and Cubozoa (including the notorious sea-wasps). Cnidarians exhibit approximately the same morphological grade of organization as the ctenophores, including a nervous system in the form of a diffuse nerve net (localized nerve concentrations may be found, for example, around the mouth of polyps, and the bell of medusae), epithelio-muscle cells (nonepithelial muscle cells in certain groups are thought to have evolved convergently to those in ctenophores and bilaterians), and an alimentary canal.

Cnidarians are definitely more closely related to the bilaterians than the sponges, and although this is a tentative conclusion, they may represent the sister taxon to the bilaterians (Figure 2; Halanych, 2004; Wallberg *et al.*, 2004). This is supported by ribosomal gene sequences, information from Hox genes, and SSU secondary structure. However, their relationship to placozoans, ctenophores, and myxozoans remains contentious. Significantly, researchers are increasingly focusing on the cnidarians to understand the origin of evolutionary novelties at the base of the Bilateria, including the molecular genetic underpinnings of the establishment of the main body axes and bilateral symmetry, the mesodermal germ layer, and sensory organs. As mentioned above for sponges, the genome of cnidarians contains a set of genes involved in the elaboration of complex morphologies in

bilaterians, but without forming these complex structures themselves. Studying the functions of these genes in basal metazoans could yield precious insights into the origin of novelties during animal evolution. In contrast, cnidarian genomes also seem to have retained a set of genes from nonmetazoan ancestors, which have been lost in the bilaterians (Technau *et al.*, 2005). This hints at the role of gene loss in animal evolution and underscores the lack of correlation between phenotypic and genomic complexity in metazoans.

The last group of nonbilaterians to be discussed are the myxozoans. Until very recently they were considered to be parasitic protists. Previously invisible to most zoologists, this diverse group of obligate parasites of a host of invertebrates and vertebrates is of substantial economic importance because of the diseases they can cause in commercial fish hosts (Kent *et al.*, 2001). New morphological and molecular evidence has indicated their metazoan affinities. The myxozoan's polar capsules show striking morphological and functional similarities to cnidarian stinging capsules or nematocysts, and myxozoan spores are multicellular. It is currently unclear whether myxozoans are derived cnidarians, or basal bilaterians (Figure 2).

1.02.3.2 Acoelomorpha: Acoela and Nemertodermatida

One of the most surprising and significant advances in metazoan phylogenetics in the last few years has been our changing perception of the phylogenetic position of two groups of morphologically simple flatworm-like organisms: the Acoela and the Nemertodermatida (Figures 1 and 3). Acoels and nemertodermatids are small ciliated worms that were previously considered to be primitive members of the phylum Platyhelminthes. Chiefly based on similarities of their epidermal cilia, the two groups were united in the clade Acoelomorpha. In common with platyhelminths, acoelomorphs have a blind-ending gut that may or may not have a lumen. However, unlike most platyhelminths, acoelomorphs lack a continuous epidermal basement membrane, as well as protonephridia, and their nervous system is only weakly differentiated in the form of a nerve net with a weakly differentiated brain and several longitudinal cords. The brain in acoels has been described as commissural, rather than ganglionic, and it has been argued that a true ganglionic brain with a central neuropil evolved after the acoelomorphs diverged from the remaining bilaterians. The name Nephrozoa has been proposed for the clade of all nonacoelomorph bilaterians, based on the widespread presence of nephridia in these taxa (Jondelius *et al.*, 2002).

Currently, molecular evidence in the form of nucleotide sequences of SSU, LSU, and myosin II heavy chain, as well as amino acid sequences from several mitochondrial genes support the position of the acoelomorphs as the basal-most extant bilaterians. In addition, possession of a complement of Hox/ParaHox genes intermediate between those found in cnidarians and the remaining bilaterians appears to buttress the basal position of the acoelomorphs. However, molecular evidence has not yet provided unequivocal evidence for the monophyly of Acoelomorpha, and the acoels and nemertodermatids may be distinct taxa, branching off subsequent to each other at the base of the Bilateria.

The acoelomorphs can therefore serve the important function as a bridge to help close the architectural gap between bilaterians and the more basal metazoans. As the most basal-living bilaterians, the morphology and life cycle of the acoelomorphs is especially important for informing the primitive conditions for the Bilateria as a whole. Their lack of segmentation, coeloms, excretory organs, their relatively simple nervous systems, the absence of distinct larval stages in their life cycles, and their apparently limited set of Hox/ParaHox genes strengthen the inference that the most recent common ancestor of the Bilateria was a relatively simple, compact, direct-developing organism, with a sac-like gut (Baguñà and Riutort, 2004).

1.02.3.3 Deuterostomia

The three core phyla of the Deuterostomia are the echinoderms, the hemichordates (enteropneusts and pterobranchs), and the chordates (urochordates, cephalochordates, and craniates plus vertebrates; Figure 3). In addition, recent molecular phylogenetic research has added the enigmatic and rather simple worm *X. bocki* to the deuterostomes as well (Bourlat *et al.*, 2003). Of the three main bilaterian clades, the high-level phylogeny of the deuterostomes is currently best understood. This section will describe deuterostome phylogeny in detail, including an account of high-level vertebrate phylogeny.

1.02.3.3.1 Echinodermata, Hemichordata, and *Xenoturbella* Currently, a sister group relationship of the echinoderms and hemichordates in a clade Ambulacraria is robustly supported by both molecular and morphological evidence (Smith *et al.*, 2004; Figure 3). Previous hypotheses often considered Hemichordata not to be monophyletic, with

the enteropneusts being the sister group to the chordates, and the pterobranchs diverging more basally within the deuterostomes. Recent molecular and morphological phylogenetic analyses established the monophyly of the Hemichordata and its placement as a sister group to the Echinodermata (Cameron, 2005; Zeng and Swalla, 2005). A reinterpretation of morphological characters in these groups, as well as in the lophophorates that were previously considered to be deuterostomes, supports this hypothesis.

The exclusively marine echinoderms contain some of the most familiar invertebrates, such as sea stars and sea urchins. Their pentaradial symmetry, calcareous endoskeletal elements, and their elaborate coelomic water vascular system are diagnostic features of the phylum. Although the living species are classified into five well-demarcated groups (asteroids or sea stars, echinoids or sea urchins, ophiuroids or brittle stars, crinoids, feather stars or sea lilies, and holothuroids or sea cucumbers), the morphological diversity of extinct echinoderms yields a further 18 or so distinct echinoderm taxa.

The phylogeny of the extant echinoderms is far from established, but some provisional conclusions may be drawn on the basis of currently available morphological and molecular data sets. First, the crinoids are the sister group to the remaining taxa, which are grouped in a clade Eleutherozoa. Within the Eleutherozoa, the asteroids are the most likely sister group to a clade comprising the ophiuroids, echinoids, and holothuroids. Within this clade there is robust support for a sister group relationship between the echinoids and the holothuroids in a clade Echinozoa. In contrast, support for a sister group relationship between the clade Echinozoa and the ophiuroids is not very strong.

The Hemichordates comprise the enteropneusts, or acorn worms, and the pterobranchs. Enteropneusts and pterobranchs are very easily distinguishable. Enteropneusts are large (typically between 20 and 25 cm), free-living, bottom-dwelling worms equipped with a bulbous anterior proboscis (prosome) that is clearly separated from the long posterior trunk (metasome) by a collar (mesosome) at the anterior end of which the mouth opens. Pterobranchs, on the other hand, are tiny sessile, colonial animals that carry a prominent crown of ciliated tentacles. Like the enteropneusts the pterobranchs possess a tripartite body, with coelomic cavities in each of the three subdivisions of the body, with an anterior part called the oral shield (prosome that secretes the tubes in which they live), a short middle collar (mesosome bearing the tentacles), and a sac-like trunk (metasome).

In several early morphological phylogenetic analyses Hemichordata was assumed to be monophyletic, but this assumption was not confirmed by most morphological phylogenetic analyses that separated the enteropneusts and pterobranchs as individual taxa. Hemichordate monophyly is confirmed, however, by phylogenetic analyses of SSU and LSU sequences, and recent reinterpretation of hemichordate morphology confirms two potential hemichordate synapomorphies that had previously been hypothesized: possession of a stomochord (anterior dorsal extension of the pharynx wall into the prosome) and presence of mesocoelomic ducts that connect the coelomic cavities of the mesosome with the outside (Cameron, 2005; Ruppert, 2005).

The monophyly of the pterobranchs is not in doubt, as they share such unique features as the secretion of tubes, or coenecia, with their prosome, or cephalic shield, and the possession of tentacles on the mesosome. However, SSU and LSU evidence and phylogenetic analysis of combined molecular and morphological data suggest the possibility that the enteropneusts are paraphyletic with respect to the pterobranchs. Alternatively, pterobranchs may be the sister group to a monophyletic Enteropneusta. If pterobranchs have evolved from within the enteropneusts, this implies several rather drastic changes in body architecture probably associated with miniaturization. These changes would include simplification of the pterobranch gill skeleton, and simplification of the nervous system from possession of the well-developed dorsal nerve cord or tube in the collar of enteropneusts, to a simpler neural ganglion in the collar region of pterobranchs.

The Ambulacraria hypothesis, according to which echinoderms and hemichordates are sister taxa, has significant implications for understanding deuterostome evolution in general, and the origin of the chordate body plan in particular, because the origin of the chordate nervous system, the notochord, and pharyngeal gill slits has frequently been discussed with respect to either enteropneust anatomy or hemichordate and echinoderm larval morphology.

For example, the classic hypothesis proposed for the origin of the chordate nervous system is Walter Garstang's auricularia or dipleurula hypothesis, later amended and elaborated by other authors, most recently Thurston Lacalli. At the core of this hypothesis is the derivation of the chordate dorsal nerve tube from the fused ciliary bands of a dipleurula-type larva, as is commonly found in the life cycles of both echinoderms and enteropneusts. However, monophyly of the Ambulacraria implies that such a larval form is a possible synapomorphy

of the echinoderms and hemichordates, and is not a plesiomorphy for the chordates (Lacalli, 2005).

The recent addition of the small, morphologically simple worm *X. bocki* to the morphologically much more complex coelomate deuterostomes also has potentially important implications for understanding deuterostome character evolution. Molecular evidence indicates a possible phylogenetic position for *Xenoturbella* as the sister group to the Ambulacraria (Bourlat *et al.*, 2003; Figure 3). Since *Xenoturbella* lacks complex morphology shared by the other deuterostomes, including coelomic cavities and a gut with both mouth and anus (it has a blind-ending gut), it raises the interesting question of whether *Xenoturbella* may have become secondarily simplified, or whether it has retained its morphological simplicity from the root of the Bilateria, and as indicated by the acoelomorphs discussed above.

1.02.3.3.2 Chordata: Urochordata and Cephalochordata The monophyly of Chordata (Urochordata, Cephalochordata, and Craniata) is widely accepted (Figure 3). The urochordates, or tunicates, have long been considered to be the sister group of the clade Cephalochordata plus Craniata, with the latter two taxa also being sister groups. Chordate monophyly is principally supported by the presence of a notochord, or chorda, a dorsal neural tube, longitudinal muscles along the notochord, or its derivatives, the presence of an endostyle that secretes a mucus filter used in feeding, and the presence of special cerebral sensory organs in the form of optic and otic receptors. It should be noted that some of these features are only present in the tadpole larvae of the tunicates, not in the sessile adults (with the possible exception of the larvaceans, or appendicularians, which are likely derived from more typical ascidian ancestors). The body plan of adult tunicates is entirely different from those of the cephalochordates and craniates.

Interestingly, until recently, available molecular phylogenetic evidence did not yield any convincing support for the monophyly of the chordates. The precise placement of the urochordates turned out to be a particularly vexing issue. SSU and LSU sequences have variously suggested urochordates to be the sister group to a monophyletic clade of Cephalochordata plus Craniata, sister group to Ambulacraria, or sister group to all of the deuterostomes. More recent phylogenetic studies based on the simultaneous analysis of a larger number of different genes have instead started to provide tentative support for an unexpected sister group relationship between vertebrates and tunicates (Blair and Hedges, 2005; Delsuc *et al.*, 2006). If this turns out to be correct, it will have important consequences for understanding the origin of the vertebrates, for which cephalochordates are commonly interpreted as a stand-in of the last common vertebrate ancestor. However, straightforward comparisons between urochordates and vertebrates are very difficult, especially because of the strong modifications of the many forms that are sessile as adults.

The vast majority of tunicates are represented by the familiar sessile ascidians or sea squirts. The remaining species comprise the pelagic thaliaceans (salps, pyrosomids, and doliolids), and the appendicularians or larvaceans. A highly complex and unique cuticular exoskeleton known as the tunic, which includes free cells, has given the phylum its name, although it is not present in all tunicates. A large pharynx or branchial basket is another conspicuous organizational feature of urochordates. A similar pharynx can also be identified in the cephalochordates and the ammocoetes larva of lampreys. The characteristic tadpole larva with a bulbous body and slender muscular tail is also unique for urochordates. In fact, larvaceans look somewhat like urochordate tadpole larvae during their entire life cycle. It has been hypothesized that larvaceans have evolved by truncating development so that the original tadpole larva has now become the definitive adult (a phenomenon known as pedomorphosis). Recent phylogenetic evidence deriving both the pelagic larvaceans and thaliacians from within a paraphyletic clade of ascidians provides some support for this hypothesis (Zeng and Swalla, 2005).

The cephalochordates, or lancelets (also known as amphioxus), are widely accepted to be the sister group to the craniates. Lancelets are the most vertebrate-like of all invertebrates, and they resemble little fish in their morphology. The segmented nature of their body musculature can readily be distinguished, and shows a striking similarity to the segmentally arranged muscle blocks or myomeres found in vertebrates. These muscle blocks are derived from coelomic (somitic) pouches. Moreover, vertebrates and cephalochordates share the attainment of a certain degree of brain complexity reflected in both its ultrastructure and the expression of developmental regulatory genes, and the elaboration of special sense organs, principally an olfactory organ (corpuscles of Quatrefages in lancelets) marked by the expression of a developmental regulatory gene also expressed in craniate ectodermal placodes. However, the evolutionary significance of these features remains unclear when, as discussed

above, urochordates indeed prove to be more closely related to vertebrates.

1.02.3.3.3 Craniata and Vertebrata The last decade has witnessed remarkable progress in resolving the major phylogenetic relationships within the extant craniates and vertebrates (Rowe, 2004; Figures 4–6). Especially the application of molecular evidence from a variety of sources has contributed significantly to the emergence of the current consensus.

The basal-most extant groups within the Craniata are the hagfishes (Myxinoida) and lampreys (Petromyzontida), which together are referred to as Cyclostomata, representing the jawless agnathans. Whether Cyclostomata is monophyletic or paraphyletic with hagfishes as the sister group to the Vertebrata, including the lampreys, is currently unclear. Different analyses contradict each other. For the purpose of this article I accept cyclostomatan paraphyly as a working hypothesis, with lampreys as the sister group to the Gnathostomata, which comprises all living jawed vertebrates (Figure 4).

The basal-most split within Gnathostomata is between the sister groups Chondrichthyes (cartilaginous fishes including sharks and rays) and Osteichthyes (bony fishes and tetrapods). Within the Osteichthyes there is a basal split between the Actinopterygii (ray-finned fishes, comprising all living fishes except the coelacanths and lungfishes) and the Sarcopterygii (lobe-finned fishes: coelacanths, lungfishes, and tetrapods). Although it has commonly been thought that the coelacanths are the nearest relatives of the Tetrapoda, accumulating molecular evidence instead supports a basal split within the Sarcopterygii between the sister groups Actinistia (coelacanths) and Choanata (the lungfishes and tetrapods). The lungfishes (Dipnoi) are the sister group to the Tetrapoda, or terrestrial vertebrates (Figure 4).

An important caveat may obtain here. Although the basal vertebrate relationships described above are widely accepted, a recently published phylogenetic analysis of complete mitochondrial genomes and 18S and 28S ribosomal sequences instead suggested that a fish is a fish, and a tetrapod is a tetrapod; the tetrapods were a sister clade to a clade comprising all gnathostome fishes (Arnason *et al.*, 2004). This study provided suggestive evidence that the traditional phylogeny in which tetrapods evolve from within a paraphyletic group of fishes is an artifact of rooting the phylogeny within the gnathostomes, either with bony fishes or cartilaginous fishes. This procedure may already assume paraphyly of gnathostome fishes. Only when phylogenies are rooted instead with a non-gnathostome outgroup, such as hagfish or lamprey, can the hypothesis of the monophyly of gnathostome fishes be really tested. In this case a monophyletic clade of gnathostome fishes, including lungfishes and coelacanths, is recovered. Yet, nothing is ever unambiguous in phylogenetics, and this result was again contradicted by more recent studies published between first submission and revision of this article, which on the basis of phylogenetic analysis of a large number of nuclear protein-coding genes showed that gnathostome fishes are paraphyletic with respect to tetrapods (Blair and Hedges, 2005).

Extant tetrapods comprise three clades: Amphibia, Reptilia, and Mammalia (Figure 4). The amphibians (anurans, such as frogs; caudatans, such as salamanders; and caecilians) are the sister group to the Amniota, comprising the extant sister groups Reptilia and Mammalia (Figures 4 and 5). Although long considered to be a paraphyletic group, the reptiles are currently recognized as the monophyletic sister clade of the Synapsida, which includes the modern mammals plus extinct stem taxa. Reptilia comprises two major clades of extant reptiles: Archosauria and Lepidosauria (Lee *et al.*, 2004; Figure 5). Monophyly of Archosauria is well established, and the clade comprises the extant crocodilians and birds, as well as the extinct pterosaurs and dinosaurs.

Living lepidosaurs are the tuataras, lizards, and snakes. Lizards and snakes are grouped together in a clade Squamata, the monophyly of which is well supported, but the internal relationships of which remain uncertain (Lee, 2005; Vidal and Hedges, 2005). Monophyly of the Lepidosauria is generally believed to be well supported as well, but several recent molecular phylogenetic analyses of nuclear genes contradict morphological and mitochondrial sequence support for a monophyletic Lepidosauria, suggesting instead a paraphyletic Lepidosauria with squamates as sister group to all remaining extant reptiles.

The perennially problematic phylogenetic position of turtles has not yet been convincingly resolved (Figure 5). On the basis of morphological and paleontological evidence turtles were either allied with a group of extinct marine lepidosaurian reptiles, or were placed outside all other living reptiles. However, the application of molecular sequence data has yielded no support for any of these hypotheses. Instead, these data surprisingly suggest that turtles are closely related to archosaurian reptiles (Rest *et al.*, 2003; Lee *et al.*, 2004). So

far no anatomical support for this relationship has been recovered, and a recent morphological phylogenetic study places turtles as a sister group to Lepidosauria (Hill, 2005).

The congruence of diverse molecular evidence has recently generated a new consensus of mammalian phylogeny that is significantly at odds with traditional ideas established on the basis of morphological and paleontological evidence (Murphy *et al.*, 2004; Springer *et al.*, 2004). The basal divergences among the mammals are in agreement with traditional ideas. The Monotremata (duck-billed platypus, echidnas) and their fossil stem group are together called Protheria, and Protheria is the sister group to the marsupial and placental mammals and their stem groups. The marsupials and their stem group are called Metatheria, while the Placentalia and their stem group are called Eutheria. Metatheria and Eutheria are sister groups within a clade Theria.

The emerging molecular picture of placental mammal phylogeny is strikingly at odds with traditional ideas (Figure 6). Four main clades are recognized: Xenarthra, Afrotheria, Euarchontoglires, and Laurasiatheria. The latter three clades were not recognized previously. Euarchontoglires and Laurasiatheria are sister clades with northern hemisphere origins. Xenarthra is sister group to this superclade, and the divergence between Afrotheria and the remaining placental mammals represents the basal-most split. The xenarthrans and afrotherians had a southern hemisphere origin.

Afrotheria includes morphologically very disparate forms such as elephants, hyraxes, manatees, golden moles, elephant shrews, tenrecs, and aardvarks. Xenarthra includes sloths, armadillos, and anteaters. Euarchontoglires unites rodents, lagomorphs (rabbits, pikas) together with flying lemurs, tree shrews, and primates. Laurasiatheria includes bats, certartiodactyls (artiodactyls and cetaceans), perissodactyls, carnivores, pangolins, and insectivores such as moles, hedgehogs, and shrews. One of the most striking implications of the new placental mammal phylogeny is the extent of parallel morphological evolution between afrotherians and laurasiatherians. Both clades show independent radiations of similar adaptive forms, including mole-like animals (golden mole and mole), hedgehog-like animals (tenrec and hedgehog), shrew-like animals (elephant shrew and shrew), anteaters (aardvarks and pangolin), and fully aquatic forms (manatees and dolphins).

The implications of the new mammal phylogeny for understanding phenotypic evolution are enormous, and many received wisdoms need to be freshly scrutinized. Humans turn out to be more closely related to mice than to cows or dogs, ungulates or hoofed mammals do not form a clade, and the traditional order Insectivora is equally spread out across the mammalian tree.

The enormous range of morphological variation characterizing clades such as Afrotheria also poses a real challenge to those who wish to reconstruct their ancestral phenotype. For example, so far just a single morphological synapomorphy has been proposed for the clade Afrotheria (Carter *et al.*, 2006), a situation reminiscent of the difficulty of establishing morphological synapomorphies for the large invertebrate clade Lophotrochozoa (see below). Moreover, current evidence suggests that morphologically very distinct taxa, such as hyraxes, elephants, and sirenians, and which form a clade within Afrotheria, may have radiated very rapidly, establishing their distinctive body plans in a short amount of time (Nishihara *et al.*, 2005). Evidently, a satisfactory synthesis of the molecular phylogeny with morphology and fossil evidence is the daunting challenge still before us.

1.02.3.4 Ecdysozoa

Monophyly of the clade Ecdysozoa was one of the major surprises of the molecular phylogenetics of the Metazoa because it implied that the segmented arthropods and annelids were merely distant relatives, with the former a member of Ecdysozoa, and the latter a member of Lophotrochozoa. Ecdysozoa unites the arthropods (e.g., insects, crustaceans, myriapods, and chelicerates), onychophorans (velvet worms), tardigrades (water bears), nematodes (roundworms), nematomorphs (horsehair worms), priapulids, kinorhynchs (mud dragons), and loriciferans (in the absence of molecular sequence data, this latter taxon is included solely on the basis of morphological evidence; Figure 7). Molecular support for Ecdysozoa derives from an increasing number of sources, including SSU, LSU, elongation factor-1α, elongation factor-2, myosin heavy-chain type II, Hox genes, and sodium-potassium adenosine triphosphatase (ATPase) α-subunit. Additionally, proposed morphological synapomorphies of the ecdysozoans include the covering of the body with a cuticle, which is ultrastructurally similar across ecdysozoans, and which is periodically moulted (presumably under hormonal control, although that has so far only been investigated in any detail in the arthropods and nematodes), the loss of motile epidermal cilia, a terminal mouth, and the loss of intestinal cilia. Finally, the neural expression of horseradish peroxidase

immunoreactivity has also been proposed as an ecdysozoan apomorphy. The failure of various phylogenomic studies to confirm the monophyly of Ecdysozoa may be due to insufficient taxon sampling (limited to those taxa whose genomes have been sequenced), and the potentially highly modified nature of the genome of the nematode *C. elegans* (Copley *et al.*, 2004; Philippe *et al.*, 2005).

With the conspicuous exception of the arthropods and nematodes, not many molecular sequences have so far been generated for the other ecdysozoan phyla. Consequently, on the basis of molecular evidence alone ecdysozoan phylogeny is not yet well resolved. However, on the basis of combined molecular and morphological evidence three likely clades can be hypothesized: Panarthropoda (arthropods, tardigrades, and onychophorans), Scalidophora (priapulids, kinorhynchs, and loriciferans), and Nematoida (nematodes and nematomorphs). The most recent molecular and combined molecular and morphological phylogenetic analyses suggest a sister group relationship between the Nematoida and Panarthropoda, with Scalidophora as a sister group to this clade (Figure 7). However, robust support for this topology remains to be discovered.

1.02.3.4.1 Panarthropoda: Arthropoda, Onychophora, and Tardigrada Onychophorans are terrestrial, 15–150 mm long, somewhat cigar-shaped animals that are readily identifiable by their 13–43 pairs of walking limbs known as lobopods, and a prominent pair of antennas that adorns the head. This undoubtedly monophyletic group has been at the center of evolutionary speculations ever since the last quarter of the nineteenth century, when onychophorans were considered an important evolutionary link between annelids and arthropods.

Tardigrades, or water bears, are small (mostly less than 1 mm) animals. Their generally roundish bodies are carried on four pairs of stubby legs that identify four body segments. They are well known for their ability to survive for extended periods in a state of extreme inactivity while being virtually completely dehydrated, a phenomenon called cryptobiosis. This ability is an obvious advantage to the many tardigrades that live in the thin and ephemeral films of water that surround terrestrial lichens and mosses. Unfortunately, their small size makes it very difficult to reconstruct their phylogenetic position with morphological evidence alone because their lack of certain important features uniquely shared between the larger-bodied onychophorans and arthropods may have been secondarily lost, rather than primitively absent. This might be true, for example, of metanephridia that are restricted to a reduced coelom called a sacculus, and a dorsal heart with openings called ostia, two characters shared by onychophorans and arthropods.

The Arthropoda is an amazingly diverse group of animals, with about 1 million described species, and total estimates suggesting up to 10-fold higher diversity. About two-thirds of described invertebrate species are arthropods. They are characterized by the possession of a more or less rigid, articulated chitinous exoskeleton that covers the entire body surface, and the possession of articulated limbs.

There are four extant arthropod groups: (1) Hexapoda or Insecta (note that there is some variation in the use of these terms in the literature, principally by considering the insects as a subgroup of the Hexapoda); (2) Myriapoda (millipedes and centipedes); (3) Crustacea (crabs, lobsters, etc.); and (4) Chelicerata (horseshoe crabs, spiders, scorpions, etc.). The literature on arthropod phylogeny is one of the most voluminous in systematic zoology, and chronicles a rich history that extends back to the very beginning of evolutionary biology. These extensive efforts to reconstruct the phylogenetic relationships of arthropods are classically rooted in morphological (including fossil) information, but the recent application of an increasingly diverse array of molecular data has yielded several important advances in our understanding.

In contrast to traditional ideas that united the myriapods and the hexapods, molecular evidence instead supports a close relationship of the crustaceans and hexapods, either as sister groups, or with hexapods derived from within the paraphyletic crustaceans. In addition, and more controversially, various nuclear and mitochondrial genes support chelicerates and myriapods as closest relatives in a clade that has been named Paradoxopoda or Myriochelata (Mallatt *et al.*, 2004; Pisani *et al.*, 2004). However, whether myriapods and chelicerates indeed form a clade, or whether chelicerates represent the earliest diverging extant taxon (Wheeler *et al.*, 2004; Giribet *et al.*, 2005), and whether hexapods and crustaceans are really closest relatives awaits further study.

The monophyly of Panarthropoda is widely accepted (Figure 7), but at present its support is mainly morphological. Comprehensively sampled molecular sequences, mainly those of SSU and LSU, provide decidedly less support for panarthropod monophyly, or relationships within Panarthropoda. Morphological phylogenies either support the onychophorans or the tardigrades as the sister group to extant arthropods, and a combination of SSU and LSU sequences has recently

suggested a clade of tardigrades and onychophorans. Before a robust phylogeny of the Panarthropoda and the Arthropoda can be achieved, more sequence data is needed, the choice of morphological characters used in phylogenetic analyses should be better justified, and the crucial role of fossils should be seriously considered. The panarthropods have a rich fossil record, and it has been shown that the incorporation of fossils into a phylogenetic analysis combining both molecular and morphological information may yield dramatically different phylogenies. For example, inclusion of just seven Paleozoic fossils into a monster phylogenetic analysis of arthropods, including almost 250 taxa, more than 800 morphological characters, and more than 2 kilobases of molecular sequence data, changes support for a clade of hexapods and crustaceans to the traditional Atelocerata hypothesis uniting hexapods and myriapods (Wheeler *et al.*, 2004). Such phylogenetic instability due to the inclusion of different sources of data needs to be more fully explored in future work.

1.02.3.4.2 Scalidophora: Priapulida, Kinorhyncha, and Loricifera The priapulids (penis worms), kinorhynchs (mud dragons), and loriciferans are thought to be a clade called Scalidophora, referring to their most conspicuous synapomorphy: the possession of an anterior end or introvert with spines or scalids, which are epidermal specializations with ciliary receptors. Molecular phylogenetic analyses based on ribosomal sequences support priapulids and kinorhynchs as sister groups, but so far no sequence data has been obtained from loriciferans.

Priapulids are carnivorous worms that possess a large introvert adorned with many spines, and they include both tiny meiobenthic (about 0.5 mm) and larger (up to 40 cm) macrobenthic species. Although only 18 extant species of priapulids have been described, their fossil record extends back to the Cambrian, showing that priapulids were once a speciose and important component of benthic communities. However, considering that within the last 30 years no less than 10 new species of priapulids have been described (with several collected new species still awaiting description), including the most recent description of a giant Alaskan species (up to 40 cm), the group may be more diverse than previously suspected.

All kinorhynchs are tiny (generally less than 1 mm) denizens of the marine meiobenthos. They have relatively narrow and slender bodies that are subdivided into 13 segment-like units called zonites, and their spiny heads covered with scalids make them easy to recognize. Despite superficial similarities to the somites or segments of the arthropod body plan, segmentation of the kinorhynch integumentary, muscular, and nervous systems is generally interpreted to support only the monophyly of the phylum.

The first species of loriciferan was described in 1983. Loriciferans are tiny (between 0.1 and 0.5 mm), and exclusively marine and meiobenthic. Loriciferans are encased in a vase-like exoskeleton called a lorica that is composed of six cuticular plates. From the lorica sprouts forth an introvert with a narrow mouth cone surrounded by spiny scalids similar to those that decorate the kinorhynch head.

Although the monophyly of the Scalidophora seems established (Figure 7), the internal phylogenetic relationships are not yet robustly supported. Most morphological cladistic analyses support kinorhynchs and loriciferans as sister taxa, but some zoologists favor a sister group relationship between priapulids and loriciferans. Sometimes the meiofaunal loriciferans are hypothesized to be the pedomorphic descendants of the larger-bodied priapulids.

1.02.3.4.3 Nematoida: Nematoda and Nematomorpha The Nematoda is a highly successful phylum. Nematodes or roundworms are small and very slender noncoelomate worms, and they inhabit every part of the world that is even marginally inhabitable. This realization has led to a staggering image: if the entire earth except nematodes were to become invisible, we would still be able to make out most of the outline of the planet surface, including mountains, and most organisms, both plants and animals, which serve as hosts for a fantastic diversity of roundworms. Interestingly, parasitic nematodes specialized for a range of plant and animal hosts have evolved multiple times convergently.

The nematomorphs or horsehair worms are thin, very long worms (up to 1 m, while being only 1–3 mm wide). The adults are free-living, but earlier life-cycle stages are all parasitic, mostly on arthropods. The Nematomorpha strongly resemble mermithid nematodes in both morphology and life-cycle characteristics, and some zoologists have regarded these features as homologous. However, recent information strongly suggests that these correspondences have evolved convergently, in the monophyletic Nematoda and Nematomorpha.

Nevertheless, a sister group relationship between the nematodes and nematomorphs in a clade Nematoida is generally accepted on the basis of morphological evidence (Figure 7). Synapomorphies

include the presence of longitudinal epidermal cords with nerve cords, a basal cuticle layer with thick, crossing collagenous fibers, and the absence of circular body wall muscles. Molecular phylogenies do not always support Nematoida, but a recent analysis of combined SSU and LSU sequences strongly supported Nematoida (Mallatt *et al.*, 2004).

1.02.3.5 Lophotrochozoa

The clade Lophotrochozoa (Figure 8) was originally proposed on the basis of SSU sequence data to designate the lophophorates (brachiopods, phoronids, and ectoprocts), annelids, and mollusks. Further investigations have increased lophotrochozoan membership also to include the echiurans, sipunculans, entoprocts, platyhelminths, nemerteans, gnathostomulids, rotifers, acanthocephalans, cycliophorans, micrognathozoans, and possibly gastrotrichs and chaetognaths. Lophotrochozoan monophyly is furthermore robustly supported by additional molecular evidence, including LSU data, Hox gene data, mitochondrial gene sequence and gene arrangement data, myosin heavy-chain type II data, intermediate filament data, and sodium-potassium ATPase α-subunit sequences.

The abundant molecular evidence for lophotrochozoan monophyly stands in sharp contrast to the resolution of its internal phylogeny and the strength of its morphological support. Using SSU sequences, for example, the monophyly of even morphologically well-defined phyla, such as Mollusca, Ectoprocta, or Nemertea, may not be supported, and, with the exception of a few possible clades, relationships within the Lophotrochozoa remain largely unresolved with molecular evidence. As a consequence, resolution of lophotrochozoan phylogeny is currently heavily dependent on morphological evidence analyzed either in isolation, or in combination with molecular sequence data.

1.02.3.5.1 Lophophorata: Phoronida, Brachiopoda, and Ectoprocta Adult phoronids are slender worm-like animals that live in secreted tubes, from which they protrude their ciliated feeding tentacles (lophophore) that surround the mouth. Like brachiopods and ectoprocts they possess a U-shaped gut, and some zoologists think that both ectoprocts and brachiopods have evolved from a phoronid-like ancestor. However, available phylogenetic evidence does not support this hypothesis.

Brachiopods, or lampshells, are sessile animals enclosed in a bivalved shell. However, their similarity to bivalves is only superficial since brachiopods are flattened dorsoventrally while bivalves are flattened laterally. Somewhat simplistically, architecturally brachiopods can be regarded as shelled phoronids. Similar to ectoprocts and phoronids, brachiopods possess a crown of ciliated feeding tentacles called a lophophore.

With the exception of two genera, one solitary and one nonsessile, ectoprocts are sessile colonial animals, with individuals (called zooids) that measure less than 0.5 mm in length, and that may be box-shaped, oval, or tubular. Ectoprocts superficially resemble entoprocts with their ciliated crown of tentacles commonly referred to as a lophophore (but see below), and the possession of a U-shaped gut. When the zooids in a colony extend their lophophores, the colony looks a little like a patch of moss, which has led to their general name moss animals.

As noted above, brachiopods, phoronids, and ectoprocts possess ciliated feeding tentacles traditionally referred to as a lophophore. However, the structural and functional uniqueness of the ectoproct tentacles has served as the basis for denying their homology with those of the brachiopods and phoronids, lessening morphological support for Lophophorata. Indeed, available morphological and molecular evidence only supports a close relationship between brachiopods and phoronids (Figure 8), either as sister taxa or with phoronids derived from within brachiopods (Cohen and Weydmann, 2005). SSU sequences and Hox gene data suggest that ectoprocts are at least lophotrochozoans, but of very uncertain phylogenetic position. So far, morphological evidence has not helped to pinpoint the phylogenetic position of the ectoprocts.

1.02.3.5.2 Eutrochozoa: Nemertea, Mollusca, Sipuncula, Annelida, Echiura, Siboglinidae, and Myzostomida Nemerteans, or ribbon or proboscis worms, are all (with the exception of one species) characterized by the possession of an eversible proboscis enclosed by a fluid-filled coelom, the rhynchocoel. The proboscis apparatus is used in prey capture, and the proboscis can be rapidly everted to coil around the prey. Secreted toxins will help subdue the prey that mainly consists of small crustaceans and annelids.

On the basis of molecular phylogenetic evidence alone it can be concluded that nemerteans are lophotrochozoans, but their precise position remains unclear. In contrast, most phylogenetic analyses based on morphology alone or in combination with molecular evidence place nemerteans together with the neotrochozoans: mollusks, sipunculids, echiurans, and annelids (plus groups derived from

within the annelids) (Figure 8). This hypothesis is supported, among others, by the shared possession of mesodermal bandlets derived from a 4d cell, and the derivation of a schizocoelic coelom from these bandlets (Jenner, 2004b). Although nemerteans are generally thought to lack trochophore larvae, in contrast to the neotrochozoans, the recent discovery of a hidden trochophore larva in the life cycle of a basal nemertean (Maslakova *et al.*, 2004) implies that the trochophore larva may be an additional synapomorphy of nemerteans and neotrochozoans.

Mollusca is a highly diverse but clearly defined group of animals. Familiar mollusks include snails, slugs, bivalves, chitons, and cephalopods. Molluscan synapomorphies include a coelomic pericardium, a mantle that secretes the shells, and a radula used in feeding. Mollusks have adapted to a wide range of marine, freshwater and terrestrial habitats, and can be considered one of the most successful groups of animals on earth.

The phylogenetic position of the mollusks remains unclear. On the basis of morphological evidence various sister groups have been suggested, including entoprocts, sipunculans, or a clade of varying membership, including neotrochozoans and panarthropods. Molecular evidence has so far not allowed the resolution of this problem, and broadly sampled rDNA sequences have frequently failed to support even a monophyletic Mollusca.

Sipunculans, or peanut or star worms, form a well-demarcated phylum. They are nonsegmented, coelomate worms that are denizens of marine benthic communities. Their bipartite body has a posterior widened trunk, an anterior slender part called the introvert, with a terminal ciliated tentacle crown that is arranged as a star around the mouth.

The precise phylogenetic position of sipunculans remains unclear. Morphology suggests an affinity to the mollusks, or to a clade of variable membership that includes various neotrochozoans and panarthropods. Molecular evidence suggests sipunculans are closely related to annelids.

The annelids, or segmented worms, are among the most familiar invertebrates, and they principally comprise the polychaetes and the clitellates. The polychaetes encompass a great diversity of forms, and molecular phylogenetic evidence suggests that they probably also include the vestimentiferans and pogonophores (now united as Siboglinidae), and the echiurans. Clitellates include the oligochaetes, including earthworms, and the parasitic hirudineans, or leeches.

The phylogenetic position of annelids on the basis of morphology remains contentious, because of the difficulty of interpreting the characters related to segmentation in the annelids and the arthropods. Traditionally their shared segmentation has been interpreted to support a close relationship. In contrast, molecular evidence firmly places the annelids within the Lophotrochozoa and the arthropods in the Ecdysozoa, suggesting the possibility that their segmentation is not homologous. The sister group to the annelids remains unknown. However, molecular evidence has suggested a larger membership of the Annelida than was sometimes suspected on the basis of morphology alone. Molecular evidence now supports the inclusion of the echiurans, vestimentiferans, and pogonophores (together siboglinids) within the polychaete annelids as well.

Adult echiurans, or spoon worms, are unsegmented, coelomate worms. They share the chaetae characteristic of annelids, and they possess a characteristic flat or grooved proboscis that can be stretched to extreme lengths in the search for food while the rest of the animal remains hidden in a protective burrow or rock crevice.

The lack of body segmentation has caused morphological phylogenetic analyses to place the echiurans outside the annelids, frequently as its sister group. In contrast, accumulating molecular evidence from various sources now support the inclusion of echiurans in the polychaete annelids. This implies that echiurans have secondarily lost such characters as parapodia and pronounced metamery of the body.

Pogonophores and vestimentiferans are now united on the basis of both molecular and morphological evidence as the family Siboglinidae that is placed within the polychaetes. They are long worms that live in secreted tubes. They have an occluded gut, and highly modified and reduced segmentation. Perhaps the most familiar examples are the large vestimentiferans (up to 1.5 m), which live associated with deep-sea hydrothermal vents. They harbor chemoautotrophic symbiotic bacteria from which they derive a significant amount of nourishment.

Myzostomids are peculiar worms that look like little pancakes with stubby legs with which they cling on to their echinoderm hosts on which they parasitize. They seem to lack a coelom, but they do exhibit signs of segmentation, and they possess several characters unique to polychaetes or subgroups of polychaetes, including parapodia adorned with modified chaetae reminiscent of annelid chaetae, internal chaetae (called aciculae and functioning as support rods inside the parapodia), a nectochaete larva, and nearly identical innervation patterns of the parapodia and cirri (marginal sensory organs) in polychaetes and myzostomids.

The phylogenetic position of the myzostomids remains contentious (Jenner, 2003), and affinities with polychaete annelids, platyhelminths, and cycliophorans have been suggested on the basis of morphological, molecular, or combined evidence. However, sampling of molecular sequences is sparse and should be extended.

1.02.3.5.3 Platyzoa: Platyhelminthes, Gnathostomulida, Rotifera, Acanthocephala, Entoprocta, Cycliophora, and Micrognathozoa Platyzoa is a clade recently proposed on the basis of molecular sequence data and some combined analyses of molecular and morphological evidence (Figure 8). Platyzoa encompasses noncoelomate lophotrochozoans. Although minimally proposed to include platyhelminths, gnathostomulids, rotifers, and acanthocephalans, the broadest definition of this clade additionally includes entoprocts, cycliophorans, and micrognathozoans, and potentially gastrotrichs and myzostomids. However, phylogenetic support for this clade is currently far from robust, and the exact membership and interrelationships of Platyzoa remain to be determined.

Platyhelminths, or flatworms, are typically dorsoventrally flattened bilaterians that come in a diverse array of forms with an even more exuberant range of life cycles that may contain multiple hosts for parasitic species. About a quarter of described flatworm species are grouped as Turbellaria, which forms a paraphyletic group of mainly free-living species from which the highly specialized parasitic groups such as cestodes and trematodes have been derived. Recent evidence suggests that the acoelomorphs (acoels and nemertodermatids) are not platyhelminths, but basal crown group bilaterians. Instead Platyhelminthes now comprises Catenulida and Rhabditophora, the latter taxon containing the bulk of extant species, including all parasitic groups.

Gnathostomulids are minute noncoelomate worms that live in the interstitial spaces of marine sands (meiofauna). Their common name jaw worms refers to the set of complex cuticularized jaw elements that are found in all gnathostomulids. Gnathostomulids are invariably described as enigmatic in evolutionary studies.

Rotifers are small (most are not more than 1 mm) and common animals in marine zooplankton, freshwater, and in association with terrestrial moss plants. Their common name, wheel animals, refers to the presence of a ring of cilia at the anterior end present in many species that is used for feeding and locomotion. When the cilia beat, the ciliated band superficially resembles a rotating wheel. Rotifers possess a cuticularized pharyngeal jaw apparatus (trophi) that is very similar ultrastructurally to gnathostomulid jaw elements.

The acanthocephalans or spiny-headed worms are generally small endoparasites (not more than a few millimeters, although one species of up to 80 cm is known) that derive their name from the possession of a spiny proboscis with which they attach inside their host. All recent phylogenetic studies (based on both morphology and molecules) support a close relationship between the generally free-living rotifers and parasitic acanthocephalans, either as sister groups, or with acanthocephalans nested within a paraphyletic Rotifera. The key synapomorphy is a syncytial epidermis with an intracellular skeletal layer, which unites these taxa as Syndermata. The possession of a syncytial epidermis may be an adaptation to living in osmotically challenging environments. Many rotifers live in freshwater and acanthocephalans live as parasites inside other animals. A syncytial skin would then provide an effective tight seal to prevent osmotic stress.

Entoprocta comprises a well-demarcated monophylum. Entoprocts are tiny animals, frequently not longer than 1 mm. Entoprocts are sessile, solitary, or colonial, and resemble little stalked cups with a crown of ciliated tentacles that protrude into the water column where they filter out small food particles. Entoprocts exhibit a superficial similarity to the ectoprocts, and together they have traditionally been grouped as Bryozoa.

Another phylum of very tiny animals (less than 1 mm in length) is Cycliophora (described as a single species, *Symbion pandora*, but at least one other species awaits description). *Symbion* was described in 1995 from specimens attached to the mouthparts of the Norwegian lobster, *Nephrops* (Funch and Kristensen, 1995). One of their unique features is a very complex life cycle, with sessile and motile stages, feeding and nonfeeding stages, dwarf males and giant females, and enigmatic chordoid and Pandora larvae. The external shape of the sessile feeding stages hints at an entoproct affinity, being shaped like a minute urn attached to a stalk that ends in an adhesive disk. Ciliated tentacles, however, are lacking. Instead the animal has a bell-shaped buccal funnel with a mouth surrounded by cilia that produce a feeding current.

The first complete description of Micrognathozoa was published in October 2000, and is based on one species, *Limnognathia maerski*, which is one of the smallest metazoans ever described, measuring some 100–150 μm in length. Strikingly, its discoverers were the same zoologists who had first described Cycliophora 5 years previously (Kristensen and Funch, 2000). Its complex cuticularized jaw

apparatus is very similar to both rotifer trophi and gnathostomulid jaws.

Although the precise relationships between the platyzoans remain uncertain at the moment, several tentative conclusions can be drawn (Halanych, 2004; Jenner, 2004b; Funch *et al.*, 2005). Accumulating molecular evidence suggests that platyhelminths (catenulids and rhabditophorans) are undeniably lophotrochozoans, but neither molecular nor morphological evidence, or a combination thereof, has reliably identified a sister group of the platyhelminths.

The striking similarities of the jaw elements in syndermates and gnathostomulids support a clade Gnathifera (Figure 8). Based on the possession of similar complex cuticularized jaws and shared ultrastructural similarities of the epidermis with the syndermates, Micrognathozoa may also be a member of Gnathifera. Available nuclear and mitochondrial sequence data paint a more ambiguous picture. They provide no unambiguous support for the monophyly of Gnathifera. Depending on data source and analysis parameters, the syndermates are sometimes the sister group of Cycliophora, a clade of Cycliophora, Micrognathozoa, and Gnathostomulida, or a clade of gastrotrichs and platyhelminths. Placement of the other platyzoan taxa is equally sensitive to changes in data source and analysis parameters.

1.02.3.5.4 Gastrotricha and Chaetognatha

Gastrotrichs are microscopic, meiobenthic animals (generally <1 mm), including both freshwater and marine species. Their general biology and evolution remain poorly known. Gastrotrich monophyly is well supported, with one of the most conspicuous autapomorphies being an extracellular cuticle that covers the entire body surface, including the locomotory and sensory cilia. Their name refers to the presence of a ventral creeping surface adorned with cilia.

The phylogenetic position of the gastrotrichs in the Metazoa remains highly contentious. Morphological evidence has been used to argue for a relationship with ecdysozoans, frequently as the sister group to the remaining ecdysozoans, or the scalidophorans and nematoidans if the Ecdysozoa was not supported as a clade. The use of molecular evidence, or a combination of morphology and molecules, has not greatly clarified their phylogenetic position either, and they have been variously allied with platyhelminths, gnathostomulids, acanthocephalans, nematomorphs, or placed basal in the Bilateria. Clearly, significant research is required to resolve the phylogenetic affinities of these poorly understood creatures.

The unique morphology of chaetognaths makes it a well-demarcated group. Chaetognaths are slender marine predators between 2 and 120 mm long, with fins lining their sides and tail. Their heads are equipped with a formidable array of grasping spines used in prey capture. This general morphology and their jerky or darting swimming behavior explain their common name of arrow worms.

The possession by chaetognaths of a mixture of characters traditionally conceived as broadly protostomian, such as the organization of the nervous system and the composition of the cuticle, and broadly deuterostomian, including radial cleavage and the mode of coelom development, have ensured enduring debate about their phylogenetic relationships. Recent morphological cladistic analyses have variously placed chaetognaths at the base of the Bilateria, Ecdysozoa, or Deuterostomia, while molecular (SSU, LSU, Hox genes, and mitochondrial genome) and combined morphological and molecular analyses have recently placed them among ecdysozoans, within Lophotrochozoa, as a sister group to onychophorans or nematodes, as a basal protostome, or a basal bilaterian. Thus the phylogenetic position of the chaetognaths remains profoundly puzzling.

1.02.4 Conclusion and Future Progress

High-level metazoan phylogeny is in a state of flux. This is a time of unparalleled research activity and funding possibilities. Reconstructing the tree of life has been afforded similar priority status as the various genome-sequencing projects. As a result, the next decade will witness a great expansion and refinement of our developing views about the phylogenetic history of life. The overview presented in this article should thus be considered as merely a tentative sketch, parts of which will be consolidated as new data accrue, while other parts will inevitably be significantly altered. The monophyly of Protostomia, the phylogeny within Lophotrochozoa, and the sequence of divergences of nonbilaterians are some of the most pressing questions of high-level metazoan phylogenetics. However, similar unanswered questions also prevail on lower levels, ranging from the phylogeny of the basic gnathostome clades within the Vertebrata, to the phylogeny of extant birds. Irrespective of what the final topology of the animal tree of life will look like, we are certain to learn a lot in the next few years.

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1.03 Phylogenetic Character Reconstruction

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Glossary

adaptation A feature or phenotype or trait that evolved to serve a particular function or purpose.

anagenesis The origin of evolutionary novelties within a species lineage by changes in gene allele frequencies by the processes of natural selection and/or neutral genetic drift.

character polarity The temporal direction of change between alternative (primitive and derived) states of a character.

character state reconstruction The process of estimating the ancestral or primitive condition of a character at a given node (branching point) in a phylogenetic tree.

clade A complete branch of the tree of life. A monophyletic group.

cladogenesis The origin of daughter species by the splitting of ancestral species; may or may not occur under the influence of natural selection.

cladogram A branching tree-shaped diagram used to summarize comparative (interspecific) data on phenotypes or gene sequences. In contrast to a phylogeny, a cladogram has no time dimension.

comparative method The study of differences between species.

continuous trait A quantitatively defined feature with no easily distinguished boundaries between phenotypes (e.g., size, cell counts, and gene expression levels).

convergence Similarity of structure or function due to independent evolution from different ancestral conditions.

discrete trait A qualitatively defined feature with only a few distinct phenotypes (e.g., polymorphism; presence vs. absence).

homology Similarity of structure or function due to phylogeny (common ancestry).

homoplasy Similarity of structure or function due to convergence, parallelism or reversal.

monophyletic A systematic category that includes an ancestor and all of its descendants; a complete branch of the tree of life; a 'natural' taxon; a clade.

node An internal branching point in a phylogenetic tree.

optimization Methods for estimating ancestral trait values on a tree. Commonly used optimization criteria are: maximum parsimony (MP) which minimizes the amount of trait change, and maximum likelihood (ML) which maximizes the likelihood of a trait at a node given likelihood values for trait evolution.

parallelism Similarity of structure or function due to independent evolution from a common ancestral condition.

paraphyletic A systematic category that includes an ancestor and some but not all of its descendents (e.g., 'invertebrates', 'agnathans', 'fish', and 'reptiles' (*sans* birds)).

parsimony	A principle of scientific inquiry that one should not increase, beyond what is necessary, the number of entities required to explain anything.
phenotypic evolution	Change in the developmental program descendents inherit from their ancestors.
phylogenetic character	A homologous feature or phenotype or trait of an organism or group of organisms.
phylogenetic systematics	A method for reconstructing evolutionary trees in which taxa are grouped exclusively on the presence of shared derived features.
phylogenetic tree	Genealogical map of interrelationships among species, with a measure of relative or absolute time on one axis. Also called a tree of life or a phylogeny.
phylogeny	The evolutionary history of a species or group of species that results from anagenesis and cladogenesis.
polyphyletic	A systematic category that includes taxa from multiple phylogenetic origins (e.g., 'homeothermia' consisting of birds and mammals).
reversal	Change from a derived character state back to a more primitive state; an atavism. Includes evolutionary losses (e.g., snakes which have 'lost' their paired limbs).
synapomorphy	A shared, derived character used as a hypothesis of homology.
taxon	A species or monophyletic group of species (plural taxa).
trait evolution	The sequence of changes of a feature or phenotype on a phylogeny.

1.03.1 Introduction to Character State Reconstruction and Evolution

Comparisons among the features of living organisms have played a prominent role in the biological sciences at least since the time of Aristotle. The comparative approach takes advantage of the enormous diversity of organismal form and function to study basic biological processes of physiology, embryology, neurology, and behavior. This approach has given rise to the widespread use of certain species as model systems, based on what has become known as the August Krogh Principle: "For many problems there is an animal on which it can be most conveniently studied" (Krebs, 1975).

From an evolutionary perspective, interspecific (between species) comparisons allow for the systematic study of organismal design. Rensch (1959) conceived of phylogeny as being composed of two distinct sets of processes: anagenesis, the origin of phenotypic novelties within an evolving species lineage (from the Greek *ana* = up + *genesis* = origin), and cladogenesis, the origin of new species from lineage splitting (speciation) (from the Greek *clado* = branch). Anagenetic changes arise within a population by the forces of natural selection and genetic drift. Cladogenesis may or may not arise from these population-level processes, and in fact many (or perhaps most?) species on Earth are thought to have their origins from geographical (allopatric) speciation under the influence of landscape and geological processes (Mayr, 1963; Coyne and Orr, 1989).

Because species descend from common ancestors in a hierarchical fashion (i.e., from a branching, tree-like process of speciation) closely related species tend to resemble each other more than they do more distantly related species. Patterns in the diversification of phenotypes have therefore been described as mosaic evolution, in which different species inherit distinct combinations of traits depending on the position of that species in the tree of life (McKinney and McNamara, 1990). Under this view, character evolution is regarded as a process of historical transformation from a primitive to a derived state, and study of this process necessarily presumes knowledge of primitive or ancestral conditions. In other words, because character evolution is perceived as trait change on a tree, it is necessary to estimate 'ancestral trait values'.

Direct observations of ancient phenotypes may be taken from fossils, which provide unique information on entirely extinct groups of organisms, and are usually associated with stratigraphic information pertaining to relative and absolute geological ages (Benton, 1993). Nonetheless, the fossil record has many well-known shortcomings, including the famously incomplete levels of preservation, and usually very limited information about the nature of soft tissues such as nerves and brains (but see Edinger, 1941; Stensiö, 1963). Paleontological information on ancient physiological and behavioral traits is even more scanty (but see Jerison, 1976; MacLeod and Rose, 1993; Rogers, 2005).

Recent years have seen great advances in the formulation of comparative methods to estimate or infer ancestral phenotypes from extant (living) species (Garland *et al*., 1992, 1999; Martins, 2000). These methods use patterns in the mosaic of traits present among species in the context of an explicit hypothesis of interrelationships. These methods also address new topics, such as whether rates of

phenotypic evolution have differed among lineages (clades), the circumstances in which a phenotype first evolved, the selective and developmental mechanisms underlying the origin of new phenotypes, and the evolutionary lability of phenotypes (Albert *et al.*, 1998; Blomberg *et al.*, 2003; Blackledge and Gillespie, 2004).

In this article, I summarize the major recent developments in phylogenetically based methods of studying character evolution, with the goals of explaining both the strengths and weaknesses of alternative methods. Most of the empirical examples cited are among animals with the most complex central nervous systems (e.g., vertebrates) in which neurological and behavioral evolution has been (arguably) most extensively studied. A major goal of this article is to highlight some of the most exciting new developments in the study of character evolution now being explored in this fascinating area of comparative neurobiology.

1.03.2 Basic Concepts

1.03.2.1 Homology: Similarity Due to Common Ancestry

All methods of ancestral character state reconstruction make explicit assumptions about the homology of the traits under study. In comparative biology the term 'homology' refers to similarity in form or function arising from common ancestry. In other words, homologous features among organisms can be traced to a single evolutionary origin. In the language of Garstang (1922), a homologous trait is a unique historical change in the developmental program of an evolving lineage. Homologous similarities may be observed in any aspect of the heritable phenotype, from properties of genetic sequences (e.g., base composition and gene order), through aspects of development, including cellular, tissue, and organismal phenotypes, to aspects of behavior that emerge from the organization of the nervous system. Homology in behavioral traits has been examined in a number of taxa, and in a variety of contexts (de Queiroz and Wimberger, 1993; Wimberger and de Queiroz, 1996; Blomberg *et al.*, 2003). Taxa are individual branches of the tree of life, and may include species or groups of species that share a common ancestor (the latter are also referred to as clades or monophyletic groups).

It is important to note that developmental, structural, positional, compositional, and functional features of phenotypes are all useful in proposing hypotheses of homology. Yet by the evolutionary definition employed above, only features that can be traced to a common ancestor in an explicitly phylogenetic context are regarded as homologues. Because phylogenies are the product of comparative analyses using many traits, it is in fact congruence in the phylogenetic distribution of characters that serves as the ultimate criterion for homology. By this criterion homologous characters are said to have passed the test of congruence. In other words, congruence in the phylogenetic distribution of numerous character states is regarded to be the ultimate evidence for homology (Patterson, 1982; see Primate Brain Evolution in Phylogenetic Context, Electric Fish, Electric Organ Discharges, and Electroreception).

1.03.2.2 Homoplasy: Convergence, Parallelism, and Reversal

All other forms of phenotypic similarity that arise during the course of evolution are referred to collectively as homoplasy (similarity due to causes other than homology). Homoplastic characters may arise from several sources: convergence due to similar functional pressures and natural selection, parallel (independent) evolution to a common structure or function from organisms with similar genetic and developmental backgrounds, or convergent reversal to a common ancestral (plesiomorphic) condition. Some well-known examples of convergent evolution in the nervous system include: image-forming eyes of cephalopod mollusks (e.g., squids and octopods) and vertebrates (Packard, 1972), and the evolution of G-protein-coupled receptors as odorant receptors in many animal phyla (Eisthen, 2002). Examples of parallel evolution in the nervous system of vertebrates have been summarized in several recent reviews (Nishikawa, 2002; Zakon, 2002). These include: electric communication in mormyriform (African) and gymnotiform (South American) electric fishes (Albert and Crampton, 2005), prey capture among frogs (Nishikawa, 1999), sound localization among owls (Grothe *et al.*, 2005), and thermoreception in snakes (Hartline, 1988; Molenaar, 1992).

Reversals are among the most common forms of homoplasy, and are often the most difficult to detect even in the context of a resolved phylogenetic hypothesis of relationships (Cunningham, 1999). The reason for this is the phenotypes of some reversals may be quite literally identical, as in the case of convergent loss of structures (e.g., the derived loss of paired limbs in snakes and limbless lizards).

1.03.2.3 Character State Polarity

A central task of ancestral character state reconstruction is determining the direction or polarity of evolutionary change between alternative states of a character. The ancestral state is referred to as

plesiomorphic or primitive, and the descendent state is referred to as apomorphic or derived. Establishing the polarity of a character state transformation is critical to understanding the functional significance of that event. Phenotypes determined to be primitive simply mean they precede the derived state in time and are not necessarily functionally inferior. It is often, although by no means always, the case that characters evolve from more simple to more complex states, or from the absence of a particular state to the presence of that state.

There are several methods in use to determine character state polarity. The most widely used method is the so-called outgroup criterion, which employs conditions observed in members of clades other than the clade in which the derived state is present. The basic idea of the outgroup criterion is that for a given character with two or more states within a group, the state occurring in related groups is assumed to represent the plesiomorphic state. In other words, the outgroup criterion states that if one character is found in both ingroup and outgroup, this character is then postulated to be the ancestral state (plesiomorphic). Of course, it is always possible that a given outgroup exhibits an independently derived state of a given character, which is why the condition in several outgroup taxa is regarded as a more reliable test of the plesiomorphic condition.

1.03.2.4 Character or Trait Data

Methods for estimating ancestral character states and analyzing phenotypic evolution may treat trait data either as continuous (quantitative) or discrete (qualitative) (Zelditch *et al*., 1995; Rohlf, 1998; Wiens, 2001). Continuously distributed trait values have no easily distinguished boundaries between phenotypes. Examples of continuous traits include the sizes of brains and brain regions (e.g., nuclei), the number of cells in a brain region, pigment intensity, amplitude or timing of communication signals, and the amount of gene expression in a tissue. Continuous phenotypic variation typically reflects the additive effects of alleles at multiple loci and is frequently also influenced by environmental factors. Patterns of intraspecific (within species) continuous variation are often analyzed using parametric statistics, including such devices as the population mean and standard deviation. Methods for the analysis of interspecific (between species) continuous traits are useful for assessing the quantitative relationships among variables to address questions regarding, for example, the trade-offs and constraints among correlated traits.

Discontinuous traits have only a few distinct phenotypes. In many cases alternative alleles generate phenotypes that differ from each other in discrete steps, such that each phenotype can be clearly distinguished from the others. Many classes of phenotypic data are inherently discrete, such as meristic counts (e.g., number of body segments, rhombomeres, and cortical visual maps), and genetic polymorphisms (e.g., left- vs. right-handedness). Nucleotide bases at a locus are discrete states of a character. The presence (or absence) of derived traits on a phylogenetic tree also constitutes a class of discrete phenotypes. Such derived traits that underlie or explain subsequent evolutionary events are referred to as key innovations. Some widely cited examples of putative key innovations in the comparative neurosciences include arthropod cephalic tagmosis (Strausfeld, 1998), cephalopod eyes (Hanlon and Messenger, 1996), craniate neural crest (Northcutt and Gans, 1983), and ray-finned fish genome duplication (Taylor *et al*., 2003; Postlethwait *et al*., 2004). Each of these novelties is thought to have been critical in the diversification of the taxon in which it originated.

1.03.2.5 Adaptation

One of the most widely applied uses of ancestral character state reconstruction is in the study of adaptation. The word adaptation is derived from the Latin *ad* (to, toward) and *aptus* (a fit), and is used to imply a feature or phenotype that evolved to serve a particular function or purpose. For example, the function or purpose of an animal central nervous system is to coordinate sensory information and motor output patterns; that is to say, a centralized brain is an adaptation for sensory-motor coordination. Adaptation is therefore used both as a noun to describe the features that arose because of natural selection, and as a verb, the process of natural selection through which the features originated. In an evolutionary context, an adaptation is not only a static description of the match between form and function, but is also an explanation for the origin of that relationship (Russell, 1916).

It is important to distinguish among several distinct uses of the word 'adaptation' in the biological sciences. A physiological adaptation is an organismal response to a particular stress: if you heat up from the sun you may respond by moving into the shade (a behavioral adaptation), or you may respond by sweating (a physiological adaptation). In an evolutionary context, adaptation is also a change in response to a certain problem, but the change is genetic. Evolutionary adaptations that

result from the process of natural selection usually take place over periods of time considerably longer than physiological timescales. Traits are referred to as adaptations only when they evolved as the solutions for a specific problem; that is, for a particular function or purpose. A physiological response can itself be an adaptation in the evolutionary sense.

In reconstructing ancestral phenotypes it is important to bear in mind the primitive condition may be more or less variable than the conditions observed in living species. In some cases physiological or developmental plasticity is itself an evolutionary (genetic) specialization that permits organisms to adapt physiologically or behaviorally. For example, many species are characterized as eurytopic, or tolerant of a wide variety of habitats. Other species are stenotopic, or adapted to a narrow range of habitats. Similarly, individual characters may be more or less variable within a species, and this variability may itself be subject to evolutionary change. Flexible phenotypes may be more adaptive in a variable environment and stereotyped phenotypes more adaptive in a stable environment (van Buskirk, 2002).

1.03.2.6 Phylogenetic Trees

Implicit in all phylogenetic methods for studying character evolution is a tree-shaped branching diagram, alternatively called a dendrogram, cladogram, phenogram, or tree, depending on the methods used to construct the diagram, and the information content it is intended to convey. It is important to note that each of the many alterative methods for building trees that are currently available was designed to communicate different kinds of information. The methods grouped formally as 'phylogenetic systematics' (cladistics) exclusively use derived similarities (synapomorphies) to hypothesize genealogical relationships. This is to be contrasted with phenetic methods which use measures of overall similarity to group taxa, including both primitive and derived aspects of similarity. Cladistic methods generate branched diagrams referred to as cladograms, which should be viewed as summary diagrams depicting the branching pattern most consistent with a given data set (morphological or molecular). It is important to distinguish raw cladograms from phylogenetic trees; there is no time dimension to a cladogram *per se*, and the branch lengths are simply proportional to the minimum number of steps required to map all the character states onto that tree. A robust phylogenetic tree is usually the result of several or many phylogenetic analyses. The geological time frames associated with branching events are usually estimated from external paleontological, molecular, and biogeographic sources of information.

Figure 1 provides a conceptual overview for how phylogenetic trees may be used to study phenotypic evolution. All comparative approaches begin by assuming (or building) a hypothesis of genealogical interrelationships among the taxa of interest. There are many methods, even whole philosophies, of tree building, and the reader is referred to Page and Holmes (1998) for an introduction to this literature. Phylogenetic methods are then used to optimize character states at internal nodes of the tree; these nodes or branching points are hypothesized speciation events. Comparisons of trait values at ancestral and descendant nodes of the tree allow the history of phenotypic changes to be traced. The distribution of these phenotypic changes (also known as steps or transformations) can then be assessed, qualitatively or quantitatively, depending on the types of data examined and the analytical methods employed.

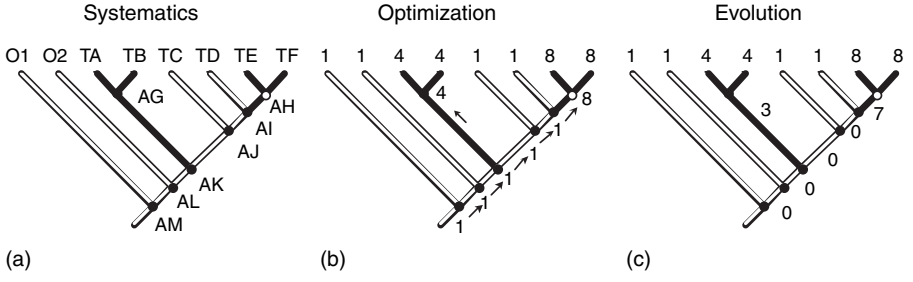

Figure 1 Summary of the comparative approach for inferring phenotypic evolution. a, Phylogenetic systematics (i.e., tree building): reconstruction of genealogical interrelationships among taxa (extant and/or fossil) using morphological and/or molecular sequence data. Taxa are species or clades (monophyletic groups of species): phylogeny includes six ingroup terminal taxa (TA–TF) and two outgroup taxa (O1 and O2). b, Character state optimization at internal nodes (branching points or hypothesized speciation events). Observed trait values at tips of the tree. Seven internal tree nodes represented by ancestral taxa (AG–AM) with trait values estimated by linear parsimony. c, Evolution: tracing the history of phenotypic changes along branches of the tree. Numbers indicate absolute amount of trait change on the branch.

A tree-shaped branching diagram conveys two kinds of information (whether they are intended or not): the tree topology, or the sequential order in which the taxa branch from one another, and the lengths of the individual branches (Figure 2). These two aspects of a tree correspond to the cladogenesis and the anagenesis of Rensch (1959). The tree topology (branching order) is reconstructed from the distribution of shared–derived traits among taxa. The traits examined may be morphological novelties or nucleotide substitutions. Branch lengths may be reconstructed from one or more sources of information, including alternative models (or modes) of character evolution, or from empirical data. Under models of constant (or near constant) evolution (e.g., molecular clocks), all terminal taxa are treated as equidistant from the root (or base) of the tree. Terminal taxa are those at the tips of the tree, as opposed to ancestral taxa at internal nodes (branching points) within the tree. Under models of punctuated equilibrium, all (or most) character evolution occurs at branching points (nodes), and all branches are therefore of equal (or almost equal) length. Branch lengths derived from empirical data sets may be treated as proportional to the amount of character state change on that particular tree topology, or from stochastic models of evolution assuming that DNA nucleotide substitutions occur at an equal rate (Sanderson, 2002). The constant evolution and punctuated equilibrium models represent extremes of branch-length heterogeneity, between which branch lengths derived from empirical data sets usually fall. Branch lengths for clades with known fossilized members can also be estimated from the geological age of these fossils (Benton *et al.*, 2000; Near and Sanderson, 2004). Calibrations based on molecular sequence divergence or fossil data can take one of two forms: assignment of a fixed age to a node, or enforcement of a minimum or maximum age constraint on a node. The latter option is generally a better reflection of the information content of fossil evidence.

It is important to recognize an analytical difference in the two kinds of information represented in a phylogeny: whereas the tree topology is transitive, the branch lengths are not. In the language of formal logic, 'transitive' means that a relationship necessarily holds across (i.e., it transcends) the particularity of data sets. In the case of phylogenetic trees, the branching order derived from analysis of one data set is expected to predict the branching order of independent data sets (e.g., those derived from different genes, genes and morphology, osteology and neurology). Branch lengths, however, are intransitive, meaning the branch length values derived from one data set are not expected to predict those of other data sets. The reason for this is that we believe there has been a single phylogenetic history of life; a unique sequence of speciation events that gave rise to the species richness of the modern world. This single history underlies the evolution of all aspects of organismal phenotypes. There are, however, no such expectations of homogeneity in the rates of phenotypic (or gene sequence) evolution; in fact,

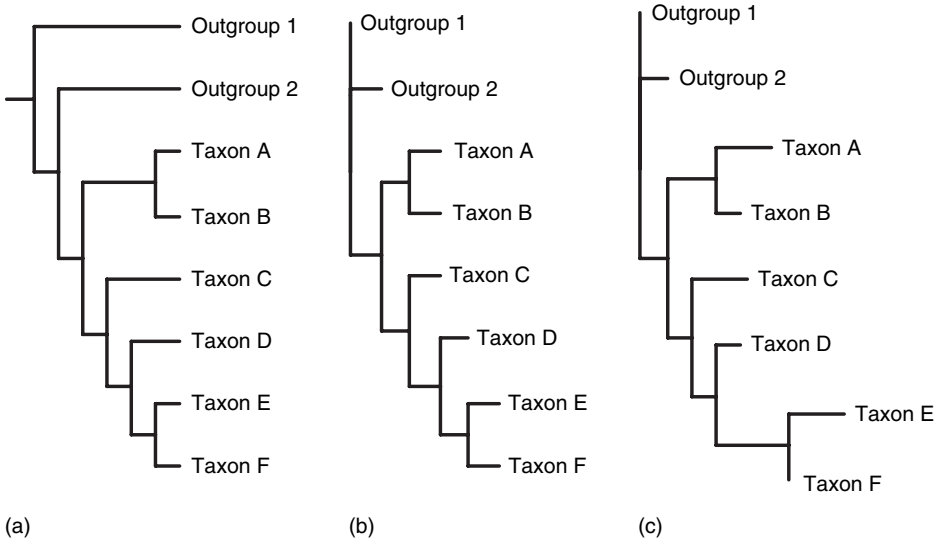

Figure 2 Alternative branch length models. a, Molecular clock: all terminal taxa equidistant from root to from an ultrametric tree. b, Equal branch lengths: all character evolution (anagenesis) occurs at branching events, as in punctuated equilibrium. c, Empirical: branch lengths proportional to amount of character evolution and/or geological ages determined from fossils. Note: tree topology is transitive; branch lengths are not.

the differential effects of directional and stabilizing selection on different phenotypes may be expected to result in longer or shorter branches for some traits than others.

1.03.3 Methods

1.03.3.1 Parsimony Optimization of Discrete Traits

The principle of parsimony (i.e., Occam's razor) is widely used in the natural sciences as a method for selecting from among numerous alternative hypotheses. The principle of parsimony underlies all scientific modeling and theory building. The basic idea is that one should not increase, beyond what is necessary, the number of entities required to explain anything. In this context, parsimony means that simpler hypotheses are preferable to more complicated ones. It is not generally meant to imply that Nature itself is simple, but rather that we as observers should prefer the most simple explanations.

Maximum parsimony (MP) is a character-based method used in phylogenetic systematics to reconstruct phylogenetic trees by minimizing the total number of evolutionary transformations (steps) required to explain a given set of data. In other words, MP minimizes the total tree length. The steps may be nucleotide base or amino acid substitutions for sequence data, or gain and loss events for restriction site and morphological data. MP may also be used to infer ancestral states of a character within a phylogenetic tree (this is discussed in the following).

1.03.3.2 Binary and Multistate Characters

Discrete characters may be characterized as either binary (coded into two mutually exclusive alternative states) or as multistate (a transformation series of three or more discrete states). The alternative states of a binary character are generally (although not necessarily) explicit hypotheses of the primitive and derived (advanced) states of a single evolutionary transformation event, such as the origin (or loss) of a novel feature. A multistate character is a more complex intellectual device with many more interpretations of meaning. Multistate characters may be presented as many stages of a long-term phylogenetic trend (e.g., larger relative brain size, larger body size) or as independent alternative trends from a common ancestral plan (e.g., large brains evolving from enlargement of the cerebellum in chondrichthyans vs. the telencephalon in mammals). An ordered transformation series models a preconceived phylogenetic sequence of changes, such that in the series 1–2–3, state 3 is only permitted to be derived from state 2. In an unordered transformation series, state 3 may be derived from either of states 1 or 2. Following a similar logic, reversals (e.g., from 2 to 1) may be allowed, penalized, or prohibited, depending on the preconceptions of the investigator. Of course, building *a priori* conceptions of order or reversibility into an analysis of character state change precludes the use of that analysis as an independent test of those assumptions. To summarize this section, treating all characters as unpolarized and unordered means that all transitions among states are regarded as equally probable.

1.03.3.3 Squared-Change and Linear Parsimony

There are two general types of MP widely used in tracing the evolution of continuous traits: squared-change parsimony and linear parsimony. Squared-change algorithms (Rogers, 1984) seek to minimize the amount of squared change along each branch across the entire tree simultaneously, using a formula in which the cost of a change from state x to y is $(x-y)^2$. Squared-change parsimony assigns a single ancestral value to each internal node to minimize the sum of squares change over the tree (Maddison, 1991). When using squared-change parsimony, the absolute amount of evolution over the whole tree is not necessarily minimized, and some degree of change is forced along most branches. Linear parsimony reconstructs ancestral node values by minimizing total changes (Figure 3). Linear-parsimony algorithms (Kluge and Farris, 1969) seek to minimize the total amount of evolution and consider only the three nearest nodes when calculating the ancestral character states. In linear parsimony the cost of a change from x to y is $|x-y|$. The result of this local optimization is that changes are inferred on very few or single branches. Linear parsimony therefore permits the accurate reconstruction of discontinuous events, or of large changes in trait values on a tree. Although evolutionary change is often thought of as gradual, large changes on a tree may result from a variety of real biological processes, not the least of which is the extinction of taxa with intermediate trait values (Butler and Losos, 1997).

1.03.3.4 Maximum Likelihood and Bayesian Optimization

Maximum likelihood (ML) methods for tracing character evolution select ancestral trait values with highest likelihood on a given phylogenetic

Taxon	*Character*
Outgroup 1	1
Outgroup 2	1
Taxon A	4
Taxon B	4
Taxon C	1
Taxon D	1
Taxon E	8
Taxon F	8

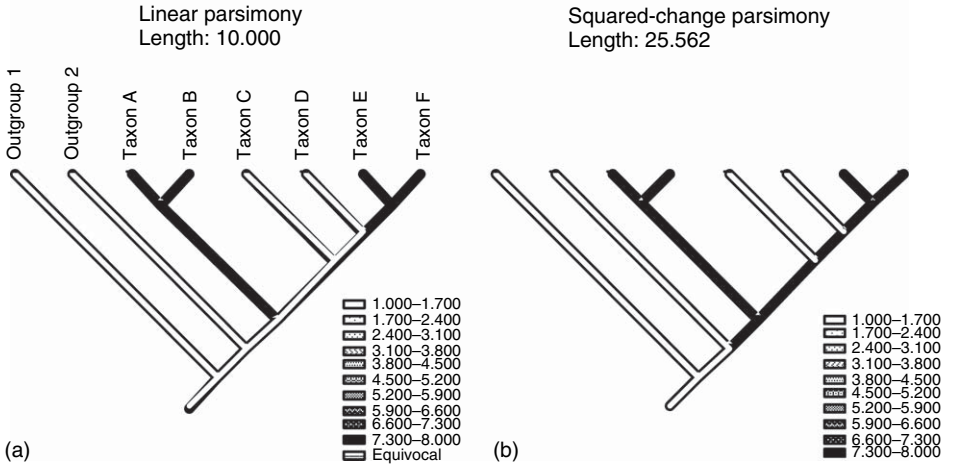

Figure 3 Alternative methods for estimating ancestral character states. a, Linear parsimony. b, Squared-change parsimony. Character state data by taxon reported in the table.

hypothesis given a model of trait evolution (defined by user). Bayesian analysis (BA) selects the ancestral trait value with the highest posterior probability, given the probabilities of priors (external evidence) and assumptions of trait evolution (defined by user). Because they are model-based approaches, ML and BA optimization methods are more commonly used in the analysis of gene sequence data, using explicit models of changes between nucleotide bases (Liò and Goldman, 1998; Sullivan *et al*., 1999). ML has been used in the analysis of continuous character evolution where the models may vary from very simple (e.g., Brownian motion) to quite complex; there is a large literature regarding methods to test the validity of using particular models (Diaz-Uriarte and Garland, 1996; Oakley, 2003).

1.03.3.5 Which Optimization Approach to Use?

Empirical studies using simulated data sets and those derived from evolution in a test tube have concluded that model-driven approaches like ML and BA give more accurate results than MP when the modeled parameters (i.e., likelihood or probability of nucleotide substitutions) are known, but can be positively misleading when the parameters are unknown (Hillis *et al*., 1992; Oakley and Cunningham, 2000). MP often provides less resolution (more interior tree nodes reconstructed with ambiguous states), than ML or BA methods, which usually give very precise estimates with high confidence levels even under circumstances in which available data are insufficient to the task. In this regard, MP methods are regarded as more conservative, with lower risk of recovering false positives (Webster and Purvis, 2002).

Most studies on the evolution of neural characters use MP approaches because, unlike molecular sequence data, it is not straightforward how to pose or parametrize models on the evolution of complex phenotypes. Continuously varying aspects of neural features, like the size or shape of structures, have been modeled as simple Brownian motion or random walk processes, under the assumptions that the trait has not experienced selection and that there are no constraints on variance through time (Butler and King, 2004). Whether or not the assumptions of Brownian motion or any other specific model are satisfied by real neural or behavioral data is almost completely unknown.

A general conclusion reached by a number of review studies is that, under most circumstances faced by comparative morphologists, linear parsimony is the most conservative method for reconstructing ancestral trait values (Losos, 1999). Unlike squared-change parsimony, linear parsimony does not average out change over the interior nodes of a tree, but rather permits

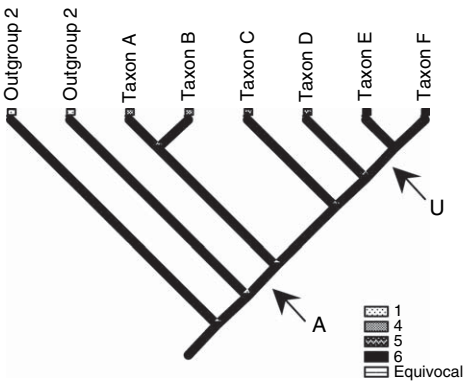

Figure 4 Ambiguous (A) vs. unambiguous (U) optimizations.

discontinuous changes along a branch. This has the advantageous effect of not forcing gradual trait evolution on the tree, and also of not forcing unnecessary trait reversals (Figure 3). A methodological advantage of linear over squared-change parsimony is that it permits the reconstruction of ambiguous ancestral character state reconstructions (Figure 4). This is a desirable property in cases where the available data are in fact insufficient to resolve the trait value at a specified internal nodes (Cunningham, 1999). A methodological disadvantage of linear parsimony is that, computationally, it requires a completely resolved tree topology in which all branching events are divided into only two daughter clades. Unfortunately, fully resolved trees are unusual in most studies with many (>30) species. By contrast, squared-change parsimony can be calculated on a tree with unresolved multichotomies (also called polytomies), and therefore often becomes the method of choice by default. One alternative to using squared-change parsimony when faced with an incompletely resolved tree is to use linear parsimony on numerous (100, 1000) arbitrarily resolved trees, and then report statistics (e.g., minimum and maximum) of the trait values obtained. Software for this procedure is available in the freely available Mesquite software package (see 'Relevant Website').

1.03.3.6 Correlative Comparative Methods

Ordinary least-squares regression allows one to investigate relationships between two variables in order to ask if change in one of these variables is associated with change in the other. One may ask, for example, how is variation in brain size related to body size, ecological role (predator vs. prey), climate, life history mode, or locomotion (Albert *et al.*, 2000; Safi and Dechmann, 2005). The least-squares fitting procedure is commonly used in data analysis in comparative studies, and conventional regression analysis has been one of the main tools available to comparative neurobiology and ecological physiology to study form–function relationships and adaptation (Garland and Carter, 1994). However, it is now widely recognized that interspecific observations generally do not comprise independent and identically distributed data points, thus violating fundamental assumptions of conventional parametric statistics (Felsenstein, 1985, 1988; Pagel and Harvey, 1989; Harvey and Pagel, 1991).

Phylogenetically based statistical methods allow traditional topics in comparative neuroanatomy and physiology to be addressed with greater rigor, including the form of allometric relationships among traits and whether phenotypes vary predictably in relation to behavior, ecology, or environmental characteristics (Brooks and McLennan, 1991; Frumhoff and Reeve, 1994; Losos, 1996). In a conventional regression analysis the data points represent terminal taxa. In a phylogenetic regression the data points represent sister-taxon comparisons (Grafen, 1989). These two methods are compared in Figure 5, in which identical data are analyzed using conventional and phylogenetic regression methods. The phylogeny of Figure 5 includes six terminal taxa (TA–TF) and two outgroup taxa (O1 and O2), which are represented by two continuously distributed characters (C1 and C2). The tree topology has been determined from data other than characters 1 and 2, and the branch lengths are treated as equal (under a model of punctuated equilibrium). There are seven internal tree nodes represented by ancestral taxa (AG–AM) with trait values estimated by least-square parsimony. By removing psuedorepilcates, the phylogenetic regression compares fewer taxa, has fewer degrees of freedom, and has a lower correlation coefficient (R^2 value) than does the conventional regression. The phylogenetic regression, therefore, provides a better quantitative measure of correlated evolution between the two traits, and is a more conservative measure of the strength of adaptive pressures.

Relationships between brain size and the volume of frontal and visual cortices in mammals have recently been studied using the methods of phylogenetic regression analysis (Bush and Allman, 2004a, 2004b). These studies found that size has a profound effect on the structure of the brain, and that many brain structures scale allometrically; that is, their relative size changes systematically as a function of brain size. They also conclude that the three-dimensional shape of visual maps in anthropoid

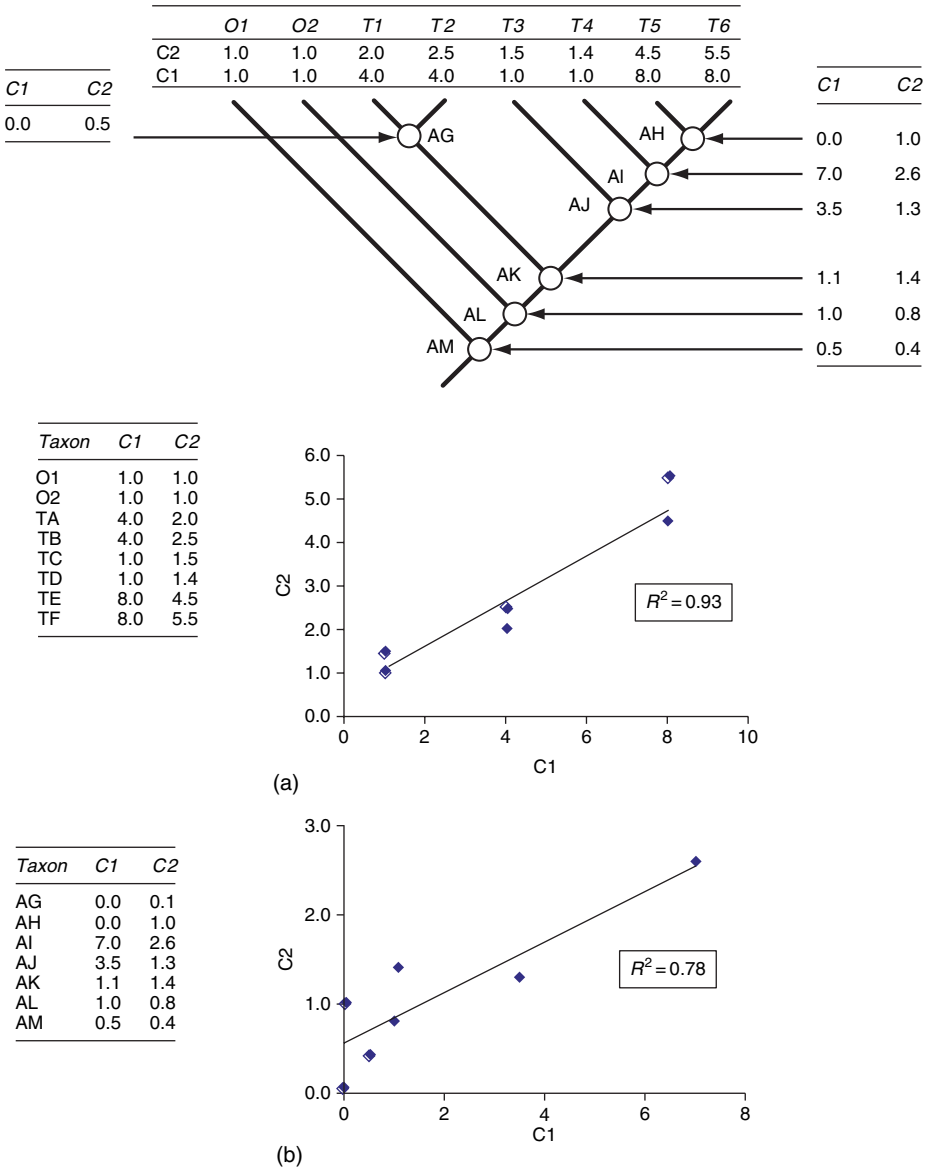

Figure 5 Comparison of conventional and phylogenetic regression analyses. Phylogeny of six terminal taxa (TA–TF) and two outgroup taxa (O1 and O2), represented by two continuously distributed characters (C1 and C2). Tree topology determined from data other than characters 1 and 2, and branch lengths treated as equal. Seven internal tree nodes represented by ancestral taxa (AG–AM) with ancestral trait values estimated by least-squares parsimony. a, Conventional regression of trait values from terminal taxa. b, Phylogenetic regression of trait values at internal tree nodes using the method of independent contrasts. Note that by removing psuedoreplicates, the phylogenetic regression compares fewer taxa, has fewer degrees of freedom, and has a lower correlation coefficient (R^2 value) than does the conventional regression. The phylogenetic regression, therefore, provides a more conservative quantitative measure of correlated evolution between the two traits.

primates is significantly longer and narrower than in strepsirrhine primates. Using conventional regression analyses, von Bonin (1947) showed that frontal cortex hyperscales with brain size, and humans have "precisely the frontal lobe which [we deserve] by virtue of the overall size of [our] brain." These are, of course, precisely the qualitative conclusions arrived at by Bush and Allman using analysis of phylogenetic regressions. In fact, many studies reviewing the uses of phylogenetic methods for reconstructing ancestral states conclude that all methods will recover a very strong historical signal (Losos, 1999).

1.03.4 Limitations of Methods

The accuracy of ancestral reconstructions has been investigated by comparisons with known phylogenies (e.g., viruses, computer simulations; Oakley and Cunningham, 2000). It is well known

that all phylogenetically based methods perform poorly when taxon sampling is low and when rates of evolution in the character of interest are unequal among branches of the tree (Garland *et al*., 1993; Sullivan *et al*., 1999; Hillis *et al*., 2003). Further, all methods for studying character evolution on a tree make certain assumptions about the capacity of trees to faithfully record the actual history of character change. These include the assumptions that: phenotypic diversification results largely from speciation and that the effects of extinction have not erased the signal, that taxon sampling faithfully represent the history of diversification, and that genealogical history is largely or entirely bifurcating (vs. multifurcating or converging). Of course, all methods assume we know the 'true' (or 'nearly true') tree topology. In addition, each of the optimization methods makes assumptions about critical parameters, including branch lengths, models of character evolution, absolute rates of evolution, homogeneity (vs. heterogeneity) of evolutionary rates, reversibility (or the lack thereof), and the orderedness (or unorderedness) of multistate characters.

The accuracy of ancestral trait reconstruction also depends strongly on parameter estimation (e.g., tree topology, branch lengths, and models of trait evolution). ML and BA perform well when model assumptions match real parameters. ML and BA are positively misleading when model assumptions are violated. MP is more conservative, recovering fewer false positives than ML and BA when biological parameters are not known. Squared-change parsimony, ML, and BA minimize large changes, spreading evolution over the internal tree branches. Linear parsimony permits reconstructions at ancestral nodes with no change, and permits ambiguous reconstructions. 'Independent contrasts' assumes that selection operates in the origin but not maintenance of derived traits.

Both conventional and phylogenetic correlations of interspecific character data make assumptions about critical parameters. These assumptions are often of unknown validity, and in some cases are known to be incorrect. Conventional statistics assume that each terminal taxon (tips of the tree) may be treated as independent sample of the relationship under investigation. This means that the character value (phenotype) observed in that taxon evolved independently (without inheritance) from the values in other taxa in the analysis. In an evolutionary context, this is equivalent to assuming that trait values result primarily from stabilizing selection in each species that acts to maintain trait values, rather than from directional selection at the origin of the trait in an ancestral species (Hansen, 1997). In other words, conventional statistics assume traits to be highly labile and without significant phylogenetic inertia. Phylogenetic correlations make converse assumptions, that trait values are due largely or entirely to directional selection at the origin of a feature and that the influence of stabilizing selection is negligible. Phylogenetic correlations also must make particular assumptions about branch lengths and models of trait evolution.

1.03.5 Conclusions

As in all aspects of historical inquiry, the study of character evolution is exceptionally sensitive to the amount of information that has actually survived up to the present. The reality of neural evolution was in most cases almost certainly very complex, and may be reliably regarded to have included vastly more numbers of independent transformations than has been recorded in the distribution of phenotypes preserved among living species. The signature of many historical events has been overwritten by reversals and convergences, or eliminated altogether by extinctions. Paleontologists estimate that more than 99% of all species that have ever lived are now extinct (Rosenzweig, 1995). This figure, of course, includes higher taxa (e.g., trilobites, placoderms, plesiosaurs) that are now entirely extinct, bringing up the aggregate percentage of extinction for all taxa. The proportion of living species that persists within certain targeted taxa may be much higher (e.g., Lake Victoria cichlid fishes). Nevertheless, in comparative studies of neural, physiological, or behavioral phenotypes, it is rare to have information on all extant species. Whether it is from extinction or incomplete surveys, taxon sampling remains one of the greatest sources of error in phylogenetic estimates of character evolution (Sullivan *et al*., 1999; Zwickl and Hillis, 2002).

Despite all these reservations, we must continue to estimate ancestral traits in order to study phenotypic evolution. None of the methods reviewed in this article should be regarded as a magic bullet, but rather there are advantages and disadvantages of each method as they are applied under different circumstances. All the methods reviewed here have proved to be useful tools in the phylogenetic toolbox. As in other aspects of science, it is important to make our assumptions explicit, and to use reasonable assumptions. Further, as in other aspects of evolutionary biology, critical insights into the evolution of neural characters will come from a better understanding of the biology of the phenotypes themselves, and the organisms in which they have evolved.

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Relevant Website

http://mesquiteproject.org – A modular system of evolutionary analysis. Version 1.06, Maddison, W. P. and Maddison, D. R. 2005.

1.04 Basic Nervous System Types: One or Many?

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Glossary

Bilateria A monophyletic group of metazoan animals that is characterized by bilateral symmetry. This group comprises all of the Metazoa except for the Radiata (Ctenophores and Cnidaria) and the Parazoa (sponges).

blastopore The site of gastrulation initiation.

coelom Fluid-filled body cavity found in animals that is lined by cells derived from mesoderm tissue in the embryo and provides for free, lubricated motion of the viscera.

Deuterostomia (From the Greek: *mouth second*) A major group of the Bilateria including echinoderms and chordates. In deuterostomes, the first opening (the blastopore) becomes the anus and the mouth derives from a secondary invagination.

Ecdysozoa Major group of protostome animals, including the arthropods (insects, arachnids, crustaceans, and relatives), roundworms, and several smaller phyla, which are characterized by a trilayered cuticle, composed of organic material, which is periodically molted as the animal grows by a process called ecdysis.

Gastroneuralia A subdivision of the Bilateria defined by the location of the nerve cord, Gastroneuralia are characterized by a ventral nerve cord and include most protostomes except the Tentaculata.

homeodomain A 60-amino-acid part of proteins that corresponds to the homeobox sequence found in homeobox genes that are involved in the regulation of the development (morphogenesis) of animals, fungi, and plants.

homology Correspondence or relation in type of structure because of shared ancestry.

Lophotrochozoa Major group of protostome animals, including mollusks, annelids, nemerteans, brachiopods, and several other phyla characterized either by the production of trochophore larvae, which have two bands of cilia around their middle, or by the presence of a lophophore, a fan of ciliated tentacles surrounding the mouth.

Notoneuralia A subdivision of the Bilateria defined by the location of the nerve cord, Notoneuralia are characterized by a dorsal nerve cord and include most deuterostomes except the Echinodermata, Chaetognatha, and Enteropneusta.

phylogeny The origin and evolution of a set of organisms, which reveals ancestral relationships, such as monophyly (common origin) or polyphyly (independent origin), among known species.

Protostomia (From the Greek: *first the mouth*) A major group of the Bilateria including the Lophotrochozoa and the Ecdysozoa. In protostomes, the mouth forms at the site of the blastopore and the anus forms as a second opening.

Urbilateria The animal that preceded all recent bilateral symmetric animals.

1.04.1 Introduction

The diversity of nervous systems is enormous. In terms of structural and functional organization as well as in terms of levels of complexity, nervous systems range from the simple peripheral nerve nets found in some of the basal invertebrate taxa to the centralized nervous systems and highly complex brains that characterize vertebrates and cephalopods. Starting in the eighteenth century, numerous attempts were undertaken to reconstruct the evolutionary origin of the diverse nervous system types found in the animal kingdom (see Origin and Evolution of the First Nervous System). However, initially none of these attempts resulted in consensus, in part because of the uncertain and ambiguous nature of the postulated phylogenetic relationships among the various animal groups considered (see Metazoan Phylogeny). At the beginning of the twentieth century, it became evident that the bilaterally symmetrical animals, the Bilateria, could be phylogenetically subdivided into two major branches (Fioroni, 1980). This subdivision of the Bilateria into the protostome and the deuterostome animals remains valid (Brusca and Brusca, 1990) and has been confirmed by molecular analyses (e.g., Adoutte *et al.*, 2000).

Do the general nervous system types that characterize the protostome and deuterostome animals also follow this binary subdivision? Classical neuroanatomical and embryological studies suggest that this is the case, at least in part. Accordingly, most bilaterian animals can be subdivided into two major groups with different central nervous system (CNS) morphologies. These are the Gastroneuralia, which are characterized by a ventral nerve cord and include major protostome groups such as arthropods, annelids, and mollusks, and the Notoneuralia, which are characterized by a dorsal nerve cord and include all (deuterostome) chordates (e.g., Nielsen, 1995). The two groups often manifest different modes of CNS development. In gastroneuralians such as arthropods, the ganglionic masses detach from the ventral neuroectoderm to form a rope-ladder nervous system of connectives and commissures, whereas in notoneuralian chordates the neuroectoderm folds inwardly as a whole to form a neural tube (Figure 1; see A Tale of Two CPGs: Phylogenetically Polymorphic Networks). As a result of the Gastroneuralia/Notoneuralia subdivision, the notion of an independent evolutionary origin of the CNS of protostomes versus deuterostomes gained general acceptance and accordingly a polyphyletic origin of bilaterian nervous systems was proposed.

The alternative notion, namely, that bilaterian nervous systems might have a common evolutionary origin, was rejected precisely because of the evident dissimilarities in the mode of development, topology, and adult morphology of the nervous systems in major protostome versus deuterostome groups.

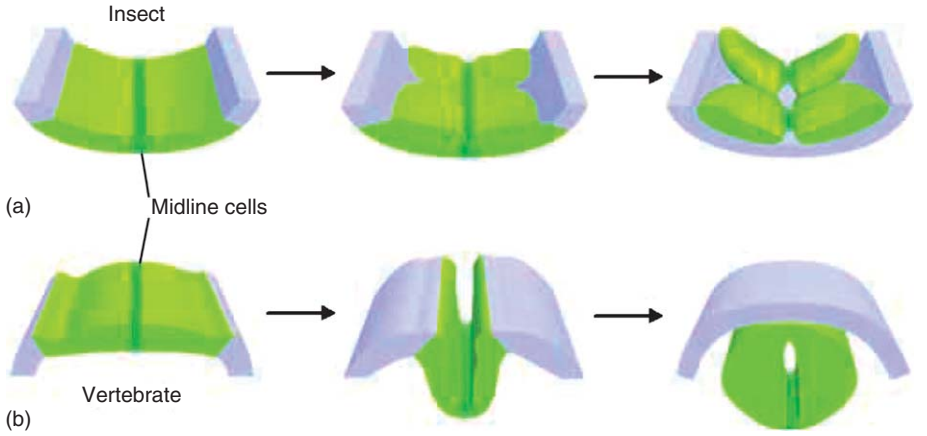

Figure 1 Morphogenesis of the ventral nerve cord in a prototype insect (a) and of the dorsal neural tube in a prototype vertebrate (b). Arrows indicate ontogenetic sequences; yellow-green, neurogenic ectoderm; blue, epidermal ectoderm. Reproduced from Arendt, D. and Nübler-Jung, K. 1999. Comparison of early nerve cord development in insects and vertebrates. *Development* 126, 2309–2325, with permission from The Company of Biologists Ltd.

However, starting in the 1980s, a number of key findings resulting from developmental biological analyses of animal body axis formation began to call into question the validity of the Gastroneuralia/Notoneuralia subdivision and, in doing so, provided initial support for the idea of a monophyletic origin of the bilaterian nervous system. In a nutshell, these findings demonstrated that the molecular genetic mechanisms of anteroposterior axis formation are shared among all bilaterians and that the molecular genetic mechanisms of dorsoventral axis formation in vertebrates are similar to those that operate in insects, only that their dorsoventral topology is inverted, upside-down. If dorsal in vertebrates corresponds to ventral in insects, might not the dorsal nerve cord of Notoneuralia in fact correspond to the ventral nerve cord of Gastroneuralia?

This axial inversion hypothesis was remarkable not only because it was based on unequivocal molecular genetic evidence, but also because it provided support for an old and much-derided view that emerged in the early nineteenth century. Its first proponent was the French zoologist Geoffroy Saint-Hilaire, in opposition to his countryman, the comparative anatomist Cuvier. Both engaged in a debate about a fundamental issue in the biological sciences, namely, whether animal structure ought to be explained primarily by reference to function or rather by morphological laws. At the heart of this debate was the question of whether a common structural plan, or Bauplan, underlies all animal development, thus indicating homology of structures across different animal phyla. Contemporary developmental biological studies based on analyses of expression and function of homologous regulatory control genes in various animal model systems have revived this fundamental question and contributed novel insight into the issue of homology of nervous systems. In this article, we will begin with this famous debate, consider the impact of molecular developmental genetics on a bilaterian nervous system Bauplan, and then discuss the current data for and against a common evolutionary origin of the nervous system. Though our main emphasis will be on conserved mechanisms of anteroposterior and dorsoventral patterning of the nervous system in insect and vertebrate model systems, we will also consider gene expression studies in invertebrates such as hemichordates and cnidarians.

1.04.2 The Cuvier–Geoffroy Debate

1.04.2.1 A Common Bauplan for Animal Development?

In 1830, a series of eight public debates were held at the Académie Royale des Sciences in Paris. The two opponents, George Cuvier (1769–1832) and Étienne Geoffroy Saint-Hilaire (1772–1844), were prominent and internationally renowned scientists. Both had made major contributions in many areas of natural history, including comparative anatomy and paleontology. Cuvier divided the animal kingdom into four completely separate branches or *embranchements*: vertebrates, articulates (largely arthropods and annelids), mollusks (which at the time meant all other soft, bilaterally symmetrical invertebrates), and radiates (echinoderms, cnidarians, and various other groups). According to Cuvier, there was no affinity whatsoever between the four *embranchements*. Any similarities between organisms were due to common functions, not to common ancestry. Function determines form; form does not determine function. Thus, even within these divisions, he allowed structural similarity to result solely from the same functional demands.

Geoffroy, by contrast, insisted that function was always dependent on structure and by no means sufficed to determine structure. What counted were the interconnections between parts; structures in different organisms were the same if their parts were connected to one another in the same pattern. Eventually Geoffroy developed the doctrine of unity of composition, applicable at least within each class of animals. Each animal is formed from a structural blueprint based on a common plan, and although animal structure is modified extensively because of functional requirements, the modification is constrained by the unity of composition (which later came to be known as the basic Bauplan). This doctrine of Geoffroy's came to be known as philosophical anatomy and was founded on analogy between structures (homology in modern terminology). Geoffroy's main criterion for determining true analogies was the connectivity between structures and this could often be better determined from the embryo rather than from the adult. The value of the theory of analogues was that it offered a scientific explanation for differences in structure.

Initially, these ideas related primarily within each class of animals or *embranchements*, but Geoffroy imagined that the principle could be extended to the animal kingdom as a whole. After having established a common scheme for vertebrates, he extended this principle across the boundaries of Cuvier's four *embranchements* to articulates. In 1822, Geoffroy published a paper entitled *Considérations générales sur la vertèbre*, in which he proposed that the ventral side of arthropods was analogous to the dorsal side of the vertebrates. This dorsoventral axis inversion hypothesis was based on a dissected crayfish that he had placed upside down

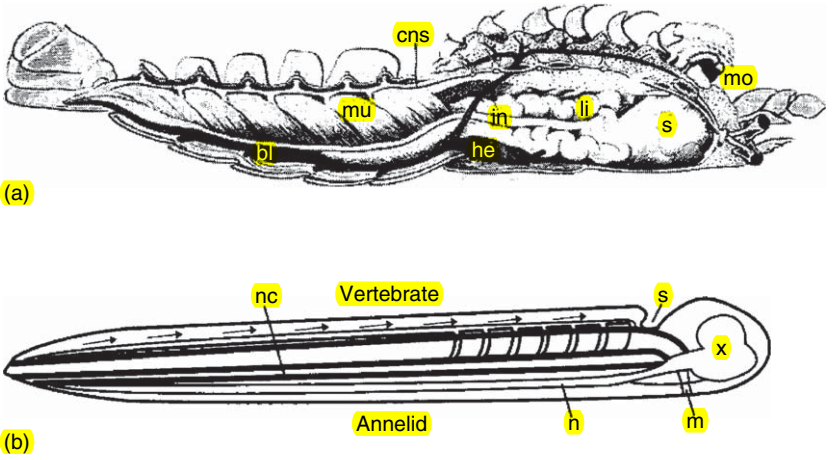

Figure 2 The dorsoventral inversion hypothesis. a, Geoffroy Saint-Hilaire's dissected lobster. In this dissection, the animal is presented in the orientation opposite to the orientation that it would normally have with respect to the ground. The central nervous system (cns) is at the top and is traversed by the mouth (mo). Below this is the digestive tract with the stomach (s), liver (li), and intestine (in). Below the gut are the heart (he) and main blood vessels (bl). Muscles (mu) flank the CNS. In this orientation, the body plan of the arthropods resembles that of the vertebrate. b, Inverted relationship of the annelid and vertebrate body plans; only the mouth changes position with inversion, making a new opening in the chordate lineage. m, mouth; n, nerve cord; nc, notochord (only in chordates); s, stomodeum (secondary mouth); x, brain. Arrows show direction of blood flow. a, Reprinted by permission from Macmillan Publishers Ltd: *Nature* (De Robertis, E. M. and Sasai, Y. 1996. A common plan for dorsoventral patterning in Bilateria. *Nature* 380, 37–40), copyright (1996). b, Modified textbook diagram; see, for example, Romer and Parsons (1977).

and, as he noted, in this orientation the organization of the main body system of the lobster resembled that of a mammal (see Figure 2). One objection readily raised against such an attempt to link arthropods and vertebrates was that the nervous system in arthropods was nevertheless found on the ventral side, whereas in vertebrates it was located on the dorsal side. Geoffroy's solution to this problem was that the definitions of dorsal and ventral were purely arbitrary, because they were based solely on the orientation of the animal to the sun. If it was assumed that the arthropod walked with its ventral side rather than its dorsal side toward the sun, then all of the organs of the arthropod would have the same topological arrangement as the organs of vertebrates.

As expected, Cuvier rejected such interpretations. For him, animals shared similar basic plans only because they carried out a similar combination of interrelated functions. Because the fundamental plan was completely different in each *embranchement,* there were no and could be no transitional forms leading from one *embranchement* to the next. Moreover, no one had ever observed the transformation of one species into another. The differences between the scientific approaches of Geoffroy and Cuvier came to a head when two young naturalists, Meyranx and Laurencet, submitted to the academy a comparison of the anatomy of vertebrates and cephalopods (squids, cuttlefish, and octopi), claiming that they were based on the same basic structural plan. Geoffroy, who was chosen by the academy to review the paper, enthusiastically adopted this claim as proof of his unity of composition shared by all animals. Cuvier could not reconcile this with the results of his careful anatomical research, and in the ensuing debates, he showed convincingly that many of Geoffroy's supposed examples of unity of structure were not accurate; the similarities between vertebrates and cephalopods were contrived and superficial. As an immediate consequence, the results of Meyranx and Laurencet never went to press (for details, see Appel, 1987).

1.04.2.2 From Unity of Composition to Unity of Nervous Systems?

Although Cuvier was considered to have won the 1830 debates, Geoffroy's philosophical anatomy remained remarkably influential during the subsequent decades. A resolution of the conflicting ideas was achieved, in part, by Darwin's evolutionary theory in which structural homology became an important criterion for establishing phylogenetic relationships. Moreover, with the advent of molecular developmental genetics, it has become clear that homology is a concept that applies not only to morphology, but also to genes and developmental processes. Indeed, and rather unexpectedly, more than 150 years after the famous debate, developmental genetics has provided experimental evidence for Geoffroy's unity of composition and

specifically for his dorsoventral axis inversion hypothesis that appeared to be so convincingly refuted by Cuvier.

The discovery that a common developmental genetic program underlies dorsoventral axis formation in both insects and vertebrates was based on the analysis of two sets of homologous genes that encode morphogens in the model systems *Drosophila* and *Xenopus* (Holley *et al*., 1995; Schmidt *et al*., 1995; De Robertis and Sasai, 1996; Holley and Ferguson, 1997). The transforming growth factor β (TGFβ) family member encoded by the *decapentaplegic* (*dpp*) gene is expressed dorsally and promotes dorsal fate in *Drosophila*, whereas its vertebrate orthologue *Bone morphogenetic protein* (*Bmp4*) is expressed ventrally and promotes ventral fate in *Xenopus*. These morphogens are antagonized by the secreted products of the orthologous genes *short gastrulation* (*sog*) in *Drosophila* and *Chordin* in *Xenopus*. Importantly, the site of action where *sog/Chordin* expression inhibits *dpp/Bmp4* signaling corresponds in both insects and vertebrates to the region of the dorsoventral body axis that gives rise to the embryonic neuroectoderm from which the nervous system derives (see below).

These results provide strong evidence that the molecular interactions that occur on the ventral side of insects are homologous (in Geoffroy's sense, analogous) to those that occur on the dorsal side of vertebrates – an observation that revitalizes Geoffroy's initial proposition of the unity of composition between arthropods and mammals and supports the hypothesis of a dorsoventral inversion of their body axes during the course of evolution (Arendt and Nübler-Jung, 1994). Moreover, these results also provide strong evidence that the molecular interactions that lead to the formation of the ventral CNS in insects are homologous to those that lead to the formation of the dorsal CNS in vertebrates, indicating a dorsoventral body axis inversion as the most parsimonious explanation for the dorsoventrally inverted topology of the CNS that characterizes Gastroneuralia versus Notoneuralia.

Comparable molecular genetic studies on other sets of homologous genes in various model systems ranging from annelids and arthropods to mammals are providing further evidence that Geoffroy's unity of composition might be the result of a developmental construction plan that is shared by all bilaterian animals. Thus, evolutionarily conserved developmental control genes act not only in dorsoventral axis specification but also in anteroposterior axis formation, segmentation, neurogenesis, axogenesis, and eye/photoreceptor cell development through comparable molecular mechanisms that appear to be conserved throughout most of the animal kingdom. The implications of these findings are far-reaching. They suggest that, although diverse in their mode of development and adult morphology, bilateral animals derived by descent from a common ancestor, the Urbilateria, which may already have evolved a rather complex body plan (De Robertis and Sasai, 1996). Accordingly, the urbilaterian nervous system may already have evolved structural features that prefigured elements of the nervous systems of the descendent bilaterian animals. If this were indeed the case, then the ventrally located arthropod nervous system may be homologous to the dorsally located chordate nervous system; the insect brain may be composed of structural units homologous to those of the vertebrate brain; the visual system of a fly may be homologous to the visual system of a mammal. The plausibility of this scenario is particularly evident with regard to the conserved mechanisms of anteroposterior and dorsoventral patterning of the nervous system that operate in insects and vertebrates.

1.04.3 Conserved Mechanisms for Anteroposterior Patterning of the CNS

1.04.3.1 *Hox* Genes Are Involved in the Regional Specification of Neuronal Identity

Along the anteroposterior axis, the insect and vertebrate neuroectoderm is subdivided into compartment-like regions, each of which expresses a specific combination of conserved developmental control genes. In both animal groups, regions of the posterior brain and the nerve cord are specified by the expression and action of homeodomain transcription factors encoded by the *Hox* genes (see Figure 3). *Hox* genes were first identified in *Drosophila* and *Hox* gene orthologues have subsequently been found in all other bilaterian animals, including mammals. During embryonic development, these developmental control genes are involved in anteroposterior patterning of features such as the morphology of segments in *Drosophila* or the morphology of axial mesoderm derivatives in mammals. *Hox* genes generally respect the co-linearity rule: they are expressed along the body axis in the same order as they are found clustered on the chromosome. Their role in anteroposterior regionalization may have evolved early in metazoan history (Carroll, 1995).

In both invertebrates and vertebrates, *Hox* gene expression is especially prominent in the developing CNS, and the nervous system may be the most

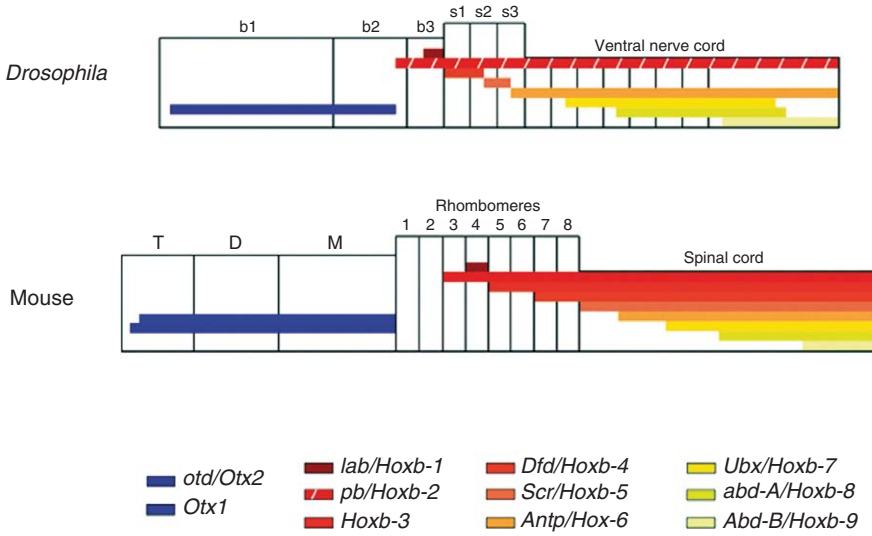

Figure 3 Conserved anteroposterior order of gene expression in embryonic brain development. Schematic diagram of *Hox* and *otd/Otx* gene expression patterns in the developing CNS of *Drosophila* and mouse. Expression domains are color-coded. (Top) Gene expression in embryonic stage 14 *Drosophila* CNS. Borders of the protocerebral (b1), deutocerebral (b2), tritocerebral (b3), mandibular (s1), maxillary (s2), labial (s3), and ventral nerve cord neuromeres are indicated by vertical lines. In contrast to the other Hox genes, *pb* is expressed only in small segmentally repeated groups of neuronal cells; this difference is indicated by a diagonally striped bar to denote the *pb* expression domain. (Bottom) Gene expression in embryonic day 9.5–12.5 mouse CNS. Borders of the telencephalon (T), diencephalon (D), mesencephalon (M), and rhombomeres are indicated by vertical lines. Reproduced from Hirth, F. and Reichert, H. 1999. Conserved genetic programs in insect and mammalian brain development. *Bioessays* 21, 677–684, with permission from John Wiley & Sons, Inc.

ancestral site of *Hox* gene action. In animal taxa investigated thus far, such as planarians (Orii *et al.*, 1999), nematodes (Kenyon *et al.*, 1997), annelids (Kourakis *et al.*, 1997; Irvine and Martindale, 2000), mollusks (Lee *et al.*, 2003), arthropods (Hirth and Reichert, 1999; Hughes and Kaufman, 2002), urochordates (Ikuta *et al.*, 2004), cephalochordates (Wada *et al.*, 1999), hemichordates (Lowe *et al.*, 2003), and vertebrates including zebra fish, chicken, mouse, and human (Lumsden and Krumlauf, 1996; Vieille-Grosjean *et al.*, 1997; Carpenter, 2002; Moens and Prince, 2002), the *Hox* gene expression patterns in the developing CNS consist of an ordered set of domains that have a remarkably similar anteroposterior arrangement along the neuraxis.

The function of *Hox* genes in CNS development has been studied through loss- and gain-of-function experiments primarily in *Drosophila*, zebra fish, chicken, and mouse. In *Drosophila*, loss-of-function studies have shown that *Hox* genes are required for the specification of regionalized neuronal identity in the posterior brain (Hirth *et al.*, 1998). Comparable results have been obtained through loss-of-function studies in vertebrates, where *Hox* genes are involved in specifying the rhombomeres of the developing hindbrain. For example, in the murine *Hoxb1* mutant, rhombomere 4 (r4) is partially transformed to r2 identity (Studer *et al.*, 1996), whereas in *Hoxa1*$^{-/-}$; *Hoxb1*$^{-/-}$ double mutants, a region corresponding to r4 is formed, but r4-specific neuronal markers fail to be activated, indicating the lack of neuronal identity of the remaining territory between r3 and r5 (Studer *et al.*, 1998; Gavalas *et al.*, 1998). This suggests that *Hoxa1* and *Hoxb1* act synergistically in the specification of r4 neuronal identity – a mode of action remarkably similar to that of their fly orthologue, *labial*, in specifying segmental neuronal identity during *Drosophila* brain development (Figure 4).

This evolutionarily conserved *Hox* gene action is underscored by experiments that show that even *cis*-regulatory regions driving the specific spatiotemporal expression of *Hox* genes appear to operate in a conserved manner in insects and vertebrates. Thus, the enhancer region of the human *Hoxb4* gene, an orthologue of *Drosophila Deformed*, can function within *Drosophila* to activate gene expression in a *Deformed*-specific pattern, whereas the enhancer region of *Drosophila Deformed* activates *Hoxb4*-specific expression in the mouse hindbrain (Malicki *et al.*, 1992). Similar results have been obtained for *Hox1* orthologues (Pöpperl *et al.*, 1995), suggesting that the expression, function, and regulation of *Hox* genes in the specification of segmental neuronal identity during CNS development may be an ancestral feature of this gene family.

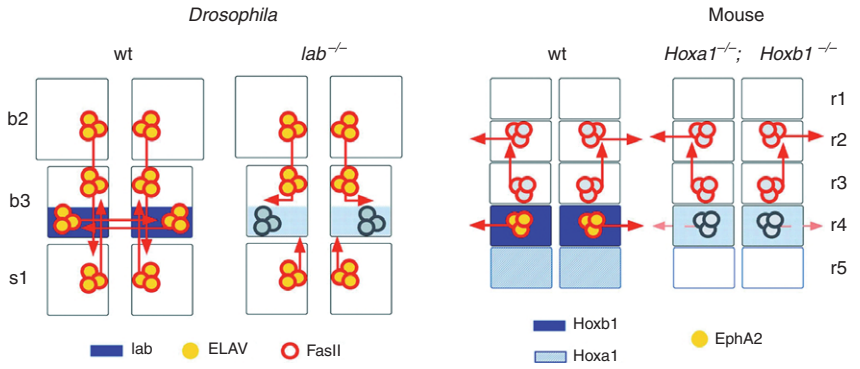

Figure 4 Comparable brain phenotypes in *lab/Hox1* loss-of-function mutants in *Drosophila* and mouse. (Left) Simplified scheme of the deutocerebral (b2), tritocerebral (b3), and mandibular (s1) neuromeres of the *Drosophila* brain. In the wild type (wt) cells in the posterior tritocerebrum express lab (blue) and also express the neuron-specific marker ELAV and the cell adhesion molecule FasII. In the *lab* null mutant (*lab*$^{-/-}$), cells in the mutant domain are present but do not extend axons and fail to express the neuron-specific marker ELAV and the cell adhesion molecule FasII, indicating a total loss of neuronal identity. Axons from other parts of the brain avoid the mutant domain. (Right) Simplified scheme of rhombomeres r1–r5 of the mouse hindbrain. In the wild type (wt) cells in r4 co-express *Hoxa1* and *Hoxb1* and also express the r4-specific marker EphA2. In the *Hoxa1*$^{-/-}$; *Hoxb1*$^{-/-}$ double homozygous mutant, cells in r4 are present but the r4-specific marker EphA2 fails to be activated in r4, indicating the presence of a territory between r3 and r5 with an unknown identity. The double mutant also exhibits multiple defects in the motor neuron axonal projections; facial motor neurons are scarce and exit randomly from the neural tube. Reproduced from Hirth, F. and Reichert, H. 1999. Conserved genetic programs in insect and mammalian brain development. *Bioessays* 21, 677–684, with permission from John Wiley & Sons, Inc.

1.04.3.2 Cephalic Gap Genes in Regionalization of the Anterior Brain: The *otd/Otx* Genes

In none of the animal species investigated to date are *Hox* genes expressed in the most anterior regions of the developing CNS. This suggests that the developing CNS is subdivided into a posterior Hox region and a more anterior non-Hox region. In both invertebrates and vertebrates, the non-Hox region of the anterior brain is characterized by the expression and action of the cephalic gap genes *tailless* (*tll*)/*Tlx*, *orthodenticle* (*otd*)/*Otx*, and *empty spiracles* (*ems*)/*Emx* (Arendt and Nübler-Jung, 1996). The most prominent example of cephalic gap genes acting in brain development is that of the *otd/Otx* genes. As is the case of the *Hox* genes, the CNS-specific expression of the *otd/Otx* genes is conserved throughout most of the animal kingdom.

otd/Otx genes are expressed in the anterior part of the developing nervous system in planarians (Umesono *et al.*, 1999), nematodes (Lanjuin *et al.*, 2003), annelids (Bruce and Shankland, 1998; Arendt *et al.*, 2001), mollusks (Nederbragt *et al.*, 2002), arthropods (Hirth and Reichert, 1999; Schröder, 2003), urochordates (Wada *et al.*, 1998), cephalochordates (Tomsa and Langeland, 1999), hemichordates (Lowe *et al.*, 2003), and vertebrates (Acampora *et al.*, 2001b; Schilling and Knight, 2001).

Functional studies, carried out primarily in *Drosophila* and mouse, have shown that *otd/Otx* gene activity is essential for the formation of the anterior neuroectoderm. In *Drosophila*, *otd* is expressed in the developing brain throughout most of the protocerebrum and adjacent deutocerebrum. In *otd* mutants, the protocerebrum is deleted due to defective neuroectoderm specification and the subsequent failure of neuroblast formation (Hirth *et al.*, 1995; Younossi-Hartenstein *et al.*, 1997). Loss-of-function analyses for *Otx* genes carried out in the mouse show that these genes are also critically required at different stages in the development of the anterior brain. *Otx2* null mice are early embryonic lethal and lack the rostral neuroectoderm that is normally fated to become the forebrain, midbrain, and rostral hindbrain due to an impairment in early specification of the anterior neuroectoderm by the visceral endoderm. *Otx1* null mice show spontaneous epileptic seizures and abnormalities affecting the telencephalic dorsal cortex and the mesencephalon, as well as parts of the cerebellum and certain components of the acoustic and visual sense organs (Acampora *et al.*, 2001b).

These essential roles of the *otd/Otx* genes in anterior brain development of insects and vertebrates suggest an evolutionary conservation of *otd/Otx* genes in embryonic brain development that extends beyond gene structure to patterned expression and function (Figure 5). A direct experimental demonstration of this functional conservation has been carried out in genetic cross-phylum rescue experiments. Thus, human *Otx* transgenes have been expressed in *Drosophila otd* mutants (Leuzinger *et al.*, 1998) and, conversely, the murine *Otx1* and

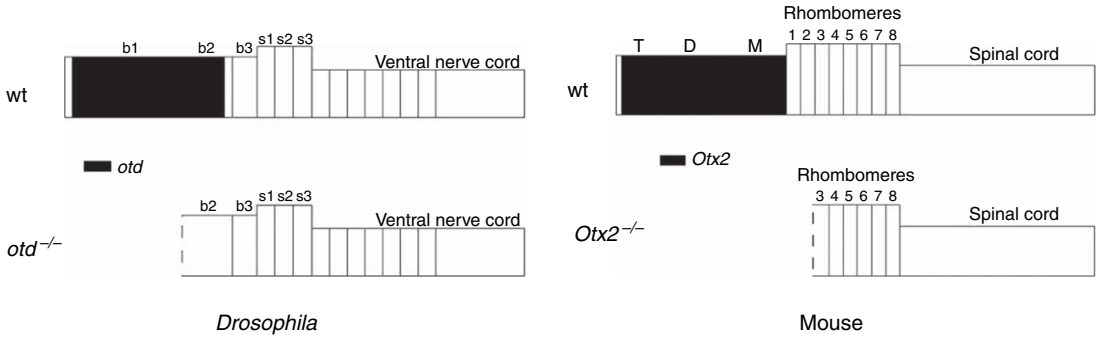

Figure 5 Conserved expression and function of the *otd/Otx2* genes in embryonic brain development. Schematic diagram of *otd* and *Otx2* gene expression patterns and *otd* and *Otx2* mutant phenotypes in the developing CNS of *Drosophila* and mouse. (Top) *otd* gene expression in the wild type (wt) and brain phenotype of *otd* null mutant in embryonic stage 14 *Drosophila* CNS. Borders of the protocerebral (b1), deutocerebral (b2), tritocerebral (b3), mandibular (s1), maxillary (s2), labial (s3), and some of the ventral nerve cord neuromeres are indicated by vertical lines. (Bottom) *Otx2* gene expression in the wild type (wt) and brain phenotype of *Otx2* homozygous null mutant in embryonic day 12.5 mouse CNS. Borders of the telencephalon (T), diencephalon (D), mesencephalon (M), and rhombomeres are indicated by vertical lines. Reproduced from Hirth, F. and Reichert, H. 1999. Conserved genetic programs in insect and mammalian brain development. *Bioessays* 21, 677–684, with permission from John Wiley & Sons, Inc.

Otx2 genes have been replaced with the *Drosophila otd* gene in the mouse (Acampora *et al*., 1998a, 2001b). Intriguingly, despite the obvious anatomical differences between mammalian and *Drosophila* brains, the human *Otx1* and *Otx2* genes complemented the brain defects in *otd* mutant *Drosophila* and, similarly, the *//*$^{-}$ *Drosophila otd* gene was able to rescue most of the CNS defects of *Otx1*$^{--}$ and *Otx2*$^{-}$ mutant mice (Acampora *et al*., 1998a, 1998b, 2001a; Leuzinger *et al*., 1998).

1.04.3.3 A Tripartite Organization of the Insect and Chordate Brain?

The conserved expression and function of *otd/Otx* and *Hox* genes suggest that invertebrate and vertebrate brains are all characterized by a rostral region specified by genes of the *otd/Otx* family and a caudal region specified by genes of the *Hox* family. However, in ascidians and vertebrates, a *Pax2/5/8* expression domain is located between the anterior *Otx* and the posterior *Hox* expression regions of the embryonic brain (Holland and Holland, 1999; Wada and Satoh, 2001). In vertebrate brain development, this *Pax2/5/8* expression domain is an early marker for the isthmic organizer positioned at the midbrain–hindbrain boundary (MHB), which controls the development of the midbrain and the anterior hindbrain (Liu and Joyner, 2001; Rhinn and Brand, 2001; Wurst and Bally-Cuif, 2001). The central role of this MHB region in brain development together with the conserved expression patterns of *Pax2/5/8* genes in this region have led to the proposal that a fundamental characteristic of the ancestral chordate brain was its tripartite organization characterized by *Otx*, *Pax2/5/8*, and *Hox* gene expressing regions (Wada *et al*., 1998).

An analysis of brain development in *Drosophila* has uncovered similarities in the expression and function of the orthologous genes that pattern the vertebrate MHB region (Hirth *et al*., 2003). Thus, a *Pax2/5/8* expressing domain was found to be located between the anterior *otd/Otx* expressing region and the posterior *Hox* expressing region in the embryonic brain. In *Drosophila*, as in vertebrates, this *Pax2/5/8* expressing domain is positioned at the interface between the *otd/Otx2* expression domain and a posteriorly abutting *unplugged/Gbx2* expression domain. Moreover, inactivation of *otd/Otx* or of *unplugged/Gbx2* results in comparable effects on mispositioning or loss of brain-specific expression domains of orthologous genes in both embryonic brain types. These developmental genetic similarities indicate that the tripartite ground plan, which characterizes the developing vertebrate brain, is also at the basis of the developing insect brain (Figure 6). This, in turn, has led to the suggestion that a corresponding, evolutionarily conserved, tripartite organization also characterized the brain of the last common ancestor of insects and chordates (Hirth *et al*., 2003).

1.04.4 Conserved Mechanisms for Dorsoventral Patterning of the CNS

1.04.4.1 Antagonistic Activity of Dpp/BMP-4 and sog/Chordin

As briefly mentioned above, among the significant molecular control elements involved in the embryonic establishment of the dorsoventral body axis are

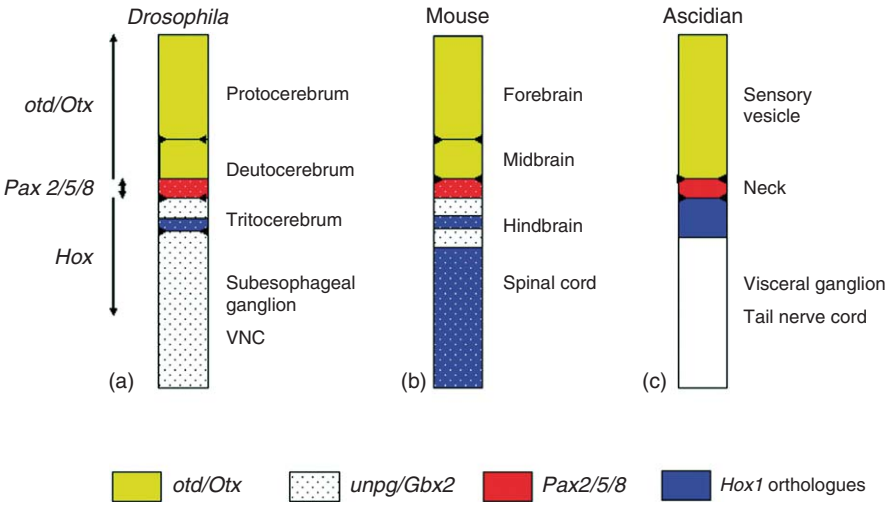

Figure 6 Tripartite organization of the (a) *Drosophila*, (b) mouse, and (c) ascidian brain, based on expression patterns of orthologous genes. The expression of *otd/Otx2*, *unpg/Gbx2*, *Pax2/5/8*, and *Hox1* gene orthologues in the developing CNS of (a) stage 13/14 *Drosophila* embryo, (b) stage E10 mouse embryo, and (c) neurula ascidian embryo. In all cases, a *Pax2/5/8*-expressing domain is located between an anterior *otd/Otx2* expressing region and a posterior *Hox* expressing region in the embryonic brain. Moreover, in *Drosophila*, as in mouse, a *Pax2/5/8*-expressing domain is positioned at the interface between the *otd/Otx2* expression domain and a posteriorly abutting *unplugged/Gbx2* expression domain. This *otd/Otx2–unpg/Gbx2* interface displays similar developmental genetic features in both *Drosophila* and mouse. Reproduced from Hirth, F., Kammermeier, L., Frei, E., Walldorf, U., Noll, M., and Reichert, H. 2003. An urbilaterian origin of the tripartite brain: Developmental genetic insights from *Drosophila. Development* 130, 2365–2373, with permission from The Company of Biologists Ltd.

signaling molecules of the TGFβ family such as Dpp, studied most extensively in *Drosophila*, and BMP-4, one of the vertebrate homologues of Dpp (De Robertis and Sasai, 1996). These proteins establish dorsoventral polarity in the insect embryo and in the vertebrate embryo. In both cases, they are restricted in their spatial activity by antagonistically acting extracellular signaling proteins. These antagonists are Sog in *Drosophila* and its homologue Chordin in vertebrates. The two groups of interacting signaling molecules, Dpp/BMP-4 and Sog/Chordin, act from opposing dorsoventral poles in both insects and vertebrate embryos (Holley *et al.*, 1995). Remarkably, in *Drosophila*, Dpp exerts its activity on dorsal cells and Sog on ventral cells, whereas in vertebrates BMP-4 acts on ventral cells and Chordin activity is found in dorsal cells. In both cases, it is the region of the embryo that attains neurogenic potential and forms neuroectoderm in which Sog/Chordin is expressed and inhibits the action of invading Dpp/BMP-4 signals.

Thus, despite the morphological differences between embryos of the two species, the *Sog/Chordin* gene is expressed on the side from which the CNS arises, whereas the *dpp/Bmp-4* gene is expressed on the opposite side of the embryo where it promotes ectoderm formation. This functional conservation of the Sog/Chordin and the Dpp/BMP-4 morphogens suggests an evolutionarily conserved, homologous mechanism of dorsoventral patterning. This suggestion is further substantiated by experimental studies showing that injection of *Chordin* RNA (from *Xenopus*) promotes ventralization of cell fates in *Drosophila* embryos, including the formation of ectopic patches of CNS. Correspondingly, injection of *sog* RNA (from *Drosophila*) causes dorsal development in *Xenopus*, including the formation of notochord and CNS (Holley *et al.*, 1995; Schmidt *et al.*, 1995). Thus, the function of *sog/Chordin* is reversed in insects and vertebrates; in both cases, injection of the gene product promotes the development of the side of the embryo that contains the CNS: dorsal in vertebrates, ventral in insects. This pervasive equivalence of gene structure and function points to an essential role of *Sog/Chordin* and *Dpp/BMP-4* in CNS induction/specification in insects and vertebrates, irrespective of the location along the dorsoventral axis at which the CNS forms (Figure 7).

1.04.4.2 vnd/Nkx, ind/Gsh, and msh/Msx: Specification of Longitudinal Columns

Beyond the mechanisms of early neuroectoderm formation, a further set of genetic elements involved in early dorsoventral patterning of the CNS appears to be evolutionarily conserved (Cornell and Ohlen, 2000). These genetic regulatory elements are three

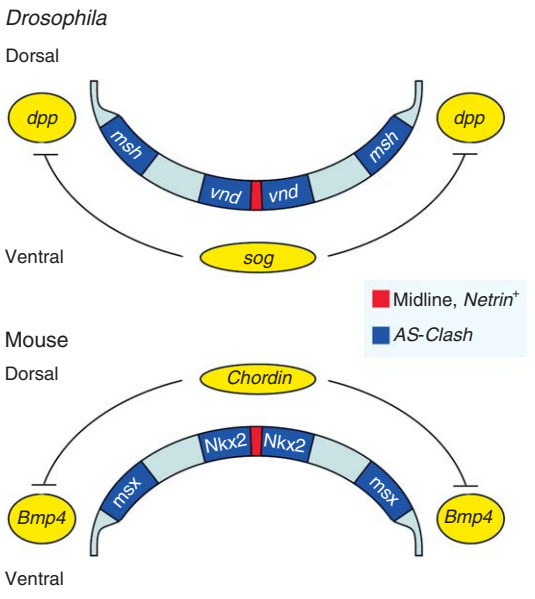

Figure 7 Transverse sections through the *Drosophila* and mouse CNS primordia showing similar dorsoventral regulation of pattern by the *sog* (*short gastrulation)/Chordin*, *dpp* (*decapentaplegic)/BMP4*, *Msx/msh*, *Nkx2/vnd, AS-C* (*achaete–scute complex)/ash* (*AS-C homologues*), and *Netrin* gene families. Reproduced from Sharman, A. C. and Brand, M. 1998. Evolution and homology of the nervous system: Cross-phylum rescues of *otd/otx* genes. *Trends Genet.* 14(6), 211–214, with permission from Elsevier.

sets of homeobox genes that control the formation of columnar dorsoventral domains in the ventral neuroectoderm of *Drosophila*; their homologues may act in a similar fashion in dorsoventral patterning in the neural plate of vertebrates (Figure 7). In *Drosophila*, the homeobox genes are *ventral nerve cord defective* (*vnd*), *intermediate nerve cord defective* (*ind*), and *muscle-specific homeobox* (*msh*) and they are expressed in longitudinal stripes along the ventral (*vnd*), intermediate (*ind*), and dorsal (*msh*) columns in the neuroectoderm (Isshiki *et al.*, 1997; McDonald *et al.*, 1998; Chu *et al.*, 1998; Weiss *et al.*, 1998). In each column, expression of the appropriate homeobox gene is required for neuroblast formation and for specification of columnar identity. Comparable expression patterns have been reported for the beetle *Tribolium* (Wheeler *et al.*, 2005).

In vertebrates, homologues of the *Drosophila* columnar genes that belong to the *Nkx* (*vnd*), *Gsh* (*ind*), and *Msx* (*msh*) gene families have been identified. These genes are expressed in columnar domains in the neural plate and neural tube of the embryonic CNS. (Invagination of the vertebrate neural plate to form the neural tube results in translocation of the lateromedial position into the dorsoventral position.) In vertebrates, several *Nkx* family members are expressed in ventral regions of the neural tube and at least one of these is expressed earlier in the corresponding medial region of the neural plate (Qiu *et al.*, 1998; Pera and Kessel, 1998; Pabst *et al.*, 1998; Shimamura *et al.*, 1995). Similarly, expression of vertebrate *Msx* family members is seen in the lateral neural plate, which later forms the dorsal neural tube (Wang *et al.*, 1996). Finally, vertebrate *Gsh* family genes are expressed at dorsoventrally intermediate levels in the neural tube (Valerius *et al.*, 1995; Hsieh-Li *et al.*, 1995). Functional studies suggest that some of these genes are involved in controlling regional identity along the dorsoventral axis of the neural tube (Briscoe *et al.*, 1999; Sussel *et al.*, 1999). These findings indicate that in the developing CNS of insects and vertebrates, the expression domains of columnar genes in the neuroectoderm/neural plate are comparable (Figure 7). This, in turn, has led to the proposal that the medial, intermediate, and lateral neurogenic columns of the *Drosophila* embryonic neuroectoderm correspond to the medial, intermediate, and lateral columns of the vertebrate neural plate, albeit in dorsoventral inverted orientation (D'Alessio and Frasch, 1996; Weiss *et al.*, 1998).

1.04.4.3 The CNS Midline: Pattern Formation and Axonal Guidance

In the nervous systems of bilaterians, specialized cells located at the midline of the neuroectoderm play an essential role in organizing the development of the CNS (Tessier-Lavigne and Goodman, 1996; Dickson, 2002). In insects and vertebrates, cells of the CNS midline are known to represent inductive centers for the regional patterning of the neuroectoderm. Moreover, the CNS midline represents an important intermediate target where growing axons either cross and project contralaterally or remain on the same side of the body. The midline cells express at their surface membrane-bound guidance molecules and secrete diffusible factors that act as attractive or repulsive guidance cues and guide growing axons from a distance; under the influence of these molecules, some axons avoid the midline, whereas others grow toward it and cross it once.

The developmental control genes that specify these midline cell populations appear to differ between insects and vertebrates. In *Drosophila*, formation of midline cells requires the specific expression of the *single-minded* gene (Nambu *et al.*, 1990), whereas in vertebrates, the formation of midline cells requires the specific expression of *HNF3beta* (Ang and Rossant, 1994; Weinstein *et al.*, 1994). Also, the morphogens that mediate

the inductive interactions of the midline cells differ in vertebrates versus insects. In vertebrates, *Sonic hedgehog* signaling from the floor plate exerts its patterning function on the adjacent dorsal neuroectoderm (Ho and Scott, 2002), whereas in *Drosophila*, *EGF* signaling exerts patterning on the adjacent ventral neuroectoderm (Skeath, 1999).

In contrast, many aspects of midline cell-mediated axon guidance are controlled by functionally and evolutionarily conserved ligand–receptor systems that include the *Netrin*, *DCC*, *Slit*, and *Robo* gene families (Araujo and Tear, 2002; Kaprielian *et al*., 2001). Homologous *Netrin* genes encode soluble attractor molecules that are detected in the floor plate and ventral neural tube of vertebrates as well as in the midline glial cells of *Drosophila* and that serve to guide commissural axons toward the midline. In both cases, the *Netrins* are expressed at a time when first commissural growth cones, which express the homologous *frazzled/DCC* genes that encode transmembrane receptors, are extending toward the midline. *Netrin* mutant embryos exhibit defects in commissural axon projections in mice and flies, indicating similar functional roles of these attractants. Moreover, in *Drosophila* as well as in vertebrates, axonal projections away from the midline depend on the presence at the midline of a repellent molecule, which binds and interacts with axonal receptors. In *Drosophila*, the midline repellent that expels commissural axons and prevents them from recrossing is the ligand Slit, which mediates its repulsive effects via receptors of the Roundabout (Robo) family that are dynamically expressed on commissural axons. In vertebrates, three *Slit* homologues (*Slit1*, *Slit2*, and *Slit3*) and three *Robo* homologues (*Robo1*, *Robo2*, and *Rig-1*) have been identified, with expression patterns reminiscent of their *Drosophila* counterparts. The vertebrate *Slit* genes are expressed in the floor plate at the ventral midline of the spinal cord, and their corresponding *Robo1* and *2* receptors are expressed by commissural axons. Studies indicate that vertebrate commissural axons become insensitive to floor plate attraction and sensitive to *Slit*-mediated repulsion after crossing the midline; this modulation of repulsion at the midline is reminiscent of the situation in the *Drosophila* CNS.

1.04.5 Evolutionary Origin of the CNS

1.04.5.1 Molecular Phylogeny: Several Possibilities

The similarities in anteroposterior and dorsoventral patterning genes as well as their conserved relative topological expression patterns and functional roles implicate a common genetic program underlying insect and mammalian nervous system development (Hirth and Reichert, 1999; Arendt and Nübler-Jung, 1999; Reichert and Simeone, 2001). This suggests that orthologous genes were already involved in neural specification in the insect and vertebrate stem species, if not already in a common bilaterian ancestor. Does this mean that the insect and chordate CNS are homologous structures and therefore of monophyletic origin? Two alternative hypotheses, which are not mutually exclusive, can be envisaged. The first of these postulates that the ancestral bilaterian nervous system was already centralized and had its development governed by conserved genetic mechanisms that are still apparent in extant insects and mammals (monophyletic origin of the brain). The second hypothesis is that the ancestral bilaterian nervous system was controlled by conserved genetic mechanisms that still operate in arthropods and vertebrates, but that centralization of the nervous system occurred independently in protostome and deuterostome lineages (polyphyletic origin of the brain).

Based on classical phylogeny, which places acoelomates, such as platyhelminthes, and pseudocoelomates, such as nematodes, nearer to the base of the Bilateria than the coelomate protostomes and deuterostomes, the first hypothesis seems more likely (Figure 8a). Since flatworms and nematodes have a CNS with a brain and a ventral nerve cord, a comparable centralized nervous system would be likely to reflect the ancestral state for both Protostomia and Deuterostomia, and indeed for all Bilateria.

In this view, the evolutionary advance of centralizing the nervous system occurred only once. In contrast, molecular phylogenetic analyses no longer provide evidence that preferentially supports one of the two hypotheses. According to studies based on 18S rRNA sequence comparisons, there are no longer any living bilaterians that can be considered to be evolutionary intermediates between the radially (or biradially) symmetric animals and the bilaterally symmetric protostomes and deuterostomes (Figure 8b). Invertebrate lineages such as platyhelminthes and nematodes, which were considered to be near the base of the bilaterian tree in classical phylogeny, are now placed next to protostome groups with highly complex body and brain morphology such as mollusks and arthropods in the two new protostome subgroupings, the lophotrochozoans and ecdysozoans (Adoutte *et al*., 2000). Thus, although neurons and nervous systems, which are present in radiate cnidarians and ctenophores, apparently existed before the origin of bilaterian animals,

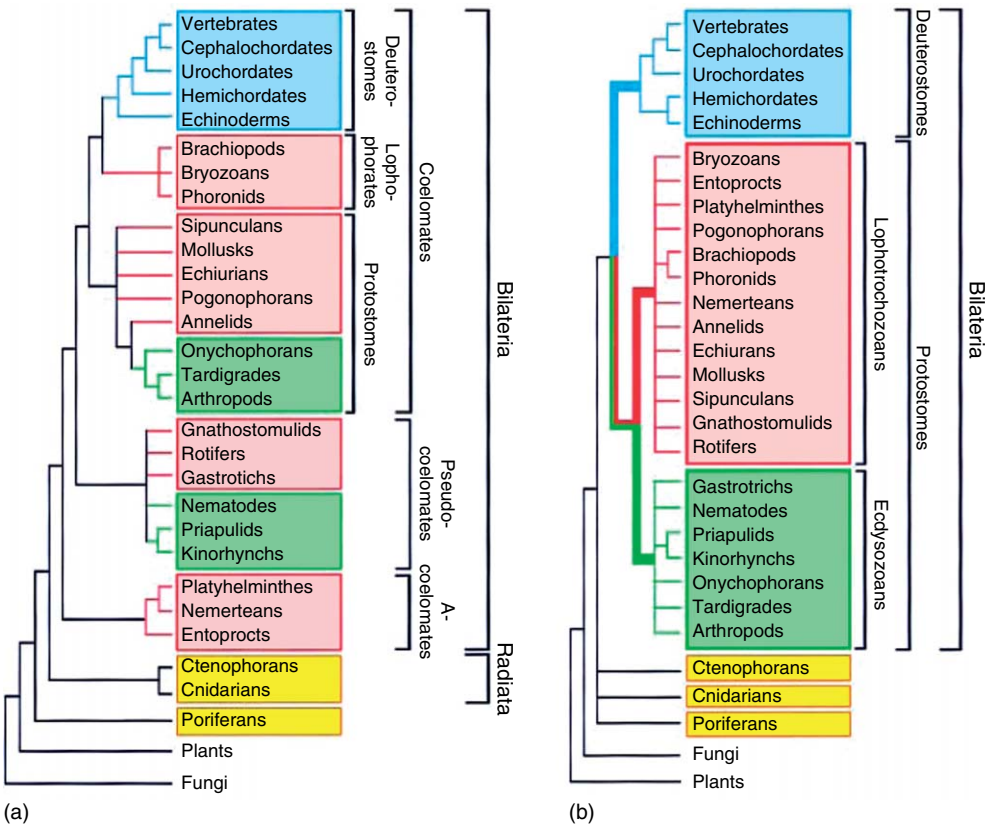

Figure 8 Metazoan phylogenies. a, The traditional phylogeny based on morphology and embryology. b, The new molecule-based phylogeny. Reproduced from Adoutte, A., Balavoine, G., Lartillot, N., Lespinet, O., Prud'homme, B., and de Rosa, R. 2000. The new animal phylogeny: Reliability and implications. *Proc. Natl. Acad. Sci. USA* 97, 4453–4456. Copyright 2000 National Academy of Sciences, USA, with permission.

the evolutionary origin of nervous system centralization and brain formation cannot be deduced from molecular phylogenetic data alone (see Origin and Evolution of the First Nervous System). This means that in terms of nervous system organization of the last common ancestor of modern bilateral animals, current molecular phylogeny is compatible with a number of possibilities (see, for example, Arendt and Nübler-Jung, 1997; Adoutte *et al.*, 2000; Gerhart, 2000; Shankland and Seaver, 2000; Meinhardt, 2002; Erwin and Davidson, 2002; Holland, 2003; and references therein).

1.04.5.2 Do Specialized Gene Expression Patterns Predict Specialized Brain Structures?

Since molecular phylogeny does not support preferentially either of the two hypotheses for the evolutionary origin of the CNS, we are left with the molecular data provided by comparative developmental genetic studies. Given the conserved molecular patterning mechanisms, or at least the conserved gene expression patterns, that characterize brain development in all bilaterians examined, what inferences can be made about the evolution of the CNS? The hypothesis of a monophyletic origin of the CNS is underscored by the notion that specialized developmental patterning mechanisms and patterned anatomical complexity evolved together (Tautz, 2003). Since comparative developmental genetics indicates that a complex set of conserved and specialized anteroposterior and dorsoventral patterning genes were operative in the nervous system of the urbilaterian ancestor of protostomes and deuterostomes, it is reasonable to assume that these genes generated an urbilaterian nervous system that also manifested complex anatomical specializations along the anteroposterior and dorsoventral axes (Hirth and Reichert, 1999; Arendt and Nübler-Jung, 1999; Reichert and Simeone, 2001). Thus, the conservation of expression and function of the dorsoventral columnar genes, including their dorsoventral inversion, provides strong evidence for the existence of an urbilaterian nervous system that was already dorsoventrally regionalized. Moreover, the observed

dorsoventral inverted expression of these genes in the CNS of insects versus vertebrates is precisely what would be predicted by the body axis inversion hypothesis, which in turn is substantiated by independent molecular evidence from gene expression data on heart development and gastrulation (e.g., Cripps and Olson, 2002; Arendt and Nübler-Jung, 1997).

Alternative scenarios for the evolution of centralized nervous systems in protostomes and deuterostomes have been proposed in which the CNSs occurred independently, after the split of the two groups, and without a dorsoventral inversion (reviewed in Gerhart, 2000; Holland, 2003; Lacalli, 2003). An implicit assumption of these proposals is that the bilaterian ancestor did not exert a dorsoventrally centralized nervous system but instead already had a structured map of patterning gene expression, which was then independently used for generating the CNS in different phyla. In the *Auricularia* hypothesis originally put forward by Garstang (1894; see also Nielsen, 1999), the evolutionary origins of the chordate nervous system are thought to be found in the ciliary bands of a deuterostome dipleurula-type larval ancestor resembling an echinoderm *Auricularia* larva. During the evolution of the chordate CNS, bilateral rows of cilia and the associated nerves were said to have converged through complex morphogenetic movements to the dorsal midline and fused to form the neural tube. Evidence for this view was found in comparative anatomical studies between echinoderms (particularly *Auricularia* larvae), hemichordates, and urochordates, and data show that a number of genes involved in chordate CNS development, including *SoxB3, Nkx2.1*, and *Otx*, are expressed in ciliary bands of larval hemichordates and/or echinoderms (Taguchi *et al.*, 2002; Takacs *et al.*, 2002; Tagawa *et al.*, 2001). Thus far, however, the ciliary band derivatives have not been shown to give rise to cells of the adult nervous system after metamorphosis. Furthermore, the *Auricularia* hypothesis does not take into account the molecular genetic similarities between the CNS of protostomes and that of chordates.

A comparative study on an enteropneust hemichordate has shown that the anteroposterior expression pattern of a large number of genes, which are involved in axial patterning of the vertebrate and arthropod CNS, is conserved in the apparently diffuse nervous system of the enteropneust acorn worm. The body-encircling basiepithelial nerve net of the directly developing hemichordate *Saccoglossus kowalevskii* expresses a complex set of regulatory genes in circumferential networks (Lowe *et al.*, 2003). Among these are the orthologues of the *otd/Otx*, *tll/Tlx*, *ems/Emx*, *unpg/Gbx*, *dll/Dlx*, *Pax*, *En*, *Lim*, *Hox*, and other highly conserved gene families, which reveal an anteroposterior order of domains that is remarkably similar to the insect and mammalian gene expression patterns (Figure 9). Unfortunately, almost nothing is known about the expression of hemichordate *dpp/BMP-4* and *sog/Chd* homologues and whether they might possess a neural/antineural antagonism that could limit and/or condense the nerve net into a CNS to one side of the body. Only in the indirectly developing hemichordate *Ptychodera flava* has a BMP2/4 homologue been described; however, no expression was observed during embryogenesis, suggesting that it is not involved in axis formation (Harada *et al.*, 2002). Moreover, little is currently known about *vnd/Nkx, ind/Gsh*, and *msh/Msx* orthologous gene expression and whether these genes might possess any early dorsoventral patterning functions in longitudinal column formation of the hemichordate nervous system. Thus far, only the expression of a hemichordate *Nkx2.1* homologue, which is specifically expressed in a ventral sector of the anterior ectoderm, is known (Lowe *et al.*, 2003).

Based on the gene expression studies in *Saccoglossus*, Lowe and co-workers have proposed that the nervous system of the deuterostome ancestor of hemichordates and chordates was also organized in a diffuse, body-encircling, basiepithelial nerve net (Lowe *et al.*, 2003). According to molecular phylogeny, this indicates that the bilaterian ancestor preceding protostomes and deuterostomes also possessed a diffuse, body-encircling, basiepithelial nerve net. Independent centralization events in protostomes and deuterostomes without dorsoventral inversion could then have resulted in anteroposteriorly oriented CNSs with similar gene expression domains (Holland, 2003).

Alternatively, the diffuse nervous system of *Saccoglossus* may represent the secondary loss of a centralized nervous system. Like cnidarians and ctenophores, hemichordates exhibit only neuroepidermal fibers without organized ganglia, brain, or other obvious specialized neural structures. Indeed, most of the data of Lowe *et al.* (2003) are equally compatible with a secondary reduction scenario, in which the ancestor of the deuterostomes would have had a centralized nervous system, which was lost in the hemichordates due to their peculiar lifestyle as sediment-burrowing worms. Moreover, the apparently simple, nerve net-like

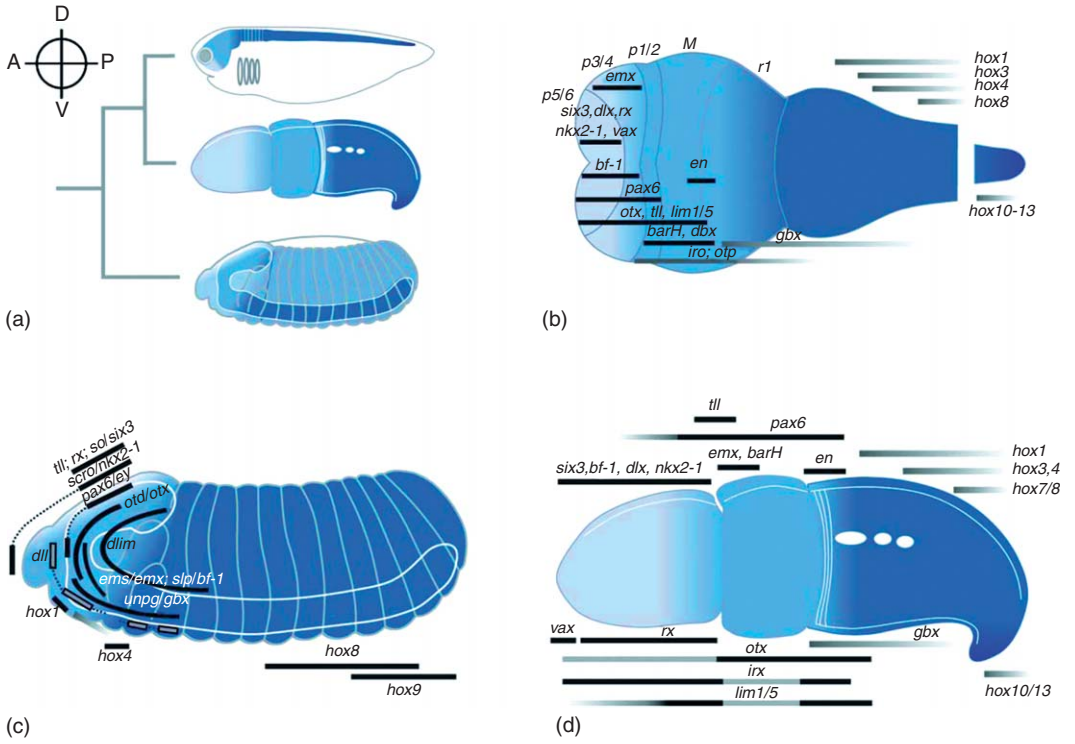

Figure 9 Comparison of the neural gene domain maps of hemichordates, chordates, and *Drosophila*. In addition to individual gene domains, the color gradient in each panel indicates general similarities of gene expression domains. a, Representation of the general organizational features of the CNSs of chordates and arthropods and the diffuse nervous system of hemichordates arranged on a phylogram. The compass indicates the axial orientation of each model. b, Representation of a dorsal view of a vertebrate neural plate (see Rubenstein *et al.*, 1998). p1/2, prosomeres 1 and 2; p3/4, prosomeres 3 and 4; p5/6, prosomeres 5 and 6; M, midbrain; r1/2, rhombomeres 1 and 2. The discontinuous domain represents the postanal territory of the nerve cord. All 22 expression domains are shown. c, *Drosophila* late stage 12 embryo model with 14 expression domains shown (lateral view, post-germ-band retraction, before head involution). All models are positioned with anterior to the left. d, The acorn worm (lateral view), with its diffuse nervous system, is shown with a blue color gradient of expression in the ectoderm; the anterior domains, the midlevel domains, the posterior domains, and the postanal territory are color matched to the anteroposterior dimension of the chordate model. Reproduced from Lowe, C. J., Wu, M., Salic, A., *et al.* 2003. Anteroposterior patterning in hemichordates and the origins of the chordate nervous system. *Cell* 113, 853–865, with permission from Elsevier.

nervous system of hemichordates may display further substructures, including CNS elements, as suggested by earlier neuroanatomical analyses: nerve fiber tracts are formed in the epithelium, including major ventral and dorsal tracts (Bullock, 1945; Knight-Jones, 1952).

1.04.5.3 A Simple Nerve Net at the Base of Nervous System Evolution?

There is some evidence that a basiepithelial, noncentralized nerve net, perhaps comparable to those found in extant hemichordates, may indeed represent the basal evolutionary state from which bilaterian nervous systems evolved. Basiepithelial nervous systems exist in some gastroneuralians, and the subepithelial nervous systems, as in insects, often go through a basiepithelial state during their development (Nielsen, 1995; Arendt and Nübler-Jung, 1999). However, the question remains of how such a simple nerve net condensed into a centralized nervous system and when this occurred in evolution. Paleontological evidence can provide a reasonable estimate of when CNSs were already formed in protostome and deuterostome animals. A conservative estimate is a date of 530–540 Mya in the early Cambrium, when a complex variety of bilaterian forms representing most of the modern major animal groups was present (Grotzinger *et al.*, 1995; Conway-Morris, 2000). These forms included arthropods such as trilobites and early agnathan-like stem vertebrates and the fossil record for both of these animal forms indicates that they already had brains and CNS with features typical for arthropods and vertebrates (Fortey, 2000; Holland and Chen, 2001). Thus, centralization of nervous systems must have occurred earlier, probably after the split between the cnidarians and the bilaterians, which is thought to have occurred between 600 and

630 Mya (Peterson *et al.*, 2004). If this is the case, then the cnidarian nervous system might be more informative of early CNS evolution in stem Bilateria than that of hemichordates.

The basic organization of the nervous system in cnidarians (and ctenophores) is that of a diffuse nerve net that can also manifest centralized elements such as nerve rings and ganglionic centers. Moreover, many of the conserved developmental control genes that operate in the insect and vertebrate nervous system are also present in Cnidaria and thus at least some of these differentiation gene batteries date to the last common ancestor of cnidarians and bilaterians (Finnerty *et al.*, 2004; Ball *et al.*, 2004; Finnerty, 2003; Galliot, 2000). Among these are anterior and posterior *Hox* genes, an asymmetrically expressed *dpp* gene, and an *Otx* gene. However, the expression patterns of these genes differ among cnidarian species and are inconclusive as far as anteroposterior or dorsoventral axis determination is concerned (Yanze *et al.*, 2001; Finnerty *et al.*, 2004). For example, the typical bilaterian head gene *Otx* is expressed along the entire primary body axis in cnidarians. In *Hydra*, the *CnOtx* gene is expressed at a low level in the ectodermal epithelial cells of the body, during early budding in the region of the parental body column from which cells will migrate into the developing bud, and *CnOtx* is strongly upregulated during reaggregation, in contrast to head or foot regeneration where it is downregulated (Smith *et al.*, 1999). In *Podocoryne*, the *Otx* gene displays two types of expression: in the gonozooid polyp at every developmental stage of the budding medusa and in the mature medusa, restricted to the striated muscle cells (Müller *et al.*, 1999). These data suggest that *Otx* is not involved in axis determination or head specification in *Hydra* and *Podocoryne*. Thus, ambiguous species-specific gene expression data in cnidarians make comparisons between cnidarian and bilateral nervous systems difficult and thus far are inconclusive concerning CNS evolution.

1.04.6 Conclusions

Contemporary experimental studies analyzing the expression and function of homologous genes in various animal model systems are reviving a fundamental question raised more than 150 years ago in the famous academic debate between Cuvier and Geoffroy Saint-Hilaire: does a common Bauplan underlie animal development, indicating homology of structures such as the ventrally located insect and the dorsally located chordate nervous system? Comparisons of the expression, function, and regulation of genes and genetic networks involved in anteroposterior and dorsoventral patterning of the insect and vertebrate nervous systems suggest that orthologous genes were already involved in neural specification in the insect and vertebrate stem species. Thus, the pervasive equivalence of the *Dpp/BMP-4* and *sog/Chd* antagonism in executing the distinction between neural and non-neural, the *vnd/Nkx*, *ind/Gsh*, and *msh/Msx* gene network involved in early dorsoventral columnar patterning, the role of the *otd/Otx* genes in anterior CNS regionalization, and the action of *Hox* genes in the specification of segmental neuronal identity are all conserved in both insect and mammalian CNS development. This strongly suggests that these molecular genetic mechanisms were already apparent in an urbilaterian ancestor and that the insect and vertebrate nervous systems evolved from a common ancestral urbilaterian brain.

However, it is also conceivable that complex gene expression characteristics pre-dated the generation of morphological complexity in the course of nervous system evolution. The analysis of developmental control gene expression in a hemichordate demonstrates that complex gene expression patterns, comparable to those observed in the CNS of insects and vertebrates, are compatible with the existence of a diffuse basiepithelial nerve net. Nevertheless, the hemichordate body plan is clearly derived and its basiepithelial nerve net may be the result of a secondary reduction or loss of an ancestral CNS. Some of the developmental control genes that operate in CNS development in arthropods and chordates are also expressed during cnidarian development. Although a diffuse, net-like nervous system is apparent in Cnidaria, the ambiguous data on orthologous gene expression in these animals impede any conclusive comparisons between cnidarian and bilateral nervous systems. The available data therefore suggest that only one ancestral, albeit rather complex, nervous system type was at the origin of bilaterian CNS evolution.

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1.05 Field Homologies

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Glossary

developmental regulatory gene A gene encoding a transcription factor or a signaling protein, which is expressed during development in specific patterns, and is able to regulate expression of other genes and control patterning and morphogenesis of specific body parts.

homologous Exhibiting biological homology; having the same relative position (topologically), embryological origin and common ancestor. For animal or body parts having the same basic organization plan, topological position of homologous morphogenetic fields and radial histogenetic divisions is generally identical; however, for identifying homologous cell groups, embryological origin is a prevalent criterion, since their topological position may differ between animals due to distinct migration.

homologue The same organ in different animals under every variety of form and function, inherited from a common ancestor. See also 'homologous'.

homology A similarity attributed to common evolutionary origin. See also 'homologous'.

homoplasy A similarity not due to common evolutionary origin, but to convergent or parallel evolution.

modularity Principle by which biological systems are made up of discrete units or modules, which are self-regulated and dynamic entities able to change independently of the rest of the body during development and evolution.

module Each unit of a modular organism or organ, from genetic cascades to cells and cell fields. A module is a self-regulated and dynamic entity that can change independently of the rest of the body without lethal consequences, producing phenotypic variations.

morphogenetic field Higher-order module, specified by a particular combination of developmental regulatory genes, that gives rise to a particular body division or subdivision. It constitutes a major unit of development and evolution, and the natural comparison character for homology considerations.

radial histogenetic division A radially arranged region or territory of the brain, whose neurons primarily derive from a specific morphogenetic field (i.e., from a restricted ventricular sector of the neural tube). The radial feature of brain histogenetic divisions is based on the predominant glial fiber-guided migration of immature neurons in their way from the ventricular zone to the mantle during development. Nevertheless, radial histogenetic divisions can contain immigrant cells coming from other fields by tangential migration. The fact that the majority of the cells in each radial

histogenetic brain division originate in a specific morphogenetic field, makes these divisions natural comparison units for field homology considerations in adult animals. This does not mean that all cells in a particular division in one animal are necessarily homologous to the cells of the same division of other animals.

telencephalon Bilateral evaginations of the rostral forebrain. It shows two major divisions in all vertebrates: pallium and subpallium.

topology Geometric configuration of any given structure (such as the brain) according to internal coordinates, which remain unaltered, independent of deformations or differential growth of subdivisions that occur during development. According to this, the topological position of any division/subdivision within the structure, and its relation to neighbors, remain the same throughout ontogeny. Further, in organisms sharing the same configuration and basic organization plan (e.g., vertebrates), the topological position of homologous divisions/subdivisions should be the same across species. In contrast, some homologous cell groups may vary their topological position by differential migration in different animals.

1.05.1 Introduction

We humans have always been intrigued by the astonishing cases of structural or functional similarities between body parts found in the animal kingdom (e.g., wings of insects and birds, eyes of squids and vertebrates, and so on). This issue was deeply investigated by the nineteenth-century naturalists and anatomists, who gained access to a large variety of new species, thanks to the European exploratory voyages to new worlds, favoring their 'intellectual voyage' in search of the archetype. Owen was one such anatomist who realized that not all similarities are equal, coining the term 'homologue' to define "the same organ in different animals under every variety of form and function" (Owen, 1843; A History of Ideas in Evolutionary Neuroscience; see also Mayr, 1982; Raff, 1996; Panchen 1999; Butler and Saidel, 2000; Puelles and Medina, 2002; Wake, 2003). He also coined the term 'analogue' to define a part or organ in one animal that has the same function as another part or organ in a different animal (Owen, 1843; Wake, 2003). Soon after the introduction of these terms, evolution came into the scene to explain why similarities exist (Darwin, 1859), and evolutionary biologists used the term 'homology', under a new perspective. For evolutionary biologists, two structures are homologous if they can be traced back to a common ancestor (i.e., homology is similarity due to inheritance from common ancestor) (Striedter and Northcutt, 1991; Raff, 1996; Gilbert and Burian, 2003; Wake, 2003). In this view, the existence of homologues is the consequence of evolution, and the study of homologies is, in essence, the study of evolution. In addition to homology, another key concept for evolutionary biologists is 'homoplasy', a term coined by Lankester to define similarity not linked to common ancestor, but due to convergent or parallel evolution (Lankester, 1870; Hall, 1999; Panchen, 1999; Wake, 2003). To discern between homology and homoplasy is not an easy task since it is difficult to know what the prevalent characters are that should be chosen for comparison. Moreover, many problems arise when trying to consider intriguing cases such as similarities not linked to common ancestor but being produced by a similar developmental pathway (i.e., sharing a common generative pathway), or similarities linked to common ancestor but produced by different developmental mechanisms (Striedter, 1998; Butler and Saidel, 2000; Puelles and Medina, 2002; see A History of Ideas in Evolutionary Neuroscience). One very useful way to approach the study of homologies is by combining developmental and evolutionary views (Gould, 1992; Raff, 1996; Gilbert *et al*., 1996; Striedter, 1998; Gilbert and Burian, 2003), which makes sense since evolution occurs as a consequence of changes in developmental mechanisms that produce phenotypic variations, which are then exposed to natural selection (de Beer, 1951; Waddington, 1966; Gilbert *et al*., 1996; Raff, 1996). This combined 'evo–devo' approach has provided unequivocal evidence for the modular nature of embryos, and for the existence of discrete fields, the morphogenetic fields, which represent major ontogenetic units (modules) able to change independently, producing variation and diversity in evolution (Raff, 1996; Gilbert *et al*., 1996; Gilbert and Burian, 2003; Gass and Bolker, 2003). This evidence has been extremely important for rescuing the concept of 'field homology', first introduced by Smith (1967) to define correspondence due to derivation from a common embryonic anlage, and used later by comparative neurobiologists to

homologize brain regions assumed to develop from homologous precursors (Northcutt, 1981; Butler, 1994a, 1994b; Striedter, 1997). Based on the importance of morphogenetic fields as major ontogenetic and phylogenetic units, the concept of field homology has recently been reformulated under the modern perspective of evolutionary developmental biology, to be used in a dynamic rather than static sense, as one of the natural comparison characters for evolutionary studies in general and, in particular, for studies of brain evolution (Puelles and Medina, 2002). Below I briefly summarize some ideas related to this concept.

1.05.2 Conceptual Considerations

Evolutionary developmental biologists try to understand the mechanisms that produce homologous and homoplastic structures, and how variation and novelties originate during development and in evolution. Understanding these principles is essential for studying evolution of a complex biological structure, such as the brain. In this section, I provide a very brief analysis of the causes and principles of evolution, for then applying these ideas to the study of brain evolution in the following section.

1.05.2.1 Causes of Homology, Convergence, and Divergence: Development, Evolution, Epigenesis

Homologous structures can show a high degree of similarity (involving static or conservative evolution) or can differ in form and/or function (involving important evolutionary changes). Nonhomologous structures can reach a high degree of similarity by way of either convergent or parallel evolution (Striedter and Northcutt, 1991). Further, many biological systems exhibit new characters or features with no counterpart in the ancestor (i.e., they have been produced as novelties during evolution) (Striedter, 2005). The reason for all of these examples of static, divergent, convergent, or parallel evolution can be found in development and in the genetic regulatory programs underlying it. Developmental mechanisms and the genetic regulatory networks involved in the formation of complex structures are often highly integrated systems subjected to constraints impeding significant changes, and random variation. Developmental and evolutionary changes are possible due to the modular nature of biological systems, from the level of genetic cascades to the levels of cells and morphogenetic fields (Raff, 1996; Gilbert *et al*., 1996). Modules, such as morphogenetic fields, are dynamic, highly stable, and self-regulated systems, able to absorb perturbations produced in the field or outside it (i.e., they are internally constrained). However, some changes in their genetic regulatory programs can alter their development and, if not lethal, can lead to phenotypic variations in the adult. The resultant phenotypes are then selected by external pressures. Genetic regulatory programs can be affected by epigenetic interactions with other modules, such extracellular signaling molecules involved in inductive interactions, and changes in these interactions can lead to phenotypic variations (Raff, 1996). In addition, genetic regulatory programs can be affected by epigenetic environmental features such as temperature, nutrition, or population density, and the phenotypic result can be different under different conditions (Raff, 1996; Hall *et al*., 2004; see Epigenetic Responses to a Changing Periphery – Wagging the Dog). This takes us to the concept of phenotypic plasticity, according to which each genome is able to produce a range of phenotypes, as a critical adaptive response to different environments (Waddington, 1956; Raff, 1996; Gilbert and Burian, 2003). The issue of developmental and evolutionary constraints, the principle of modularity, and the concept of morphogenetic field are explained in more detail in separate sections.

1.05.2.2 Internal Constraints in Development and Evolution. Epigenetic Constraints

In spite of the rich structural and functional variety of animal body forms found in nature, no more than about 35 different body plans exist, all of which appeared during the Cambrian radiation over a half billion years ago, indicating an astonishing stability of structure once integrated as a complex form (Raff, 1996). Although extensive evolutionary changes have occurred since the Cambrian, the underlying body patterns have been conserved. This means that there are constraints in evolution, which include at least physical/morphological, genetic, developmental, historical, and epigenetic constraints (Raff, 1996; Striedter, 1998, 2005). Once a body plan is assembled, the genetic regulatory programs and developmental mechanisms underlying it become tightly integrated, and significant change is severely constrained (Raff, 1996). The integration of genetic and developmental controls of a body plan during evolution is irreversible, which means that a body plan cannot be transformed into another one without fatally disrupting its ontogeny (Raff, 1996). Once an integrated body plan is established, selection favors improvements/changes within that body plan, and disfavors new body plans that are unable to compete as well as

established ones (Raff, 1996). In addition to genetic constraints due to tight integration of genetic networks making small changes lethal (this does not occur when duplications or alternative links exist), the genomic size is also a source of constraint since it affects properties such as cell size and division rate (Raff, 1996), which affect the morphology. Some simple vertebrates such as lungfish or salamanders have disproportionately huge genomes, much larger than that of humans (Raff, 1996). Larger genomes result in larger cells, and cells containing large genomes have a slower DNA replication, constraining the growth rate of the organism. In frogs and salamanders having a large genome and, as a result, larger cells, this is related to a simplification of brain morphology (Roth *et al*., 1994).

It appears that the developmental and genetic internal constraints are not equally tight during the whole development, thus allowing for some degree of variation within body plans observed during evolution. Such changes are possible due to the modular nature of development (genetic cascades and networks, transduction/signaling pathways, cells, morphogenetic fields), which allow changes in one module without affecting the rest (Gilbert *et al*., 1996; Raff, 1996; Gass and Bolker, 2003; Gilbert and Burian, 2003). In vertebrates, echinoderms, and possibly other animal groups, internal developmental constraints are maximal during the phylotypic stage, but are relatively loose during early and late development. The phylotypic stage (called pharyngula in vertebrates) represents the most evolutionarily conserved stage of development, in which embryos of different species share a comparable regional/modular organization and comparable expression patterns of developmental regulatory genes. The stability of this stage is due to the existence of tight and complex integration (interactions) between modules, which impeds variation without fatal consequences (Raff, 1996). Curiously, and paradoxically, this highly constrained stage, which later gives rise to a highly conserved body plan, can be achieved by way of very different developmental mechanisms, meaning that early development is loosely constrained. Striedter (1998) explains this paradox of many different developmental mechanisms leading to a conserved phylotypic stage by proposing the existence of attractors, represented by valleys in epigenetic landscapes. These attractors represent the different dynamic states of modules (Raff (1996); these are discussed in the following section). Integration between modules is less strict before and after the phylotypic stage, allowing evolutionary changes. When these changes occur after the phylotypic stage, they produce phenotypic variation in the adult that affect specific modules and their derivatives (mosaic evolution).

1.05.2.3 The Principle of Modularity: Genetic Cascades, Cells, Cell Fields, and Organs

As noted above, evolutionary changes during development are possible due to the modular organization of developing embryos. Developing organisms are made up of partially independent, interacting units or modules at several hierarchical levels (from genetic cascades to cell lineages and cell fields), which are able to change independently of the rest of the body, allowing mosaic evolution in both genes and morphology (Raff, 1996; Gass and Bolker, 2003; Gilbert and Burian, 2003). Modules are thus important units, in both development and evolution, that link genotype to phenotype. Within some limits (due to constraints), modules can become semi-independent entities (dissociation), allowing nonrandom evolutionary variation without fatal consequences for the embryo. Evolutionary variation can occur by changes in timing or location of regulatory gene expression or other processes (including signal transduction interaction between modules), by changes of growth rate or duration within a module, by module duplication followed by divergence (this occurs in the production of serial homologues), or by co-option (Gould, 1977; Gilbert *et al*., 1996; Raff, 1996; Gass and Bolker, 2003; Gilbert and Burian, 2003).

The correct expression of developmental regulatory genes is essential for body and body part formation. Changes in their expression (timing or location) can produce important morphological alterations, and the occurrence of such changes is considered a major cause for the production of morphological diversity in evolution (Carroll *et al*., 2001). Developmental regulatory genes, also called master control genes, constitute a small fraction of the genome, and encode transcription factors or signaling proteins, that directly (through direct DNA biding) or indirectly (through signal transduction cascades) regulate the expression of other genes and control key aspects of development and formation of specific body parts (Carroll *et al*., 2001). The coding region of these genes is extremely well conserved in evolution, but the regulatory region, containing key elements (enhancers) for gene transcription regulation, can change in evolution, producing gene expression changes in timing or location. Such evolutionary variation in gene regulatory regions is possible due to its modular organization, which allows variations such as addition of new enhancers or binding sites for new

activators or repressors, that can affect positively or negatively the gene transcription (Gilbert, 2000; Carroll *et al*., 2001). Evolutionary changes have often occurred at the level of the gene regulatory regions, as well as with the evolution of new activators, repressors, or cofactors acting directly or indirectly on the regulatory region of master control genes (Carroll *et al*., 2001). Changes can also occur at the level of the downstream targets of master control genes, also in the regulatory region of those target genes, which may produce, for example, a particular transcription factor that can act through a different downstream cascade of genes, producing a different effect and a different phenotype. The modular nature of the regulatory region of genes, of the genetic cascades, and of the embryo (in different fields) makes possible that particular master control genes can have different effects when acting, in different contexts, in different parts of the body (Carroll *et al*., 2001). Modular interaction between genetic regulatory cascades is also essential to understand how so much morphological diversity and so many different cell types can be produced from relatively few master control genes. Through such interactions, different master gene combinations defining distinct morphogenetic fields can lead to different body parts and subdivisions (Carroll *et al*., 2001).

Developmental modules are dynamic and self-regulated entities, that have specific locations in the embryo, and show specific properties (such as specific gene expression patterns) and capacities of interaction with other modules, than can change dynamically during the course of development (Raff, 1996; Gass and Bolker, 2003). Thus, developmental modules are usually transient entities (e.g., cascades of developmental regulatory genes, such as those expressing transcription factors; or morphogenetic fields) that, in general, are not found as such in the adult but produce specific adult phenotypes (e.g., expression of specific structural genes, specific sets of differentiated cells, specific regions or organs). Each state of the module (e.g., each state to pass from being a stem cell, to a neural stem cell, and from here to a forebrain neural stem cell, and so on, to finally become a differentiated cholinergic neuron in the basal telencephalon; or the transient states between a morphogenetic field and a particular adult brain division) can be considered as a 'basin of attraction' or 'attractor', and developmental genes act as regulators to make the transition between different 'basins of attraction' (between transient cell types) (Kauffman, 1987, 1993; Raff, 1996; Striedter, 1998). Of interest, these attractors are the characters or natural units of comparison between different organisms for homology considerations. If the phylogenetic continuity criterion is met (analyzed using a cladistic method), particular attractors in two animals can be considered homologous (see Phylogenetic Character Reconstruction). However, it is important to note that homology at a particular attractor state (e.g., a transient state during development) does not neccessarily mean complete homology at the final state. For example, a particular morphogenetic field can be homologous in two different animals, but its final product in one of the animals may contain cell populations homologous to similar cells in other animals, plus other nonhomologous cell populations (with no counterpart in other species or the common ancestor, as inferred using a cladistic analysis).

Although developmental modules generally lose some of their attributes after the major developmental events have occurred (e.g., when cells reach a differentiated state), there are cases of modules that either keep their properties (e.g., as stem cells) or are able to recover them in adult animals, and are responsible for cases of regeneration or continuous cell production. This would explain the continuous production of blood cells or epithelial cells in the adult (Gilbert, 2000). This also happens in the brain of different animals, including mammals, where localized foci of adult neurogenesis are found in the subventricular zone leading to the rostral migratory stream (giving rise to cells that migrate tangentially to the olfactory bulb) or in the hippocampal formation (García-Verdugo *et al*., 2002; Alvarez-Buylla and García-Verdugo, 2002; Merkle *et al*., 2004). These examples of developmental modules keeping or recovering their 'generative' properties in the adult are more common in amphibians and fish, but often are also found in reptiles (Gilbert, 2000). For example, amphibians and reptiles are able to regenerate some distal body parts, such as the tail, following amputation. The enigmatic case of 'Wolffian' regeneration in urodele amphibians (regeneration of the eye lens but from a different origin, the iris edge; Gilbert, 2000) may also be a consequence of the persistence of developmental modules with intact generative and self-regulatory properties after the major developmental events have occurred (see also Puelles and Medina, 2002).

1.05.2.4 Morphogenetic Fields as Evolutionary and Developmental Higher-Order Modules Linking Genotype and Phenotype

Among modules, morphogenetic fields constitute higher-order units of embryonic development, that are specified by particular combinations of

developmental regulatory genes (master control genes) and that give rise to specific cellular groups and structures found in the adult. They represent the major units establishing the link between genotype to phenotype, and the major units of ontogenetic and phylogenetic change (Gilbert *et al*., 1996). Further, they constitute major natural units or characters for comparison between species and for homology studies.

In the brain, morphogenetic fields are represented by segments (rhombomeres, prosomeres) and smaller divisions and subdivisions within them (Raff, 1996; Gilbert *et al*., 1996; Puelles and Rubenstein, 1993, 2003). The evidence for their existence is ample, and includes:

1. data on expression of developmental regulatory genes in different species, allowing visualization of fields as discrete expression domains characterized by specific combinations of genes (Simeone *et al*., 1992; Bulfone *et al*., 1993, 1995, 1999; Puelles and Rubenstein, 1993; Puelles *et al*., 2000; Bachy *et al*., 2002; Brox *et al*., 2003, 2004; Lumsden, 2004; see Origins of the Chordate Central Nervous System: Insights from Hemichordates);
2. knockout mutations showing specific alterations of the field and its derivatives, either by lack of formation, malformation, or transformation into an adjacent field (Schneider-Maunoury *et al*., 1993; McKay *et al*., 1994; Stoykova *et al*., 1996; Sussel *et al*., 1999; Yun *et al*., 2001, 2003; Bishop *et al*., 2002, 2003; Muzio *et al*., 2002);
3. fate map studies showing clonal restriction at least at the level of the ventricular zone (Fraser *et al*., 1990; Marin and Puelles, 1995; Cambronero and Puelles, 2000; Cobos *et al*., 2001b; García-López *et al*., 2004); and
4. in the case of brain segments, transplantation studies showing replication of the field and its derivatives when a specific rhombomere is grafted into a different position of the embryo (Kuratani and Eichele, 1993; Martínez *et al*., 1995).

During development, fields can be patterned and/or can reach a new order (including new gene expression) by interaction with other fields. This can occur by way of signaling proteins produced by cells in particular fields or organizer centers (such as Sonic hedgehog, Wnt, BMP, and FGF proteins), that are secreted to the extracellular medium and diffuse to act at a distance in a concentration-dependent manner (Carroll *et al*., 2001). The response of cells to such inductive proteins depends on both the distance from the source and their expression of appropiate receptors. Examples of such interactions are common in the central nervous system and constitute an important mechanism for patterning (Ericson *et al*., 1997; Shimamura and Rubenstein, 1997; Ragsdale and Grove, 2001). The internal order of fields can also be affected by signaling from boundaries separating the field from neighbors (Echevarría *et al*., 2003; Riley *et al*., 2004).

One important aspect of morphogenetic fields is that, as modular units, they exist in specific locations within the embryo and, for adequate comparison of these fields in different animals, it is important to understand the overall organization plan and major axis (rostrocaudal, dorsoventral) of the embryo or organ/region containing the field. This allows one to know the correct location of the field according to the internal coordinates of the embryo/organ, including its relation to neighbors (topological position; Kuhlenbeck, 1978; Nieuwenhuys, 1974, 1998a, 1998b; Puelles and Medina, 2002). Analyzing the correct topological location of the field avoids wrong comparisons of fields sharing some of the same genetic programs and producing similar cell types, such as the subpallium (in the telencephalon) and the ventral thalamus (also called prethalamus; in the diencephalon) (Figure 1), both of which express homeobox genes of the *Dlx/Distal-less* family, and give rise to GABAergic neurons, but are located in completely different positions (Puelles and Rubenstein, 1993; Bulfone *et al*., 1993; Puelles *et al*., 2000; Brox *et al*., 2003). Comparisons of field topological location between animals are useful and make sense when the compared animals, or the organs/regions where the fields are located, share a common organization plan (Kuhlenbeck, 1978; Nieuwenhuys, 1998b; Puelles and Medina, 2002). This is the case with vertebrates, and with the central nervous system of vertebrates (see Basic Nervous System Types: One or Many?, Evolution of the Deuterostome Central Nervous System: An Intercalation of Developmental Patterning Processes with Cellular Specification Processes, Origins of the Chordate Central Nervous System: Insights from Hemichordates).

Another important aspect of morphogenetic fields is that, although highly stable, they are dynamic entities. They exist at a particular place within the embryo, and at a particular time, and their internal features (given by the lower-order modules within them, such as genetic cascades and networks and cells) and interactions with other fields change dynamically during development. At the end, each morphogenetic field gives rise to a particular set of derivatives, but homology of morphogenetic fields in two animals at a particular stage of development does not necessarily mean homology of all their

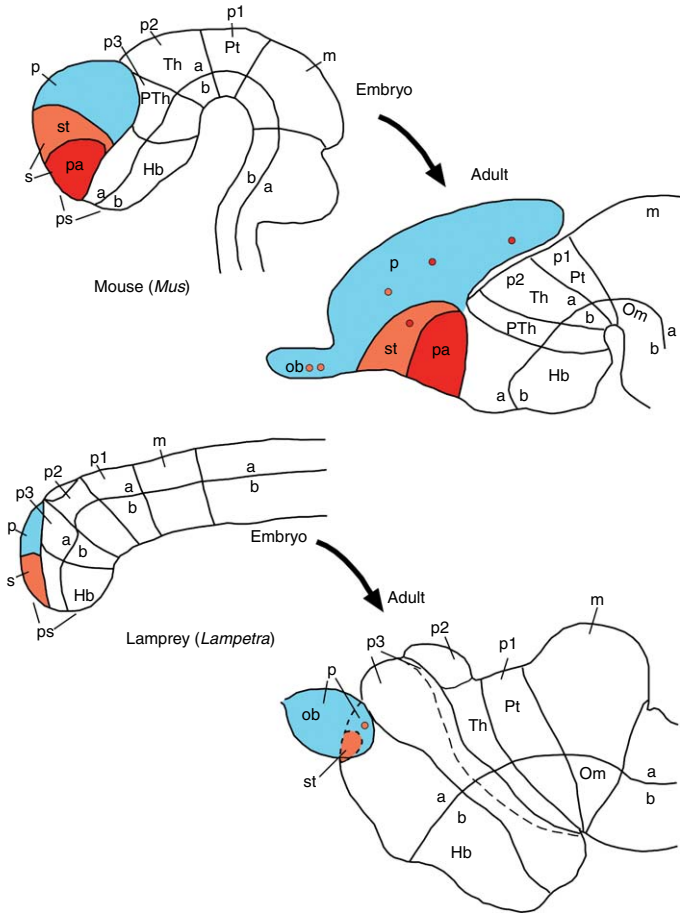

Figure 1 Schematic lateral views of the embryonic and adult brains of a mouse and a lamprey (a jawless fish close to the origin of vertebrates), showing some of the major divisions and subdivisions as understood nowadays based on available data, including data on expression of developmental regulatory genes (master control genes) during development (Pombal and Puelles, 1999; Murakami *et al.*, 2001; Puelles and Rubenstein, 2003). During development, each division and subdivision is characterized by expression of a specific combination of master control genes, and constitutes a distinct self-regulated morphogenetic field, which gives rise to a particular set of derivatives in the adult. Each morphogenetic field occupies a specific topological position within the brain basic organization plan (or brain archetype). Morphogenetic fields of two animals showing comparable molecular features (similar expression of master control genes) and comparable topological position within the general brain archetype can be considered homologous (if the phylogenetic continuity applies, which can be analyzed using a cladistic method; see A History of Ideas in Evolutionary Neuroscience). For instance, using this analysis, the alar regions of prosomere 2 (p2) of mouse and lamprey, giving rise to the thalamus (Th, or dorsal thalamus), are field-homologous. Also, the basal regions of the mesencephalon (m) of mouse and lamprey are homologous as a field, and the oculomotor neurons (Om, motor neurons of the third cranial nerve) derived from this field are homologous as well. Other cell groups found in adult basal mesencephalon may not be homologous between mouse and lamprey (or between mouse and another vertebrates), since divergent evolution may have led to the appearance of novel cell groups. In the telencephalon (colored in the schematics), two major homologous morphogenetic fields exist in the embryonic brain of both mouse and lamprey, called pallium (blue) and subpallium (orange-red), showing distinct gene expression (*Pax6* and *Emx1* in the pallium versus *Dlx* in the subpallium). In addition, mouse (as well as chicks, frogs, and zebra fish) show a smaller expression domain of the homeobox gene *Nkx2.1* within the subpallium, allowing subdivisions of this field into two smaller morphogenetic fields, called striatal field (orange) and pallidal field (red), giving rise to the striatum and pallidum, respectively, in the adult. However, lampreys do not show expression of *Nkx2.1* in the telencephalon (Murakami *et al.*, 2001; this gene is also previously called *TTF-1*), suggesting that a pallidal morphogenetic field is absent in lampreys and possibly was absent in the origin of vertebrates. This agrees with the apparent lack of a pallidum in adult lampreys (Pombal *et al.*, 1997a, 1997b). This also suggests that the pallidal morphogenetic field was a novel acquisition during the transtition from jawless to jawed vertebrates. The appearance of a new field does not only imply evolution of a novel histogenetic division, but possibly triggers changes in adjacent or distant fields due to interfield (intermodular) interactions. In the case of the telencephalon, the appearance of a new pallidal field possibly triggered changes in the striatum and even the pallium, part of which was related to the existence of tangential cell migrations from the pallidum to those divisons, involving the acquisition of new cell types (integrated as interneurons of the striatum or pallium, colored in red in schematic) and new modulatory functions on the activity of native striatal and pallial cells. The striatal field also produces part of the interneurons found in the pallium in different vertebrates (colored in orange in the schematic), and this may also be true in lampreys (Pombal and Puelles, unpublished observations). a, alar region (dorsal); b, basal region (ventral); Hb, basal hypothalamus; m, mesencephalon; ob, olfactory bulb; Om, oculomotor neurons; p, pallium; pa, pallidum; Pt, pretectum; PTh, prethalamus or ventral thalamus; ps, secondary prosencephalon; p1, p2, p3, prosomeres 1, 2, or 3; s, subpallium; st, striatum; Th, thalamus or dorsal thalamus.

derivatives at the final stage, because some evolutionary divergence may have occurred during later development, producing novel cell groups and features. Abnormal changes in the internal order of the field or in the interaction with other fields (e.g., due to mutation of master control genes, or alteration in their regulatory region) can lead to field reorganization (to reach a new order) and changes in size, sometimes involving repatterning events. This occurs in the telencephalon after knockout mutation of homeobox patterning genes such as *Pax6*, *Emx2*, *Gsh2*, or *Nkx2.1* (Sussel *et al*., 1999; Yun *et al*., 2001; Stoykova *et al*., 2000; Toresson *et al*., 2000; Bishop *et al*., 2002, 2003; Muzio *et al*., 2002). For example, knockout mutation of *Nkx2.1* in mouse produces severe malformation and size reduction of the pallidal subdivision (medial ganglionic eminence or MGE), and repatterning of most of its former primordium to give rise to a larger striatal subdivision (lateral ganglionic eminence or LGE) (Sussel *et al*., 1999). In *Emx2*/*Pax6* double mutant, the pallium is severely malformed and reduced, and its primordium is repatterned to become subpallium (Muzio *et al*., 2002). These examples indicate the importance of master control genes in the patterning and specification of morphogenetic fields, and show the self-regulatory and dynamic properties of the fields. These dynamic properties of morphogenetic fields are important for homology considerations. Thus, instead of saying that the resultant extra-large striatal subdivision found in *Nkx2.1* knockout mice (or at least the part derived from the repatterning of MGE into LGE) is not homologous to the striatal subdivision of wild-type animals because of their different origin, we should consider the LGE of both mutant and wild-type mice as an attractor homologous state (in the sense explained above; see also Raff, 1996; Striedter, 1998), no matter how this was reached.

1.05.3 Field Homology: A Useful Concept in Studies of Brain Evolution

The importance of morphogenetic fields as major units of development and evolution, and as major natural comparison characters for homology considerations makes the field homology concept very useful in evolution studies, including the brain evolution studies (Puelles and Medina, 2002). Each morphogenetic field gives rise to a specific set of derivatives found in the adult, and each organ, region, or subdivision found in the adult is the result of a specific field and its interactions with other fields, and can be formed with derivatives of several morphogenetic fields. In the brain, most cellular derivatives of each morphogenetic field remain in close association within specific radial histogenetic divisions, or in specific nuclei, areas, or cell condensations (such as patches or islands) within radial divisions. This is so because of the predominant radial glial-guided migration followed by immature neurons during development, in their way from the ventricular zone to the mantle (Rakic, 1972, 1995; Nieuwenhuys, 1998c; see 00116), although some subpopulations of cells undergo long-distance tangential migration (Anderson *et al*., 1997, 2001; Marín and Rubenstein, 2001). Another reason for the close association of cells derived from a particular morphogenetic field is that they tend to express similar combinations of cell adhesion molecules (such as cadherins) allowing their aggregation by homotypic binding, but distinct from those of adjacent fields (Redies *et al*., 2000). The prevalence of radial migration and the existence of radial histogenetic domains in the brain has been observed using fate map studies of different brain fields (Marín and Puelles, 1995; Cobos *et al*., 2001a, 2001b; García-López *et al*., 2004). This is also observed after labeling of discrete cellular clones in the ventricular zone of the embryonic telencephalon, which produces mature cells that are aligned along a narrow band extending from the ventricle to the pial surface (Striedter *et al*., 1998; Noctor *et al*., 2001). The existence of radial histogenetic domains in the adult brain, whose cellular components are mostly derived from specific morphogenetic fields, allows the use of such radial divisions as the natural comparison units for homology considerations in the adult brain. Thus, specific radial histogenetic divisions found in the adult in different animals, and having the same morphogenetic origin and topological location, such as the dorsal thalamus, the striatal domain, or the derivatives of a particular rhombomere, can be considered homologous as a field (Puelles and Medina, 2002).

However, for further homology considerations, careful analysis of the different cell groups found in the radial histogenetic division must be done, since a division may contain both homologous and nonhomologous cell groups when compared to the cell groups in the same radial division of another vertebrate (Puelles and Medina, 2002). This can be due to the fact that, as noted above, homologous morphogenetic fields may undergo partially divergent evolution during late development, producing novel cell groups and features with no counterpart in other animals or in the ancestor. This is the case of the dorsal thalamus, which becomes very large in birds and mammals and contains many cell groups with no apparent counterpart in the dorsal thalamus

of extant reptiles, or possibly in the common ancestor of extant birds, mammals, and reptiles (Butler, 1994a, 1994b). In addition, adult radial histogenetic divisions may contain immigrant cells that originate in different morphogenetic fields. These immigrant cells in a specific histogenetic region are not homologous to 'native' cells of the region, in the same animal or in other animals. However, they can be homologized to similar immigrant cells of the same region in other animals if they have an identical origin (and other similar features, such as neurotransmitter content) and can be traced back to the common ancestor (using cladistic analysis). An example of this is found in the striatum (Figure 1), a part of the basal ganglia found in the basal telencephalon of adult reptiles, birds and mammals, and derived from a homologous subpallial morphogenetic field (LGE in mammals), expressing *Dlx* family genes (Reiner *et al*., 1998; Marín *et al*., 1998; Smith-Fernandez *et al*., 1998; Puelles *et al*., 2000). The striatum in these animals contains homologous GABAergic projection neurons that originate in the LGE or LGE-like field, having specific neuropeptide contents and connections (Reiner *et al*., 1998). In addition, the striatum in these animals contains similar sets of interneurons, such as the cholinergic interneurons (Reiner *et al*., 1998). In mouse, the striatal cholinergic interneurons immigrate from a different morphogenetic field of the subpallium, characterized by expression of the homeobox gene *Nkx2.1* (Marín *et al*., 2000), and with the involvement of a *Lhx7/8*, a LIM-homeobox gene dowstream of *Nkx2.1* (Zhao *et al*., 2003). This is possibly true for the striatal cholinergic interneurons of birds and reptiles as well. The cholinergic interneurons of the striatum are neither homologous to the striatal projection neurons nor to other groups of striatal noncholinergic interneurons, but – if their identical origin is confirmed – they could be homologous to the striatal cholinergic neurons of different reptilian, avian, and mammalian species.

In a few cases, homologous cell groups occupy very different locations in the adult, in different histogenetic radial divisions, although they originate in the same morphogenetic field. This is the case of the facial branchial motoneurons, which in reptiles, birds, and mammals originate in rhombomeres 4 and 5, and their axons exit the brain in the 7th cranial nerve (facial nerve), through rhombomere 4 (Lumsden and Keynes, 1989; Medina *et al*., 1993; Medina and Reiner, 1994; McKay *et al*., 1997). However, after being born and starting to grow their axon, these motoneurons migrate caudally in reptiles and mammals (forming the facial nerve genu), to finally occupy a position at the level of rhombomeres 6–7 (Medina *et al*., 1993; Auclair *et al*., 1996; McKay *et al*., 1997). In birds, however, facial motoneurons stay within rhombomeres 4–5 and are seen at this same location in the adult (Medina and Reiner, 1994). In this case, the general segmental division derived from each rhombomere can still be homologized as a field between species, and also occupy comparable topological positions and relation to neighbors. When analyzing the specific cell groups, it is clear that, independent of their distinct position in the adult, the facial motoneurons of birds, reptiles, and mammals are homologous. Thus, for homology considerations of specific cell groups in the adult, it is extremely important to know the exact morphogenetic origin of the group (i.e., origin with respect to the morphogenetic fields found during development), and the origin is a prevalent criterion independent of the final location of the cells in the adult. Once an identical origin from homologous morphogenetic fields has been confirmed, other attributes can be considered for investigating the putative homology of specific cells found in the adult, such as neurotransmitter and/or neuropeptide content, connections, neurotransmitter receptor expression, etc. Connectivity and receptor features need to be considered with caution since these may have changed during the evolution of homologous cells (Striedter, 1998; see A Tale of Two CPGs: Phylogenetically Polymorphic Networks).

1.05.3.1 In Search of the Brain Archetype in Vertebrates: Developmental Regulatory Genes as Useful Tools for Deciphering the Archetype and Identifying Homologous Fields

As noted above, morphogenetic fields exist in specific locations within the animal or organ/region, and this also applies to the brain. The brain of vertebrates shares a common basic organization plan (or archetype; also called 'Bauplan'; see Basic Nervous System Types: One or Many?, Evolution of the Deuterostome Central Nervous System: An Intercalation of Developmental Patterning Processes with Cellular Specification Processes) where the morphogenetic fields and their derivatives are located. This basic organization plan is better appreciated during development, and developmental regulatory genes, combined with other data such as fate mapping results, are very useful tools for trying to unravel it (Puelles and Rubenstein, 1993, 2003; Puelles and Medina, 2002; see Evolution of the Deuterostome Central Nervous System: An Intercalation of Developmental Patterning Processes with Cellular Specification Processes, Origins of the Chordate Central Nervous System: Insights from Hemichordates). As noted above, these master control genes show highly evolutionarily

conserved sequences, and encode transcription factors or signaling proteins that regulate expression of other genes, thus controlling patterning and morphogenesis of specific body parts (Carroll *et al*., 2001). They also show generally conserved expression patterns in the embryonic brain, and restricted expression domains comparable across species (Smith-Fernandez *et al*., 1998; Puelles *et al*., 2000; Murakami *et al*., 2001; Bachy *et al*., 2002; Brox *et al*., 2003, 2004). These genes help in understanding important organization features of the developing brain, such as its curved longitudinal axis, the existence of longitudinal and transverse (segmental) divisions, and the existence of smaller subdivisions, each one in a specific location within the general plan. Expression of these genes indicates that, during development, the brains of different vertebrates pass through a highly similar stage (a kind of phylotypic stage), in which the embryonic brain shows clearly comparable molecularly distinct divisions and subdivisions, and a common archetype. These divisions and subdivisions represent distinct morphogenetic fields, and each one is characterized by the expression of a specific combination of master control genes. Thus, these genes are very valuable tools for locating these morphogenetic fields within the general brain archetype, and for finding homologous morphogenetic fields in the brain of different vertebrates (Puelles and Medina, 2002). Understanding this is very important for later studying the derivatives of each field in the adult, for searching cases of field homology and cell group homology in the adult, and for understanding how morphological divergence occurs in evolution.

1.05.3.2 Evolution of Homologous Fields in the Brain: The Case of the Pallium

In the brain, homologous morphogenetic fields can give rise to adult homologous structures showing a high degree of evolutionary conservation, such as the striatum (Figure 1; Reiner *et al*., 1998; Marín *et al*., 1998; see The Evolution of the Basal Ganglia in Mammals and Other Vertebrates) or, often, give rise to structures that are homologous as a field in different vertebrates, but that show a high degree of variation between species though keeping a few basic common features (e.g., the neocortex of mammals, the hyperpallium of birds, and the dorsal cortex of reptiles, all of which derive from the dorsal pallium; Medina and Reiner, 2000; see Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?, The Origin of Neocortex: Lessons from Comparative Embryology). Within the pallium, there are striking differences between animal groups. For example, the dorsomedial pallium shows an extraordinary development in mammals (in particular, its dorsal pallial derivative, the neocortex) but, in contrast, the ventrolateral pallium shows the greatest development in reptiles and especially in birds, giving rise to a large structure called dorsal ventricular ridge (Butler, 1994b; Striedter, 1997; see Evolution of the Nervous System in Reptiles, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?, The Evolution of Vocal Learning Systems in Birds, Evolution of the Amygdala in Vertebrates, The Origin of Neocortex: Lessons from Comparative Embryology). One of the main factors that may be involved in such variations is the possible existence of changes in the networks of developmental regulatory genes that operate in forebrain development (Carroll *et al*., 2001; Gilbert and Burian, 2003). Indeed, numerous developmental regulatory genes play key roles in patterning, specification, cell proliferation, and/or cell differentiation of specific parts in the central nervous system (from the spinal cord to the telencephalon), and changes in their expression (e.g., by knockout mutation producing a lack of function) lead to morphological alterations that include changes in the relative size of regions and lack of formation of particular sets of neurons, among other changes (Ericson *et al*., 1997; Briscoe *et al*., 1999; Sussel *et al*., 1999; Yun *et al*., 2001, 2003 Bishop *et al*., 2002, 2003; Muzio *et al*., 2002). An interesting observation is that the relative size of one particular region (radial histogenetic division) can be affected by both genes expressed in its own primordium during development, or genes expressed in adjacent morphogenetic fields, and it appears that several patterning genes play key opposing roles in establishing the relative size of regions. This occurs in the telencephalon, where the relative size of the pallium is enlarged by knockout mutations affecting homeobox genes expressed in the subpallium (such as *Gsh2*), and is reduced by knockout mutations affecting homeobox genes expressed in the pallium (such as *Pax6*) (Stoykova *et al*., 1996, 2000; Toresson *et al*., 2000; Yun *et al*., 2001). Any alteration in the equilibrium between genes having opposing roles in specifying subpallial versus pallial territories (either by overexpression or by lower expression of one of the two groups) may have led to the very large pallium observed in mammals and birds.

Within the pallium, the opposing roles of *Emx1/2* versus *Pax6* (expressed in opposing gradients in the pallium) can affect the relative size of caudomedial versus rostrolateral pallium and the specific areas in them (Bishop *et al*., 2002, 2003). This, in fact, is affecting the relative size of the medial pallium

(displaced caudally in mammals) and adjacent parts of dorsal pallium versus the rest of the pallium, including the ventrolateral pallium or part of it (i.e., piriform cortex and claustrum; Stoykova *et al*., 1996). In addition, some of the genes involved in internal pallial patterning and specification, such as *Emx1* and *Emx2*, also have a role in cell proliferation, thus affecting growth and size of the pallial areas where they show higher expression (Bishop *et al*., 2003), and the level of expression of these genes can be affected by signaling proteins that diffuse from organizer centers, such the cortical hem located at the edge of the medial pallium (Ragsdale and Grove, 2001; Garda *et al*., 2002). Other developmental regulatory genes (such as the LIM-homeobox genes and several LIM cofactor genes) are expressed in combinatorial patterns in the pallium and other parts of the forebrain, and – as in the spinal cord – may play key roles in the differentiation of specific sets of neurons and in the establishment of specific connections (Rétaux *et al*., 1999; Bulchand *et al*., 2001, 2003; Moreno *et al*., 2004). In addition, one of these genes (*Lhx2*) plays a role in the formation of the dorsomedial pallium and its boundary with the cortical hem, and lack of its function produces severe malformation of the dorsomedial pallium (affecting specially the medial pallium), without affecting any part of the ventrolateral pallium, which expresses the antagonist LIM protein Lmo3 (Bulchand *et al*., 2001; Vyas *et al*., 2003).

This provides an idea of only some of the complex and important networks of regulatory genes operating in pallial development (as in the development of other brain regions). Any alteration in the expression of these regulatory genes within or near the pallium may have led to the differences observed within the pallium between mammals and birds. Further, the pallium has important reciprocal connections with the thalamus, and a recent study indicates that developing thalamocortical axons releases a mitogenic factor that increases cell proliferation of pallial cells, indicating that epigenetic influences from distant regions can also modulate pallial development (Dehay *et al*., 2001). Thus, alterations affecting the number or the pathfinding of thalamocortical axons may influence the final size of cortical areas as well. Nevertheless, this does not affect the initial specification and area formation of the pallium, which is due to intrinsic factors, mediated by locally expressed developmental regulatory genes (Miyashita-Lin *et al*., 1999). Until now, developmental evolutionary neurobiologists have focused on analyzing similarities in expression pattern of a number of these regulatory genes, and this has been extremely valuable for understanding the brain archetype and finding homologous fields. In the future, we will have to center on investigating what differs in brain development of different animals, by searching for differences in expression patterns of some master control genes and by searching changes at the level of promoter/enhancer, activators, repressors, and cofactors regulating the expression of master control genes (Medina *et al*., 2005). This will help us understand what exact developmental mechanisms led to the morphological variations found in the brain of different vertebrates.

1.05.3.3 Evolution of New Fields in the Vertebrate Brain: Analysis of the Lamprey

Although most vertebrates share a basic brain archetype, it appears that early vertebrates (see Evolution of the Deuterostome Central Nervous System: An Intercalation of Developmental Patterning Processes with Cellular Specification Processes) lacked at least some of the whole set of morphogenetic fields found in the forebrain of tetrapods, suggesting that some new fields evolved after vertebrates first appeared (Medina *et al*., 2005). This is based on analysis of the lamprey, a jawless fish close to the origin of vertebrates. During development, the forebrain of these animals show some of the same molecularly distinct divisions found in tetrapods, including comparable diencephalic divisions/subdivisions, and comparable pallial and subpallial divisions in the telencephalon (Murakami *et al*., 2001). In the telencephalon of developing lamprey, the subpallium is characterized by *Dlx* expression, whereas the pallium expresses *Pax6* and *Emx1* genes, showing patterns comparable to those observed in other vertebrates. However, the subpallium does not show any expression of the pallidal marker gene *Nkx2.1*, suggesting that these animals lack the morphogenetic field giving rise to the pallidum (Murakami *et al*., 2001; this gene is also called *TTF-1*), which agrees with the apparent absence of this structure in adult lampreys (Pombal *et al*., 1997a, 1997b). In zebra fish, a teleost jawed fish, the subpallium includes both striatal and pallidal subdivisions, expressing either only *Dlx* or both *Dlx* and *Nkx2.1* orthologue genes (Rohr *et al*., 2001), suggesting that the pallidal subdivision appeared in the telencephalon as a novel morphogenetic field during the transition from jawless to jawed vertebrates (Medina *et al*., 2005). This example indicates that novel morphogenetic fields can evolve by appearance of novel expression domains of developmental regulatory genes (also involving a cascade a

downstream genes), and these novel fields can lead to the production of novel histogenetic divisions and cell groups in the adult. Understanding what exact mechanisms lead to the appearance of novel expression domains of master control genes during development will be essential for understanding evolution of novel structures in the brain.

For homology and evolutionary considerations, another way of looking at this is by analyzing the hierarchy of the fields, and how addition of a new field can affect the rest. The whole embryo is considered to be the primary field, being later subdivided into secondary and then tertiary fields, and so on (Gilbert *et al*., 1996; Carroll *et al*., 2001). If we arbitrarily consider the telencephalic vesicle as a 'primary' field (to simplify counts), then pallium and subpallium are 'secondary' fields (Figure 1). Within the subpallium, the appearance of a *Nkx2.1* expression domain divides it into two 'tertiary' fields (striatal and pallidal). (This is an oversimplification, since – atleast in tetrapods – the *Nkx2.1* expressing domain of the subpallium is further subdivided into at least two fields: a 'true' pallidal field expressing *Nkx2.1* and *Dlx* genes, and an anterior entopeduncular field expressing *Nkx2.1, Dlx* plus sonic hedgehog (Puelles *et al*., 2000; Marin and Rubenstein, 2002).) At a certain point of development, the lamprey subpallium can be considered homologous to that of jawed vertebrates as 'secondary' morphogenetic fields. They give rise to subpallial divisions in the adult that can be considered homologous as a field, but have the following major differences. After subpallial subdivision into 'tertiary' fields, the *Nkx2.1* expressing pallidal field of jawed vertebrates is not homologous to any field in the lamprey. The striatal 'tertiary' field (expressing only *Dlx* genes) of jawed vertebrates remains homologous to the single subpallial field of lamprey, and both give rise to a homologous striatum (Figure 1). Nevertheless, the striatum produced in jawed vertebrates is the result of both similar plus newly evolved interactions of its primordium with other modules, such as the novel *Nkx2.1* expressing field. For this reason, the resultant striatum of jawed vertebrates has some new features with no counterpart in the lamprey striatum. The new features of the striatum can even go further if immigrant cells coming from the newly evolved field arrive in the striatum and integrate establishing connections with native cells, as it occurs with striatal interneurons in amniotes (see above) and in some amphibians (Figure 1). This involves an evolutionary increase in both morphological and functional complexity of the striatum. Thus, novel fields can have consequences that go further than just producing a new histogenetic territory, since they can influence, and trigger, important morphological and functional changes in adjacent and/or distant fields.

1.05.4 Conclusions

The concept of field homology has recently been reformulated under the modern perspective of evolutionary developmental biology (Puelles and Medina, 2002), based on the existence of morphogenetic fields as major units of development and evolution, linking the genotype to the phenotype, which are considered as the natural comparison characters for homology considerations. Morphogenetic fields are self-regulated entities, having specific properties (such as expression of specific combinations of developmental regulatory genes), and specific interactions with other fields, which change dynamically during development. They exist in specific topological locations within the organism and give rise to specific sets or lineages of cells. In the brain, morphogenetic fields are represented by segments, and by divisions and subdivisions within them (different hierarchies). Based on the predominant radial glial-guided migration of neurons during development, the majority of their cellular derivatives stay within single radial histogenetic divisions, which therefore constitute natural comparison units for field homology considerations in the adult brain. However, homology of a specific morphogenetic field in two animals does not necessarily mean complete homology of the cell groups in the derived histogenetic divisions, since some evolutionary divergence may have occurred, involving the appearance of novel, nonhomologous cell groups in one of the animals, or in both (which may be native cells of the same field or immigrant cells from other fields).

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1.06 Reelin, Cajal–Retzius Cells, and Cortical Evolution

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Glossary

CP	Cortical plate.
Dab1	Disabled-1 gene or protein.
DC	Dorsal cortex.
DVR	Dorsal ventricular ridge.
LC	Lateral cortex.
MC	Medial cortex.
Myr	Mega-year.
MZ	Marginal zone.
VZ	Ventricular zone.

1.06.1 Introduction

One of the most fascinating questions in neurobiology concerns the evolution of the cerebral cortex, a sequence of events that leads to the development of the human cortex and its cognitive abilities (see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates). Genes that control development and growth are privileged targets of evolutionary selection (Raff, 1996), and comparative studies of cortical development may shed light on this process (see Cortical Evolution as the Expression of a Program for Disproportionate Growth and the Proliferation of Areas; Captured in the Net of Space and Time: Understanding Cortical Field Evolution). In the absence of fossil material, however, the evolution of cortical development can only be inferred from comparative studies of modern organisms (Butler and Hodos, 1996).

Studies carried out during the last decades demonstrated that basic, conserved developmental mechanisms pattern the brain in general and the telencephalon in particular (Monuki and Walsh, 2001; Grove and Fukuchi-Shimogori, 2003). Secreted factors (such as Shh, Wnts, Bmps, and Fgfs) establish morphogenetic gradients to which precursors in the neuroepithelial sheet respond by modulating expression of arrays of transcription factors (such as Pax6, Emx1/2, and Tbr1), thereby adapting neuronal cell numbers and types. Different neuronal classes migrate following different routes to colonize various structures.

The cortex is reduced to a periventricular layer in anamniotic vertebrates and increases in size and organization in amniotes. It gains prominence in synapsids, the lineage leading to mammals, and evolves explosively in primates. Despite their obvious importance, however, the molecular events that underlie cortical evolution remain mostly unknown. Work in mice identified several genes with a role in cortical development and presumably evolution (Lambert de Rouvroit and Goffinet, 2001; Monuki and Walsh, 2001). Among them, reelin and its signaling partners may be critical players in the evolution of the mammalian laminar cortical plate (CP) (Bar *et al.*, 2000). In addition, in humans, reelin signaling is essential for cortical foliation (Hong *et al.*, 2000). Comparisons of reelin expression in mammals, reptiles, and birds show that reelin-expressing cells are present in the cortical marginal zone (MZ), from the preplate (PP) stage, in all amniotes, but both the number of positive cells and their level of expression are much higher in mammals than in other lineages. In this article, we will briefly review data on comparative reelin expression during cortical development and discuss some questions that we consider of special neurobiological interest because they would be amenable to study, provided more efforts are invested to provide access to genomic sequences and high-quality embryonic material from multiple species.

1.06.2 Reelin Signaling During Cortical Development and Evolution

A schematic cladogram of evolutionary filiations from stem amniotes to modern reptiles, birds, and mammals is provided in Figure 1 (Colbert *et al.*, 2001). In all

Era	Period	Subperiod	Myr
	Quaternary		
		Neogene	22
Cenozoic	Tertiary	Paleogene	42
	Cretaceous		79.2
	Jurassic		61.5
Mesozoic	Triassic		42.5
	Permian		45
		Pennsylvanian	33
	Carboniferous	Mississippian	40
	Devonian		46
	Silurian		31
		Bala	
		Dyfed	
	Ordovician	Canadian	71
Paleozoic	Cambrian		60
	Vendian		40
Sinian	Sturtian		
Riphean			
Animikean			
Huronian			
Randian			
Swazian			
Isuan			
Hadean			

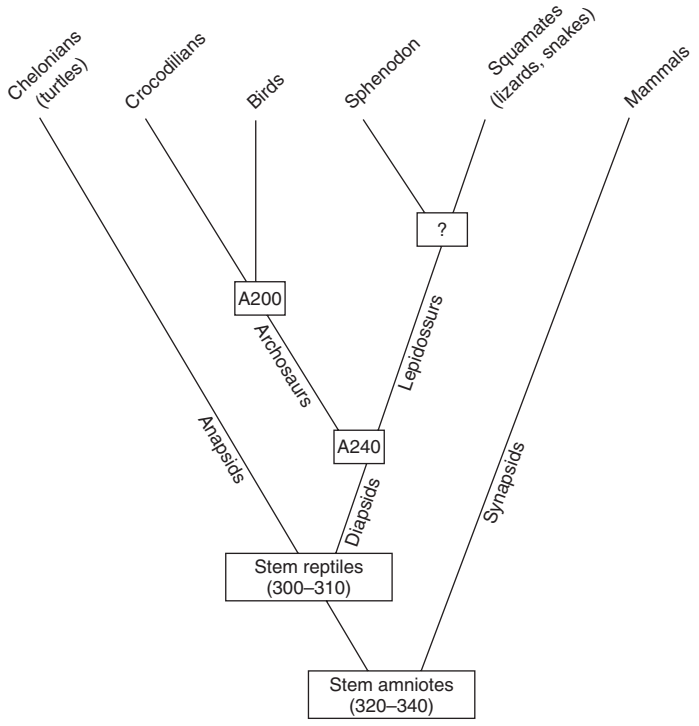

Figure 1 Summary of geological epochs, and simplified evolutionary filiations of amniotes. Numbers refer to estimated time of phyletic divergence (in mega-years (Myrs)). Adapted from Bar, I., Lambert de Rouvroit, C., and Goffinet, A. M. 2000. The evolution of cortical development. An hypothesis based on the role of the reelin signaling pathway. *Trends Neurosci.* 23, 633–638, with permission from Elsevier.

amniotes, telencephalic development follows a basic, evolutionary homologous Bauplan. This basic organization includes a medial, dorsal, and lateral cortex located at the external aspect of the ventricle, and the dorsal ventricular ridge (DVR) located ventral to the ventricle (Figure 2). There is a consensus that the medial cortex is homologous to the mammalian hippocampal formation, whereas the dorsal and lateral cortices are the precursors of the neocortex and the pyriform cortex or rhinencephalon, respectively. Views about the DVR, which is diminutive in mammals, are more controversial (Northcutt, 1981; Butler and Hodos, 1996; Fernandez *et al.*, 1998).

In mammals, cortical development begins with the appearance of the PP (Caviness, 1982; Allendoerfer and Shatz, 1994; Sheppard and Pearlman, 1997; Super *et al.*, 2000), a heterogeneous structure that contains future subplate cells, reelin-negative pioneer neurons and reelin-positive subpial cells destined for the MZ (Meyer *et al.*, 1998, 2000), and probably other cell types. The next stage is the condensation of the CP, densely populated with radial bipolar neurons. The appearance of the CP results in the splitting of the PP, elements of which are displaced in the subplate (Allendoerfer and Shatz, 1994) and in the MZ (Caviness, 1982; Sheppard and Pearlman, 1997). The CP develops from inside to outside, by migration of new neurons that cross previously established layers and settle at progressively more superficial levels. The organization of the CP is controlled by the reelin-signaling pathway (Rice and Curran, 2001; Tissir and Goffinet, 2003). Defective reelin signaling results in a loosely organized CP, with absence of PP splitting, and inverted maturation, from outside to inside (Caviness and Rakic, 1978; Rakic and Caviness, 1995). Normal reelin signaling is necessary but not sufficient for the development of the CP. For example, in mice deficient in cyclin-dependent kinase 5 (Cdk5) or its cofactors p35 and p39 (Ohshima *et al.*, 1996; Chae *et al.*, 1997; Gilmore *et al.*, 1998; Ko *et al.*, 2001), the radial organization of the early CP is preserved, yet its maturation proceeds from outside to inside as in

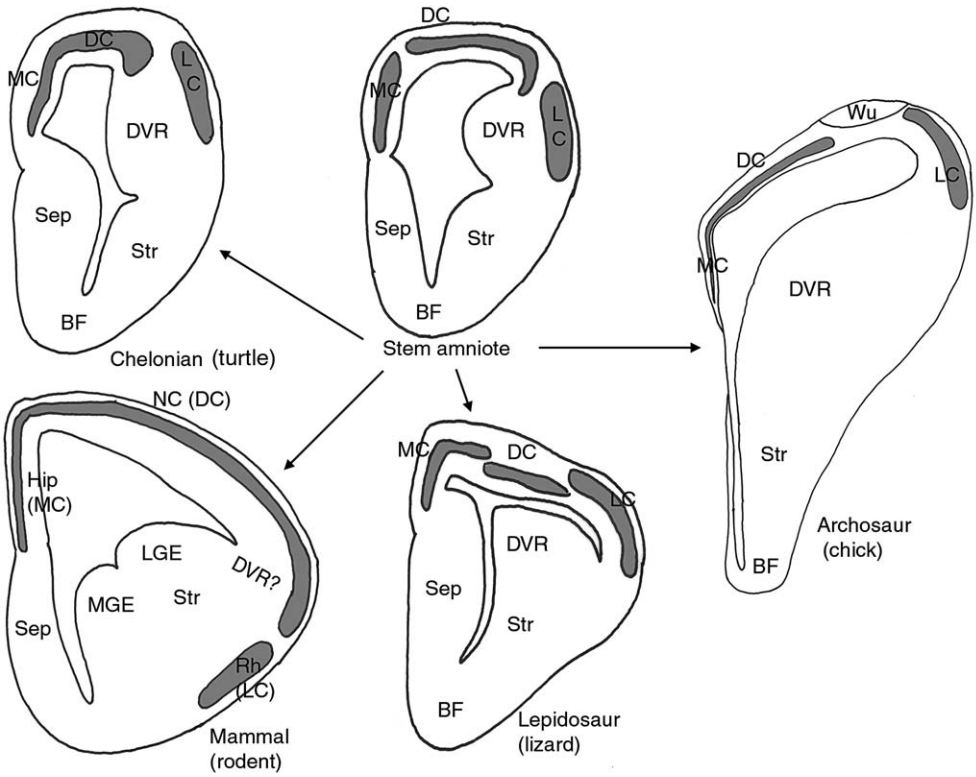

Figure 2 Schematic organization (frontal sections) of the dorsal embryonic telencephalon in putative stem amniotes, chelonians (turtles), squamates (lizards and snakes), archosaurs (birds and crocodiles), and mammals. BF, basal forebrain; DC, dorsal cortex (mammalian NC, neocortex); DVR, dorsal ventricular ridge; LC, lateral cortex (mammalian Rh, rhinencephalon); MC, medial cortex (mammalian Hip, hippocampus); MGE and LGE, medial and lateral ganglionic eminences; Sep, septal nuclei; Str, striatum; Wu, avian Wulst. Reproduced from Bar, I., Lambert de Rouvroit, C., and Goffinet, A. M. 2000. The evolution of cortical development. An hypothesis based on the role of the reelin signaling pathway. *Trends Neurosci.* 23, 633–638, with permission from Elsevier.

reelin-deficient mice. Two reelin-dependent developmental events, CP organization and inside-out maturation, were presumably also important acquisitions during cortical evolution (Bar *et al.*, 2000). Additional factors of cortical evolution include increased neuron generation in the ventricular zone (VZ) and the necessity to migrate over longer distances, requiring a sophisticated migration machinery in which Cdk5, p35/p39, and other genes such as filamin-1, *Lis1*, or doublecortin participate (Lambert de Rouvroit and Goffinet, 2001; Grove and Fukuchi-Shimogoril, 2003).

Reelin is a large extracellular glycoprotein that is secreted by Cajal–Retzius neurons in the MZ (Bar *et al.*, 1995; D'Arcangelo *et al.*, 1995). It binds to two receptors of the lipoprotein receptor family, very low density lipoprotein receptor (VLDR) and apolipoprotein E receptor type 2 (ApoER2) that are expressed at the surface of CP cells (Hiesberger *et al.*, 1999; Trommsdorff *et al.*, 1999). This triggers the activation of an intracellular signal that ultimately directs the architectonic organization of the CP. Upon reelin-receptor binding, the adapter disabled-1 gene or protein (Dab1) is tyrosine phosphorylated by Scr family kinases (Howell *et al.*, 2000), but the rest of the mechanism is still poorly understood (Bock *et al.*, 2003; Pramatarova *et al.*, 2003; Ballif *et al.*, 2004; Jossin *et al.*, 2004).

Reelin and Dab1 expression have been studied in mouse (Rice and Curran, 2001; Tissir and Goffinet, 2003), human (Meyer and Goffinet, 1998; Meyer *et al.*, 2002, 2003), turtle (Bernier *et al.*, 1999), lizard (Goffinet *et al.*, 1999), chick (Bernier *et al.*, 2000), and crocodile (Tissir *et al.*, 2003), allowing comparisons and correlations with architectonic patterns in representatives of the main amniote lineages (Figure 3; Goffinet, 1983). The expression of VLDLR and ApoER2 is supposed to overlap largely that of Dab1, but this remains to be studied. In turtles, which are considered the most closely related to stem amniotes, cortical architectonics is the most rudimentary. Reelin-positive neurons are present in the MZ of the medial and dorsal cortical fields. In addition, some less strongly labeled neurons are dispersed in the CP. The situation in the lateral cortex is different, with reelin-positive neurons scattered diffusely in the cortex (Bernier *et al.*, 1999). Dab1 is expressed in CP cells in all sectors

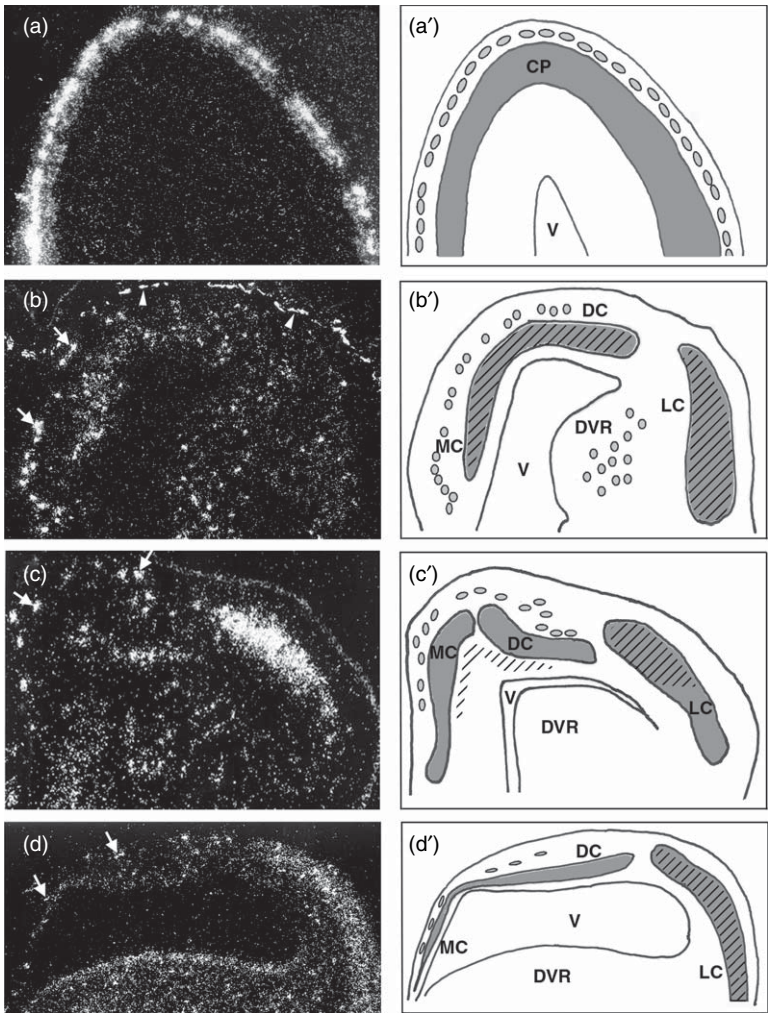

Figure 3 Comparison of reelin mRNA expression. Frontal sections in the embryonic cortex of the mouse (a, a′), turtle (b, b′), lizard (c, c′) and chick (d, d′). Expression patterns of reelin mRNA are shown in darkfield views (a–d). Schematic drawings (a′–d′) show reelin-positive zones (circles for cells in marginal zone, and hatched areas for more diffuse expression in cortical plate or subcortex). CP, cortical plate; DC, dorsal cortex; DVR, dorsal ventricular ridge; LC, lateral cortex; MC, medial cortex; V, ventricle.

a, a′, The mouse cortex is characterized by an almost continuous subpial layer of neurons that express extremely high levels of reelin. The underlying CP is reelin-negative but expresses Dab1. Detailed descriptions are provided in Alcantara *et al.* (1998) and Schiffmann *et al.* (1997).

b, b′, In the turtle cortex, reelin-positive cells (arrows) are dispersed in the marginal zones of the MC and DC, and to a lesser extent in the lateral cortex and DVR. The cortical plate in MC and DC is weakly reelin-positive and Dab1-positive. Arrowheads point to spontaneously darkfield-positive melanophores. Detailed description in Bernier *et al.* (1999).

c, c′, In the lizard MC and DC, reelin-positive neurons (arrows) are abundant in the marginal zone, and there is a second layer of reelin expression in the subplate (hatched area in c′), whereas the cortical plate is reelin-negative but Dab1-positive. The LC is diffusely Dab1-positive, and its dorsal component expresses reelin (hatched in c′). Detailed description in Goffinet *et al.* (1999).

d, d′, In the chick, subpial reelin-positive cells (arrows) are found only in the diminutive MC (hippocampus) and DC (parahippocampus), and the cortical plate is negative. There is diffuse reelin expression in the LC. Detailed description in Bernier *et al.* (2000).

(Goffinet, unpublished). In chicks (Bernier *et al.*, 2000), a CP is evident only in the medial cortex and in the adjacent, dorsal parahippocampal cortex. At this level, a few strongly reelin-positive neurons are found in the MZ, but not in the CP itself. A similar canvas of reelin-positive cells in the MZ and reelin-negative CP is found in crocodiles (Tissir *et al.*, 2003). Dab1 expression has not been studied in the chick and crocodilian telencephalon.

In lizards (squamates), an elaborate architectonic organization of the medial and dorsal cortices develops in parallel with a specific, bilaminar expression of reelin, bracketing a reelin-negative and Dab1-positive CP (Goffinet *et al.*, 1999). Large reelin-positive neurons are present in the MZ, as in other species. Unlike in other amniotes, a second layer of reelin-positive cells is found in the subcortex. As in turtles, the lateral cortex expresses both reelin and

Dab1. In turtles, lizards, and chicks, maturation of the CP proceeds from outside to inside (as in reelin-deficient mammals; Tsai *et al.*, 1981; Goffinet *et al.*, 1986). Mammals (synapsids) are characterized by a spectacular development of the CP both in terms of cell numbers and architectonic organization, and by its maturation from inside to outside (Caviness and Rakic, 1978; Rakic and Caviness, 1995). This is accompanied by an amplification of reelin production in Cajal–Retzius cells (CRc) – as estimated using *in situ* hybridization with species-specific probes and monoclonal antibodies with conserved epitopes – and anomalies in reelin-deficient mice indicate that this modification of reelin expression was necessary for the evolution of the mammalian cortex.

Although several pieces of the puzzle are lacking, such as the expression of lipoprotein receptors and the analysis of more species, these data clearly suggest that the production of reelin by early neurons in the MZ and the expression of Dab1 (and reelin receptors) by CP neurons is a feature of all amniotes. This pattern, presumably present in stem amniotes, is evolutionarily homologous. From this ancestral pattern, the expression profiles have evolved differently in divergent lineages; similar elaborate CP organizations in mammals and in some cortical areas in lizards were probably acquired by convergent evolution. In addition to the control of neuronal numbers and differentiation, and of hodological relationships, the modulation of architectonic organization is an important, hitherto neglected, parameter of cortical evolution, in which the reelin-signaling pathway plays an important role.

1.06.3 The Puzzle of Cajal–Retzius Cells

CRc are complex neuronal cells that develop early in the mammalian cortical MZ and undergo apoptotic degeneration near the end of cortical neuronal migration (Del Rio *et al.*, 1996; Mienville, 1999; Frotscher *et al.*, 2001; Grove and Fukuchi-Shimogori, 2003). They have multiple origins in telencephalic VZs and migrate in the PP by following several routes, particularly by tangential migration (Meyer and Wahle, 1999; Meyer *et al.*, 2002; Takiguchi-Hayashi *et al.*, 2004). In spite of heterogeneity of origin and migration pathways, mammalian CRc are uniquely characterized by a co-expression of reelin and the transcription factor p73, a member of the p53 family (Kaghad *et al.*, 1997; Yang *et al.*, 2000; Meyer *et al.*, 2002) that may regulate their apoptosis.

As mentioned above, reelin-positive neurons are present in the early MZ in all amniotes, indicating, as a parsimonious hypothesis, that these cells may all be evolutionary-related and could have evolved from an ancestral CRc present in stem amniotes. On the other hand, the neuronal population of the embryonic MZ is more complex than previously thought (Meyer *et al.*, 1998; Fairen *et al.*, 2002), suggesting that the evolutionary history of CRc may be more intricate than such a simple evolutionary homology. Recently, co-expression of reelin and p73 was shown to be a defining feature of embryonic CRc in humans and rodents (Yang *et al.*, 2000; Meyer *et al.*, 2002). In order to assess whether CRc are indeed evolutionarily homologous, we studied reelin and p73 mRNA expression using double *in situ* hybridization with species-specific probes in the embryonic cortex of mice, turtles, lizards, crocodilians, and chicks.

As illustrated in Figure 4, early-born neurons in the embryonic cortical MZ in turtles and crocodiles co-express reelin and p73, as they do in mammals. The two probes label the same cells and single positive cells are rare. In turtles, the reelin-positive cells scattered in the CP are p73-negative. In crocodiles, the stream of reelin mRNA-expressing cells in the intermediate zone (Tissir *et al.*, 2003) does not express p73 and thereby differs from MZ cells. Rather surprisingly, in chicks, despite the close similarity in terms of brain organization and evolutionary relationships with crocodiles, reelin and p73 are rarely co-expressed in MZ neurons. Similarly, in lizards, very few among the abundant reelin-positive MZ neurons are labeled with the p73 probes. There are several possible explanations to these findings, such as trivial problems in cloning and using p73 mRNA probes. p73 mRNA sequences are closely related to p53 and even more so to p63, and proved particularly difficult to clone in lizards. But, even with that restriction, the results at least show that expression of p73 is very low in chick and lizard and/or that reelin and p73 co-expression is rare in those species. The *p73* gene is thought to regulate apoptosis and this regulation may be less important in reelin-positive subpial cells in chicks and lizards than in other species. If this is true, the co-expression of p73 and reelin may not be the best criterion to assess putative evolutionary homologies. Reelin-positive neurons are present in the developing brain of the lamprey (Perez-Costas *et al.*, 2002), zebra fish, and *Xenopus* (Costagli *et al.*, 2002), and the *p73* gene was identified in zebra fish (Pan *et al.*, 2003; Rentzsch *et al.*, 2003). With the discovery of new molecular markers and the definition of more genomic sequences, tools should become available in the coming years to unravel the complex evolutionary history of MZ neurons.

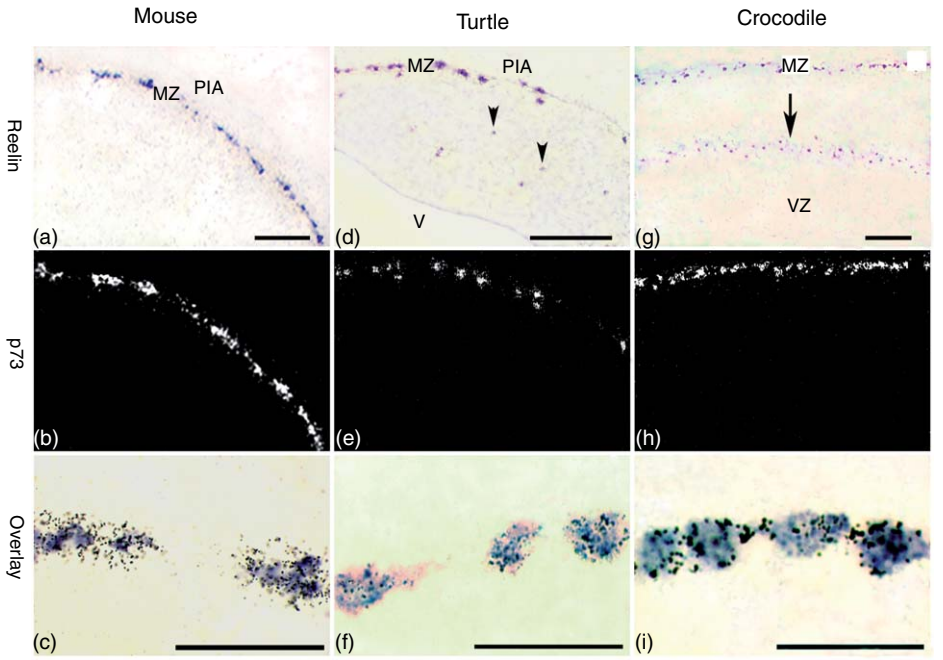

Figure 4 Co-localization of reelin and p73 mRNA in CRc. Double *in situ* hybridization studies of reelin mRNA (digoxigenin-labeled probes, first row), p73 mRNA ([^{33}P]-labeled probes, second row), and their co-localization (overlay, third row) in the embryonic cerebral cortex of the mouse (a–c), turtle (d–f), and crocodile (g–i). Almost all subpial reelin-positive neurons express p73 in all three species. In contrast, the reelin-positive cells in the turtle cortical plate (d, arrowheads) and in the crocodilian subcortex (g, arrow) are p73-negative. Note that the reelin signal illustrated in the mouse was underexposed for histological analysis and is relatively much higher than in other species. PIA, pial surface; MZ, marginal zone; V, lateral ventricle; VZ, ventricular zone. Scale bar: 100 μm (a, d, g); 50 μm (c, d, f).

1.06.4 Redundant Expression of Reelin in Mice: What Could It Teach Us about Evolution?

The reelin expression studies summarized above clearly demonstrate a drastic amplification of the mRNA and protein levels in mammalian CRc as compared to other reelin-positive neurons in the reptilian and avian MZ. It is tempting to assume that high levels of reelin are required during cortical spreading, and indeed a sustained supply of reelin-positive cells is observed in the human MZ throughout fetal development (Meyer and Goffinet, 1998). On the other hand, there is ample evidence that the concentration of reelin in the mouse cortical MZ is in large excess. The reeler (reelin-deficient) mutant phenotype only appears in brains of chimeric mice when reeler cells greatly outnumber normal cells (Mikoshiba *et al.*, 1986; Terashima *et al.*, 1986; Mullen *et al.*, 1997; Yoshiki and Kusakabe, 1998). In spite of a loss of CRc and a drastic decrease of reelin concentration, homozygous p73 mutant mice do not have a reeler-like cortex (Yang *et al.*, 2000). A normal CP develops *in vitro* in serum-free culture medium without the addition of exogenous reelin, and addition of reelin to reeler slice *in vitro* (Jossin *et al.*, 2004), or ectopic expression of low levels of reelin in the VZ in transgenic reeler mice malformation (Magdaleno *et al.*, 2002), are able to partially correct the reeler trait. These observations suggest that reelin may diffuse from sources other than CRc and that the expression of receptors and Dab1 may be more important than the site of ligand production.

Why did mammalian CRc amplify a production that appears so redundant in mice? As a way of explaining this apparent contradiction, we would like to suggest the following scenario. Amplification of reelin synthesis in CRc was necessary for the development of a foliated cortex, and stem mammals initially developed a moderately folded, not a lissencephalic, cortex. During evolution, some cortices, such as that of rodents, evolved secondarily into a lissencephalic type. Not being detrimental, elevated reelin production in CRc was not necessarily adjusted in parallel with the reduction of cortical surface. Other lineages, most notably primates, acquired increasingly more foliated cortex and, in humans, additional numbers of reelin-expressing cells became necessary. This idea is compatible with observations that cortical foliation can vary widely within closely related

lineages. For example, in monotremes, Echidna has an elaborate, highly foliated cortex, whereas Platypus is almost lissencephalic (Rowe, 1990). Similar examples can be found in other phyla, including primates. Production of a gyrated mouse cortex is artificially accomplished by elegant, yet relatively simple, manipulations (Rakic, 2004), such as germline inactivation of caspases 3 and 9 (Kuida *et al.*, 1996, 1998), increased expression of beta-catenin in transgenic mice (Chenn and Walsh, 2002, 2003), or incubation of embryonic cortex *in vitro* in the presence of lysophosphatidic acid (Kingsbury *et al.*, 2003; Price, 2004). When such an increase in foliated cortex is observed, the cortical ribbon is nearly normal and, unlike the reeler cortex, shows that the production of reelin is largely sufficient. These observations indicate that the production of a gyrated cortex does not require extensive genetic modifications and could have evolved in any phylum, for example, by acquisition of more precursors of radial units in VZs.

As no living species are closely related to stem mammals, the hypothesis proposed above will always remain somewhat speculative. However, it predicts that, in a given lineage, the density of reelin-positive cells (per cortical surface area) should be higher in the embryonic brain of representatives with a smooth cortex than in those with a gyrated cortex. The mean density of reelin-positive cells, averaged over large areas, including gyral crowns and sulci, could be compared in embryonic cortices of rodents versus animals that are thought to have evolved little, but have some cortical gyration, such as hedgehogs or spiny anteaters. Potentially the best possible test would be to compare the density of CRc in the embryonic MZ of Echidna and Platypus.

All components of the reelin and other signaling cascades were probably present in stem amniotes, available as basic building blocks for cortical evolution. Why then did significant cortical foliation occur only in mammals? Foliation correlates nicely with cortical volume and may be required to increase it beyond some threshold, but how is it achieved? Surely the amplification of reelin production in MZ cells was not the sole limiting factor, as the examples above indicate, nor was the necessary increase in the number of radial cortical units. We would propose that increased reelin synthesis and the development of an enlarged number of precursors and radial cortical units were not hard to achieve, but that the resulting increase in cortical surface did not occur widely because it was difficult to master for some unknown reason. One difficulty could be the coordinate growth of mesodermal components, such as blood vessels and the cranial envelope that must accompany brain growth. Another problem that had to be solved in order to evolve a laminarly organized and tangentially widespread cortex is that of increased neuronal excitability and susceptibility to seizures. A consequence of the highly geometrical arrangement of radial cortical columns is that it facilitates modification of the membrane potential by field effects (ephaptic interactions), largely believed to be involved in the oscillations of electrocortical rhythms such as the alpha or theta rhythms. This quasicrystalline arrangement presumably has advantages in terms of computational power, but also comes at a price, as ephaptic excitation facilitates the tangential spreading of activity and decreases the threshold for aberrant epileptic discharges (McCormick and Contreras, 2001).

1.06.5 Conclusions

Despite the huge complexity of the question, our understanding of cortical evolution has progressed recently in parallel with our understanding of developmental mechanisms. Further advances can be made by careful comparative analyses of cortical development in different species, using simple techniques such as comparative genomics, immunohistochemistry, and *in situ* hybridization. Two conditions are requisite to make this possible, however. First, genomic sequences should be available for monotremes and at least one member of other amniote lineages (chelonians, crocodilians, sphenodon, squamates). Second, a significant multi-national effort should aim at providing access to high-quality embryonic material from representatives of these lineages.

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1.07 Evolutionary Neuroethology – A Case Study: Origins and Evolution of Novel Forms of Locomotion in Hippid Sand Crabs (Malacostraca, Decapoda, Anomala)

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Glossary

A6	Abdominal ganglion 6 (fusion of at least four embryonic neuromeres); innervates the sixth abdominal segment and tailfan.
arthrodial membrane	Tough flexible cuticle occurring between skeletal elements that allows relative movement.
AT	Anterior telson muscle; an ancestral, malacostracan muscle absent in Anomala.
ATU muscle backfilling	Anterior telson–uropod muscle. Technique of staining individual neurons from their cut axons, revealing their entire structure.
electromyograms (EMGs)	Recording of the electrical activity of a muscle by placing extracellular electrodes on or in the muscle.
extensor muscles	Muscles used to straighten the abdomen after bending; straighten out limb.
flexor muscles	Muscles that decrease the angle between two segments of the body or a limb, e.g., to bend the abdomen as during a tailflip; draw a limb toward the body.
interneuron	Neuron that is neither purely sensory nor motor.
LG	Lateral giant interneurons; bilaterally paired chain of command neurons (one pair/segmental ganglion) for one form of tailflip.
LTU muscle	Lateral telson–uropod muscle.
MG	Medial giant interneurons; bilateral pair of command neurons for one form of tailflip.
MoG	Motor giant, a particularly large and specialized fast flexor motoneuron.
muscle pioneer	Mesodermal muscle founder cell hat establishes attachment points, therefore, axis of mature muscle.

neuroethology	The study of the neurobiological basis of unrestrained behavior, as performed in natural habitat.
neuromere	Subdivision in developing CNS; in arthropods, generally one neuromere/segment.
nongiant tailflipping	Repetitive tailflips produced by nongiant circuitry (not involving LG or MG).
NSR	Nonspiking stretch receptor neuron(s); transmit(s) afferent signal as graded depolarization.
propodite	Proximal segment of uropod that is attached to the sixth segment.
PS	Uropod power-stroke muscle, in Hippidae; homologue of PTU muscle in other species.
PTF muscle	Posterior telson flexor muscle; converted to uropod power stroke synergist (insertion moved to uropod) in hippid crabs.
PTU	Posterior telson–uropod muscle.
RS	Uropod return-stroke muscle; new muscle in telson of sand crabs (Albuneidae, Hippidae).
tailfan	Three-component structure (telson flanked by uropods) at posterior end of malacostracan body.
tailflip	Rapid, ventral flexion of abdomen; generates propulsive force for escape or backward swimming.
telson	Terminal appendix posterior to the sixth (terminal) abdominal segment, caudomedian element of tailfan; not a true segment.
telson–uropod muscle	The only ancestral musculature directly linking the telson and uropod propodite.
TUSR	Telson–uropod stretch receptor.
uropod	Paired appendages of sixth abdominal segment; form tailfan together with the median telson.
VTF	Ventral telson flexor muscle.

1.07.1 Background and Introduction

1.07.1.1 Why Evolutionary Neuroethology?

The question of how nervous systems and behavior evolve is a core issue in both neurobiology and evolutionary biology. How can something as extraordinarily complex as the neuronal networks underlying behavior (neurobehavioral networks) tolerate change without compromising function? The nervous systems, including the brains, of species within large taxonomic groups look remarkably similar while often producing strikingly different behaviors. Biologists, at least since Darwin, have puzzled over this conundrum of conserving neural architecture yet diversifying behavior during evolution. Current research using molecular techniques to analyze the development of nervous systems has made remarkable progress in exposing how the conserved genetic tool kit allows the generation of novel features. But are the principles derived from comparative genetic-developmental analyses sufficient to explain how behavior evolves under natural selection? "It is one thing to know the laws of Nature, and quite another to know the outcomes of those laws" (Barrow, 1998, p. 66). Evolutionary neuroethologists ask the question: How do nervous systems really evolve? In this article, I reconstruct the pedigrees of the two unusual and surprising modes of locomotion displayed by all species in one decapod crustacean family. Some aspects of the gross anatomy of these animals have diverged so much from the corresponding parts in their close relatives that at first glance they appear unrelated. Why is it important to find out how individual nervous systems have evolved? An ultimate goal of basic neuroscience research is to understand the rationale for the functional organization of neural circuits, including those in the brain. These extraordinarily complex and species-specific networks are not what an engineer would design to execute the appropriately adaptive movements or to register only relevant physical characteristics of each species' environment because they are compromises between inheritance and natural selection. Thus, neither the nervous system nor the behavior of any species is fully understandable without knowing both the neural mechanisms producing its behaviors in its natural environment (neuroethology) and the ancestry of the neurobehavioral circuitry. Motor mechanisms are fundamental to all aspects of behavior, because movements present sensory structures to changing conditions, which leads to active as well as passive sensation. Furthermore, mutations are likely to be more serious (detrimental to survival) in motor control networks, particularly for locomotion, than in sensory systems, because sensory modalities are generally used in parallel to acquire information about the outside world. From this, it is expected that neural evolution would be more conservative in motor control than in sensory systems and, therefore, result in greater conservation of ancestral organization in motor than in sensory centers in the central nervous system of any group of animals (see Relevance of Understanding Brain Evolution, A Tale of Two CPGs: Phylogenetically Polymorphic Networks).

1.07.1.1.1 Prerequisites for tracing the evolutionary history of neurobehavioral circuitry To reconstruct the evolution of a species' functional neural networks requires that four criteria be met:

1. adequately known phylogeny to allow neural differences among species to be mapped in the sequence of their appearance during speciation;
2. experimentally accessible nervous systems (presence of individually identifiable neurons in species exhibiting distinctively different behaviors that are amenable to detailed analysis by kinematic and physiological methods);
3. surrogates for ancestral systems of interest in modern species; and
4. insight into the selective forces that shaped the evolutionary changes in the nervous system under study.

1.07.1.1.2 Hippid sand crabs satisfy the prerequisites for evolutionary neurobehavioral analysis Hippid sand crabs meet the criteria outlined above. Although there are controversies about some relationships within the Decapoda, they are peripheral to the discussion of the lineage of sand crabs. Sand crabs and their close relatives are amenable to investigation by the full spectrum of experimental, anatomical, and physiological techniques (Paul, 1991, 2003). Their one drawback as subjects for evolutionary neurobiological research is the extended time of their embryonic and larval development (Johnson and Lewis, 1942; Harvey, 1993), which severely limits their potential as subjects in genetic and developmental analyses. On the other hand, a fortuitous fact in the history of neuroscience is that crayfish have long been favorite experimental subjects (Huxley, 1880; Edwards *et al.*, 1999) and have retained key traits not only of ancestral decapod skeletomusculature, but also neuroanatomical and behavioral traits that can be traced back to the earliest Malacostraca (Figures 1c and 2; Sections 1.07.4 and 1.07.5). The motor systems in crayfish are thus suitable surrogates for the ancestral motor systems from which sand crab neurobehavioral networks would have evolved.

1.07.1.2 Neurobehavioral Evolution

Novelties in evolution – such as invasion of new niches or evolution of new locomotor behaviors (which may or may not appear conjointly with the former) – are often associated with loss, reduction, or alteration of ancestral structures rather than evolution of new structures. Structural changes in a neural network, i.e., additions or deletions of neurons and changes in synaptic wiring, correlated with behavioral evolution are difficult to identify. Evolutionary losses of identified neurons have been documented in taxa whose behaviors clearly differ from those of their ancestors (Wilson and Paul, 1987; Antonsen and Paul, 2001; Paul, 2003; Faulkes, 2004), but whether they caused, resulted from, or were incidental to the behavioral change in the lineages in question is not easily recognized. The appearance of new types of neurons in a neurobehavioral network is, on the other hand, likely to be directly linked to behavioral evolution and, when identified, offers unique opportunities to question how nervous systems evolve. The rarity of examples of new neurons may reflect the fact that they are recognizable only by their absence from the homologous (similar to presumed antecedent) neurobehavioral circuit, i.e., appropriate comparative data must be available. The larger the nervous system, the more difficult it is to identify unique, functional contributions of particular neurons in individual species, let alone in related species with divergent behavior, and success in this regard has been greatest in the analysis of neurobehavioral networks in arthropods and mollusks (Katz and Harris-Warrick, 1999; Katz *et al.*, 2001).

1.07.1.3 The Basic Plan of the Decapod Central Nervous System

The basic plan of the decapod body and central nervous system was established with the appearance of Malacostraca in the Paleozoic. Of particular interest here is the architecture of the last, sixth, abdominal ganglion (A6), because it innervates the tailfan, a defining character of the class Malacostraca (Hessler, 1983). This ganglion is more complex than the anterior segmental ganglia, because it is evolutionarily and ontogenetically the fusion of as many as four neuromeres (Dumont and Wine, 1987; Scholtz, 1995; Harzsch, 2003). Nevertheless, homologous elements in A6 of different species, as well as in A6 and ganglia of anterior segments, can be inferred by their positions relative to recognizable landmarks (Dumont and Wine, 1987; Strausfeld, 1998; Harzsch and Waloszek, 2000; Mulloney *et al.*, 2003). Condensation of neural centers has been a recurrent theme in the evolution of both invertebrates and vertebrates (see Aggression in Invertebrates: The Emergence and Nature of Agonistic Behavioral Patterns). Because the complexity of the malacostracan A6 is intermediate between that of single neuromeres and that of the larger subesophageal ganglion or the brain, it is potentially informative for comparative research into the ways neural architecture is affected by condensation of neural centers as their functions adapt during morphological and

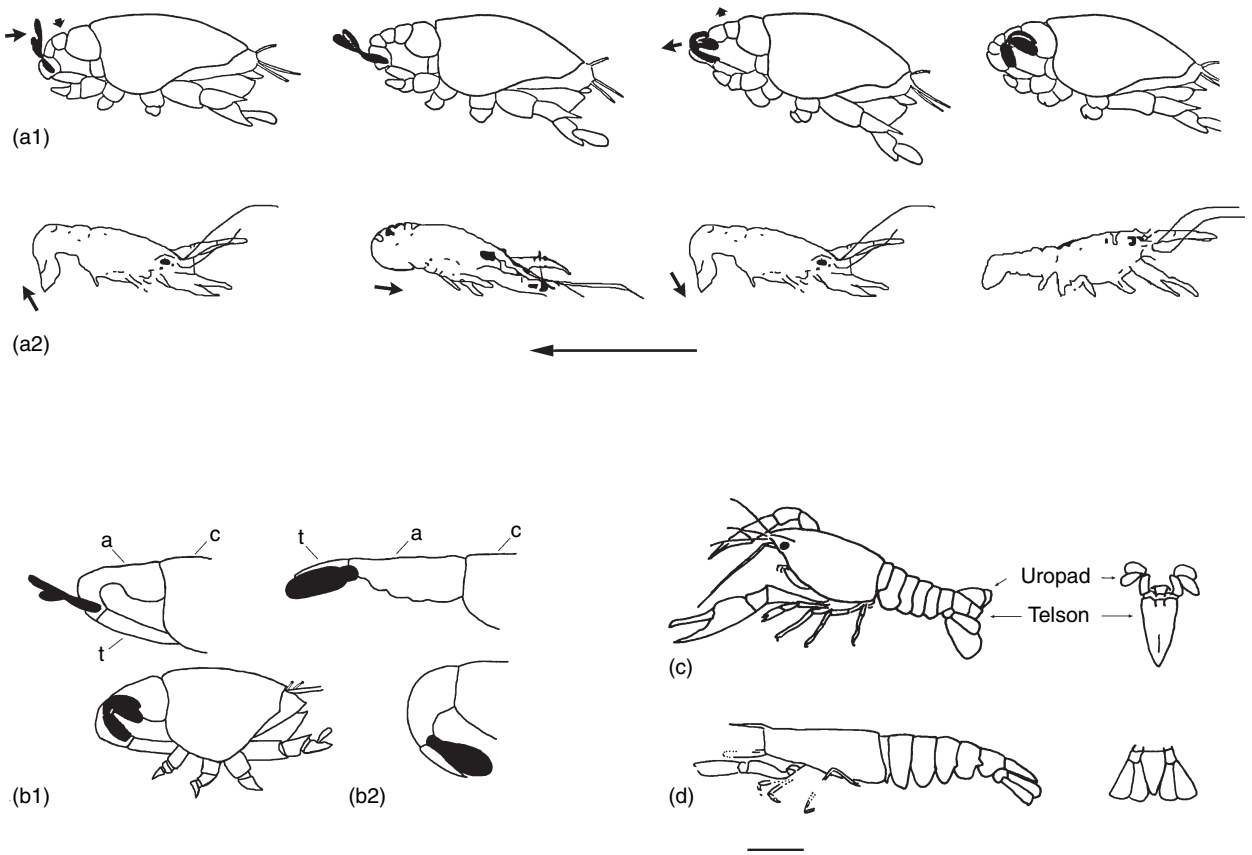

Figure 1 Comparison of swimming with the uropods and tailflipping. Direction of movement shown by long arrow. Tracings of single movie frames of (a1) a hippid crab, *Emerita analoga*, swimming by beating the uropods while keeping the telson flexed under the body and (a2) a tailflipping crayfish. Frames are selected to show form of movements and are not consecutive. Starting from rest position (right frame), hippids extend the uropods rearward (middle two frames) during the return stroke, then rapidly sweep them forward during the power stroke (left frame). When swimming hard (high frequency, large-amplitude uropod strokes), a slight extension of the anterior abdominal segments accompanies the return stroke and is reversed during the power stroke (short arrows over abdomen). Tailflip swimming (a2) begins with an extension of the abdomen (right frame; always evident in electromyograms, although its amplitude depends on the starting posture of the abdomen) prior to the powerful ventral flexion (the power stroke) of the abdomen, which carries the tailfan with it; followed by re-extension (left frame). b, The near immobility of the abdomen and telson during the large ark of the uropod stroke in hippids (b1) contrasts with the large excursion of the abdominal segments (extension–flexion) in crayfish (b2). In both behaviors, the blades of the uropod flare open just prior to the onset of the power stroke and increase surface area and hence thrust is generated. c, The uropods of sand crabs flank the abdomen (right, dorsal view of telson and uropods of *E. analoga*) rather than the telson as in crayfish, left. d, The external form, particularly of the abdomen and tailfan, of this decapod fossil from the Devonian resembles that of crayfish and other long-bodied, extant decapods. Scale bar (in d): a1, ~1 cm; a2, 3–4 cm; d, 1 cm. a1, Adapted from Paul, D. H. 1971. Swimming behaviour of the sand crab, *Emerita analoga* (Crustacea, Anomura). I: Analysis of the uropod stroke. *Z. Vergl. Physiol.* 75, 233–258. a2, Adapted from Krasne, F. B. and Wine, J. J. 1987. Evasion responses of the crayfish. In: Aims and Methods in Neuroethology (*ed.* D. M. Guthrie), pp. 10–45. Manchester University Press. d, Adapted from Schram, F. R., Feldman, R., Copeland, M. J. 1978. The late Devonian Palaeopalaemonidae and the earliest decapod crustaceans. *J. Paleontol.* 52, 1375–1387.

behavioral evolution (Paul and Macmillan, 1997; Strausfeld, 1998; Loesel *et al.*, 2002; Mulloney *et al.*, 2003).

1.07.2 Locomotion in Hippid Sand Crabs

Comparing motor patterns, muscles, and neurons in members of selected decapods has revealed that the evolution of sand crabs' novel behaviors occurred in stages and involved deleting from, adding to, and messing around with ancient neurobehavioral circuitry. Loss of certain highly specialized neurons appears to have removed constraints against modifying neuromusculature and behavior and precipitated the evolution of the Anomala by allowing body form and locomotion to change (see Section 1.07.3).

1.07.2.1 The New and Ancestral Modes of Swimming

The swimming and digging behaviors of sand crabs differ between the two families (Hippidae and Albuneidae), and both forms of locomotion are

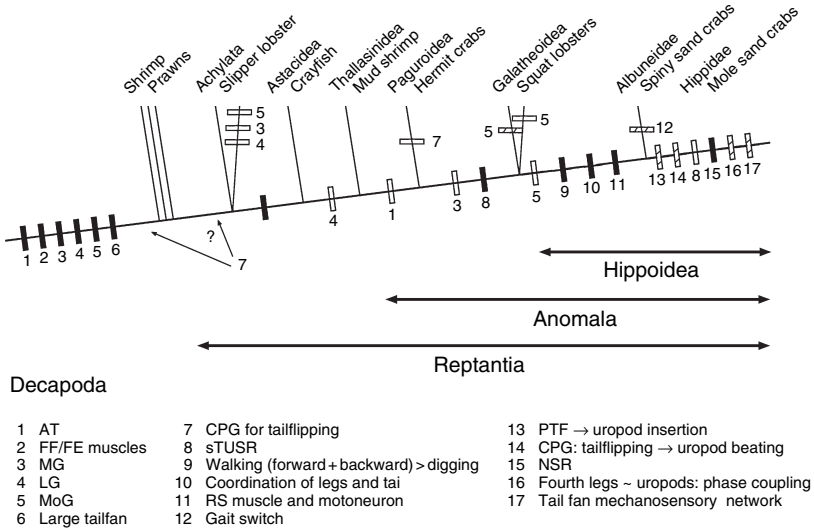

Figure 2 Neurobehavioral traits discussed in this article mapped on a partial phylogeny of decapod crustaceans; most prominent exclusion are the Brachyura, true crabs (based primarily on Scholtz and Richter, 1995; see also Martin and Davis, 2001). The organization of the tailfan musculature is the same in shrimp and prawns and members of the first three divisions of the Reptantia (except unknown for slipper lobster), but has been modified in different ways within the Anomala (also referred to as Anomura, which originally included the Thallasinidea). Character 7 (the existence of a central pattern generator (CPG) for tailflipping = nongiant tailflipping) has been demonstrated only in crayfish (Reichert *et al.*, 1981), but is generally assumed to be present in other Astacidea, all Achylata, and shrimp and prawns, and perhaps Thallasinidea. Since slipper lobsters tailflip without the giant interneurons (3, 4), either 7 appeared prior to the Achylata (and perhaps is a decapod synapomorphy) or nongiant tailflipping evolved independently in slipper lobsters. Data from Paul *et al.* (1985), Wilson and Paul (1987); Faulkes and Paul (1997a, 1997b, 1998); Paul (1981a, 1981b, 2004), Paul and Wilson (1994), and Faulkes (2004).

unlike those of any other crustaceans. Most remarkable is hippid crabs' use of the uropods to rapidly propel themselves backward (Figures 1 and 3). These animals keep their abdomen and elongate telson flexed beneath the body at all times and rapidly beat their relatively long uropods through a large arc to swim and, in conjunction with the legs, to dig into sand (Paul *et al.*, 2002). The large-amplitude movements of the uropods, independent of the abdomen and telson, contrast with the tight coupling of the uropods with axial (abdomen–telson) movements in crayfish and other species with tailfans, including albuneid sand crabs (Figure 1b).

1.07.2.2 The Hypothesis

Swimming by uropod beating almost certainly evolved from swimming by repetitive tailflipping (Paul, 1991). Three partially separate neural mechanisms are known to mediate the tailflip behaviors of crayfish. Two involve paired giant interneurons (the medial giants, MGs, and lateral giants, LGs), which mediate different forms of powerful ventral flexions of the abdomen and tailfan. The third, known as nongiant tailflipping because neither MG nor LG are involved, mediates repetitive extensions and flexions of the abdomen for rapid backward swimming (Reichert *et al.*, 1981). The giant interneurons were lost sequentially in the lineage leading to sand crabs (Figure 2), and it was the nongiant circuitry for tailflipping from which hippids' mode of swimming with the uropods is thought to have evolved. The digging behaviors of sand crabs are also unique and not identical in the two families (Hippidae and Albuneidae). They are summarized in the next section.

1.07.2.3 Digging Preceded Uropod Beating: The Mosaic Ancestry of Sand Crab Digging Behaviors

Three pairs of legs (legs 2–4, innervated by thoracic neuromeres 5–7) that other decapods use for walking have been so modified in sand crabs that they are unable to walk across the surface (Faulkes and Paul, 1997a, 1997b, 1998; Paul *et al.*, 2002). Analyses of electromyograms (muscle activity) in individual muscles for each leg joint while sand crabs were digging provided descriptions of the temporal patterns of the motor output to each leg, as well as the coordination between limbs of different body segments. From this, it became apparent that digging is a mosaic of disparate behaviors that are mutually incompatible in sand crabs' walking relatives: walking forward, walking backward, and tailflipping (Figure 4; Paul *et al.*, 2002). The fundamental similarities between the ways all sand crabs dig indicate that digging evolved in their common ancestor and later diverged in certain details following separation

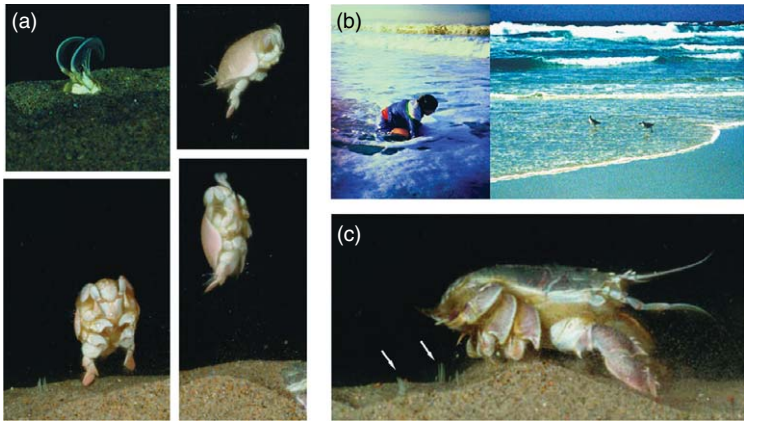

Figure 3 Sand crabs at home in aquariums and their natural habitat. a, Mole sand crabs (Hippidae; shown here *Emerita analoga*) filter-feed by extending their long, plumose second antennas above the sand (top left). The other three panels show an individual emerging from sand (bottom left), swimming (top right), and treading water, i.e., beating the uropods to keep itself suspended, but not moving relative to the substrate. To stay positioned at the appropriate level for feeding during the downwash from breaking waves, which shifts with the tide, hippid crabs emerge from the sand periodically to swim with the current (up or down the beach, depending on the direction of the tide), and rapidly dig in again before being carried too far up or down the beach. b, Hippid behaviors are superbly adapted for an active life in the turbulent physical environment of intertidal sandy beaches (Dugan *et al.*, 2000), which makes them easy to collect by hand when they are close to the sand's surface (left; see (a)); shore birds are major predators of hippid crabs (right). c, A spiny sand crab, *Blepharipoda occidentalis* (Albuneidae) moving across two *E. analoga* buried just beneath the surface with their first antennas protruding (white arrows). Carapace length of *E. analoga* = 2.0–2.5 cm and of *B. occidentalis* ~7 cm.

of Albuneidae and Hippidae (Faulkes and Paul, 1997b). The members of these families live in dissimilar niches and require different locomotor capabilities. Albuneids are subtidal and intertidal scavengers that remain buried beneath the surface and are weak swimmers. Hippids, in contrast, are intertidal filter feeders that must maintain themselves in the swash zone as the tide shifts, and to do so emerge periodically from the sand to swim up or down the beach before digging in again to feed (see Figure 3a). Their prowess in both digging through sand and swimming or treading water (Section 1.07.5.2.2) is the *sine qua non* of their existence. The abilities of hippids to disengage leg (digging) movements during swimming and to couple rhythmic movements of the digging legs with the uropods during digging presumably evolved conjointly with their peculiar mode of swimming with the uropods. Furthermore, the phase separation between their fourth legs and uropods, which cycle together, is complex and apparently critical for their ability to maintain their position on surf-swept sandy beaches (Figure 3b; Paul *et al.*, 2002). The power strokes of the left and right fourth legs and uropods remain evenly spaced as frequency changes because the phase relationship between the fourth legs shifts from bilateral synchrony to one-third out of phase as overall frequency drops during the course of a dig (Figure 4b; Faulkes and Paul, 1997a). This pattern ensures continuity in the collective force generated by the fourth legs and uropods. As their force vectors are slanted with respect to the long axis of the body (see tilt of fourth leg and uropod tip trajectories in Figure 4), this coordination maintains the rear-down slant of the body while the powerful digging legs 2 and 3 provide the propulsive force for submerging the animal beneath the sand's surface. The coupling (and uncoupling) of the hind digging legs with the uropods accounts for hippid sand crabs' seamless transitions between two such different media as water and sand. The different coupling patterns between limb segments displayed in sand crabs (Figure 4) exemplifies well the inherent functional and evolutionary flexibility of modularity in the organization of neuromotor networks (Paul *et al.*, 2002) and the developmental mechanisms that give rise to them (Section 1.07.5).

1.07.3 Neurobehavioral Mechanisms and Biomechanics are Inseparable

Tailfan skeleton musculature, tailflip behaviors, and MG and LG neurons are interrelated. Before plunging into the analysis of any central nervous system, the machinery that the particular nervous system operates must be considered. For example, cephalopod mollusks have excellent visual capabilities and manipulative skills, as do humans, but our brain in an octopus would be as helpless in controlling

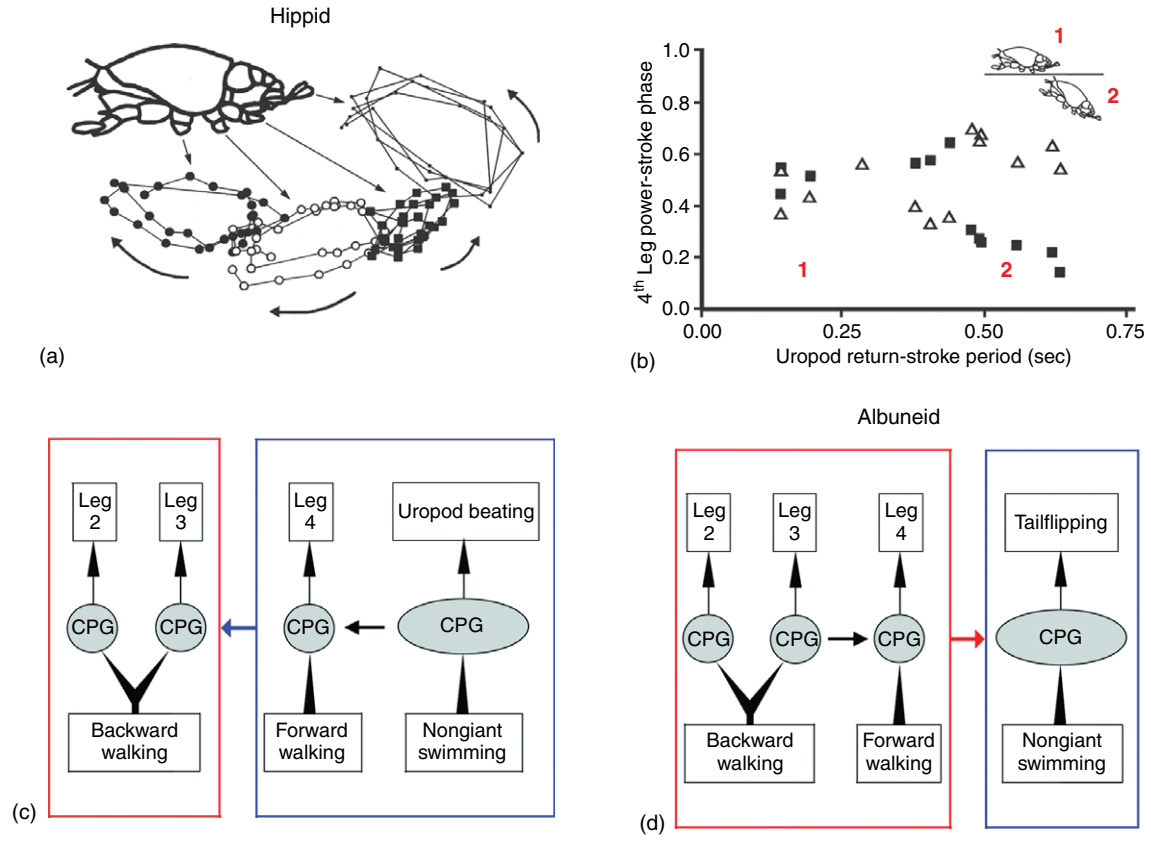

Figure 4 The mosaic ancestry of sand crab digging behaviors. Limb movements of hippids (a, c) and albuneids (d). The movements and motor patterns of the second and third digging legs resemble those used by other decapods to walk backward, while those of the fourth legs resemble patterns for forward walking in other species. a, Hippid uropods cycle in the same, bilaterally synchronous pattern during swimming and digging, but during digging the three pairs of digging legs also move rhythmically. The fourth legs cycle in the same direction as the uropods, whereas the second and third pairs cycle in the opposite direction (curved arrows). b, The coordination between the cycling of left (solid box) and right (open triangle) fourth legs with respect to the uropod period changes during the course of a dig according to the frequency of uropod beating (data are onset of electromyograms bursts recorded from unrestrained animals). The fourth legs move in unison and in antiphase to the uropods at the beginning of a dig (time 1), and drift apart when overall frequency drops until they are cycling about one-third of a cycle out of phase with each other and the uropods (time 2). Inset: position at start of dig, above (1) and when submerged below (2) surface of sand (horizontal line). c and d, Summary diagram of the neural organization of digging in hippid (c) and albuneid (d) sand crabs. The evolutionary change in coordination between limb CPGs (central pattern generators) was greater than in the CPGs themselves (see character 12 in Figure 2). Adapted from Faulkes and Paul (1997a, 1997b, 1998); see also Paul *et al.* (2002).

tentacle movements as octopus motor circuitry would be in controlling our hands and feet. What skeletal–muscular requirements must be met for a decapod crustacean to be able to swim with the uropods?

1.07.3.1 Hinged versus Single-Pivot Joints

Two basic requirements for generating propulsive force with any limb that functions as a lever are a basal joint that allows limb movement through a sufficiently large arc and musculature capable of generating powerful movement of the limb through its full trajectory while providing stabilization against counter forces. Arthropod joints are typically double hinged, with movement restricted to one plane. The uropod has just one articular point with abdominal segment 6, but in most decapods this joint is so deeply recessed between the telson and the sixth segment that elevation of the propodite (the basal segment of the uropod) above the horizontal plane is impossible and protraction and retraction in the horizontal plane are severely restricted. The joint does, however, allow uropod depression, which results in cupping of the tailfan (Figure 1b2). Hippids, by contrast, have evolved a 'socket-and-ball' uropod joint with the sixth segment that is biomechanically analogous to a vertebrate ball-and-socket joint (Figure 5d; Paul *et al.*, 1985). This allows the uropod propodite to be swung through large arcs nearly comparable to the range of motion of the human shoulder joint. Hippids swim exclusively with the equivalent of a human swimmer's backstroke.

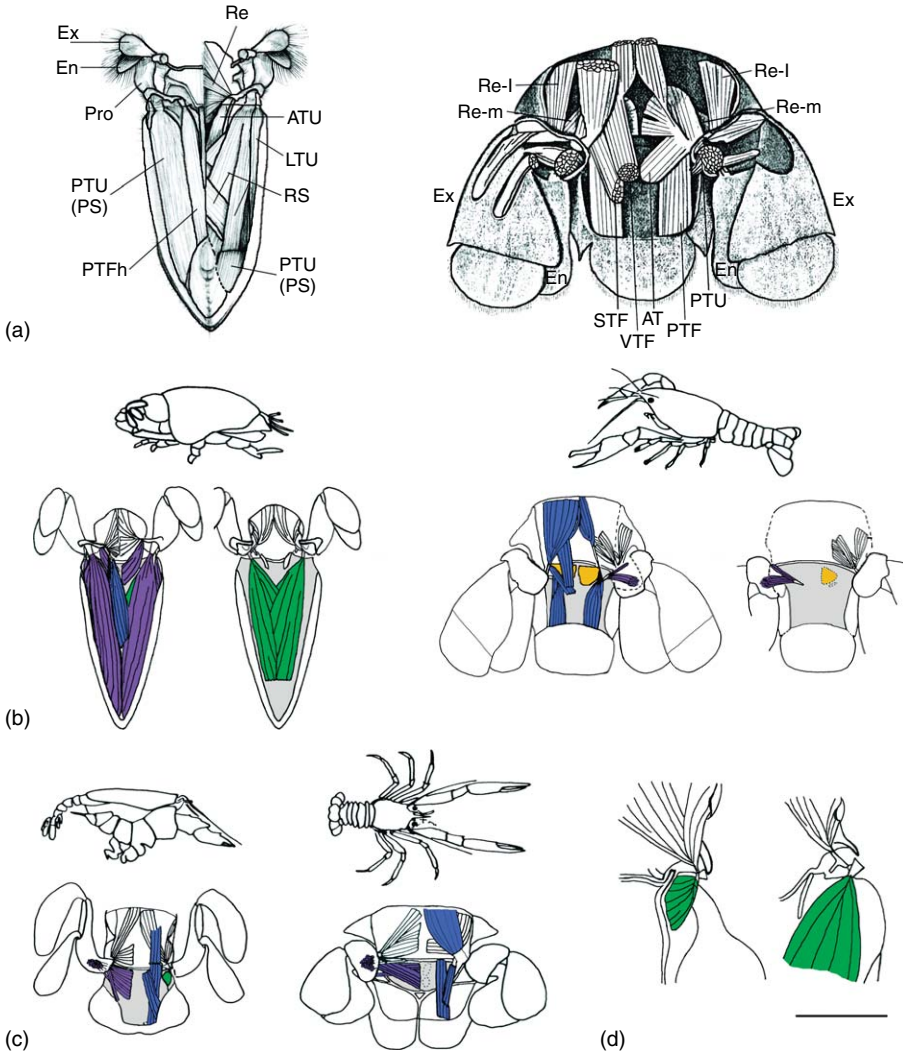

Figure 5 The divergence of telson musculature in Hippidae from that typical of the decapod telson is so extreme that no specific homologies can be suggested by visual inspection alone; analysis of the innervation is required. a, Ventral views of the tailfans of *E. analoga* (left) and crayfish (right) show the most ventral muscles in place on the left and removed on the right. Note that all of the telson muscles in *E. analoga* arise from broad areas of the inner dorsal telson and insert directly on the uropod propodite via discrete tendons. This is in marked contrast to crayfish, where nearly all of the muscles in the telson are the terminal elements of the series of abdominal fast flexor muscles, and all insert on or are linked to the flexor tendon in the posterior–lateral abdominal segment 6, which, in turn, is linked to the ventral side of the uropod. The fibers of the trio of relatively small telson–uropod muscles in crayfish (only PTU labeled) insert directly, without tendons, over the proximal ventral surface of the propodite, but are attached to the dorsal–anterior telson via a slender tendon, which is bound to the flexor tendon by connective tissue. b and c, The modifications of conserved telson muscles (blue), loss of an ancestral muscle (orange), and appearance of a new muscle (green) in the anomalen species were discovered by inspection of the tailfans of two anomalen species that continue to tailflip, *B. occidentalis* and *M. quadrispina* (Paul *et al.,* 1985; Paul, 1991; Wilson and Paul, 1987). The ventral (blue) muscles are removed from one side to expose the deeper/more dorsal muscles. b, *E. analoga* (Hippidae, left) compared with crayfish (right; the inset shows a more dissected view to reveal the three telson–uropod muscles attached to the dorsal telson by a single tendon, left side; the area of attachment of AT muscle fibers (orange oval), right side. c, *B. occidentalis* (Albuneidae, left), *M. quadrispina* (Galatheidae, middle). Muscle homologies were surmised by examination of their positions relative to each other and skeletal landmarks and confirmed by the similarities in central positions and morphologies of the motoneurons innervating them (see Figure 7). d, Detail of the dorsal insertion on the uropod of the uropod RS muscle in the albuneid *B. occidentalis* (left) and hippid *E. analoga* (right); note that it is adjacent to the tendon of the uropod remoter muscle, which arises in the sixth segment, and is similarly positioned in crayfish (shown in a). AT, anterior telson muscle; En, uropod endopodite; Ex, uropod exopodite; Pro, uropod propodite; PTF, posterior telson flexor muscle; PTU, posterior telson–uropod muscle; Re-l, lateral uropod remoter muscle; Re-m, medial uropod remoter muscle; STF, slow telson flexor muscle (present in all species, function unclear); VTF, ventral telson flexor muscle. a (right), Adapted from Schmidt, W. 1915. Die Musculatur von *Astacus fluviatilis*: Ein Beitrag zur Morphologie der Decapoden. *Z. Wiss. Zool.* 113, 165–251. b–d, Adapted from Paul, D. H. 1981b. Homologies between neuromuscular systems serving different functions in two decapods of different families. *J. Exp. Biol.* 94, 169–187; Paul, D. H., Then, A. M., and Magnuson, D. S. 1985. Evolution of the telson neuromusculature in decapod Crustacea. *Biol. Bull.* 168, 106–124.

1.07.3.2 Neuromuscular Repercussions of Increasing a Joint's Freedom of Movement

The evolution of musculature capable of generating controlled, forceful movements around such a flexible joint, while simultaneously stabilizing it against counter forces as the uropod is swept through its full trajectory, is no mean feat. The divergence of proximal tailfan musculature in hippids from that of typical decapod tailfans is so extreme that visual inspection alone gives no clues about muscle homologies. In fact, the one apparent exception turned out to be a false lead: the strikingly similar muscles in the anterior telson of crayfish and hippids, which also have approximately equivalent actions on the uropod, are not homologues, as was made evident by analysis of their innervations (Figures 5–7; Paul *et al.*, 1985). Comparison of the motoneurons showed that the muscle in crayfish (the anterior telson (AT) muscle) had been lost in the sand crab lineage long before the advent of Hippidae, and was accompanied (or followed) by expansion of a smaller muscle (the anterior telson–uropod (ATU) muscle) into the vacated space in the anterior telson. The modifications of conserved telson muscles (e.g., the ATU muscle), the loss of an ancestral muscle (the AT muscle), and the appearance of a new muscle (below) in the hippid lineage are illustrated in Figure 5 and summarized in Figure 6. Two obvious differences distinguish the tailfan muscles of hippid sand crabs and prototypical tailflipping species, such as crayfish. One is the massive uropod return-stroke muscle (RS muscle, Figures 5 and 6), which is absent from crayfish and allies. This muscle is responsible for swinging the uropod rearward preceding each power stroke, which corresponds to the extension phase in swimming by repetitive tailflipping and is equivalent to the upstroke of a wing beat in flying species. Without this muscle, swimming by uropod beating could not have evolved, for no conceivable morphological changes could have produced its biomechanical equivalent from ancestral telson muscles. The second obvious difference is the pair of ventral muscles that insert on the ventral side of the uropod propodite and are responsible for the uropod power stroke. The large uropod power-stroke muscle (PS ≡ homologue of crayfish's ATU

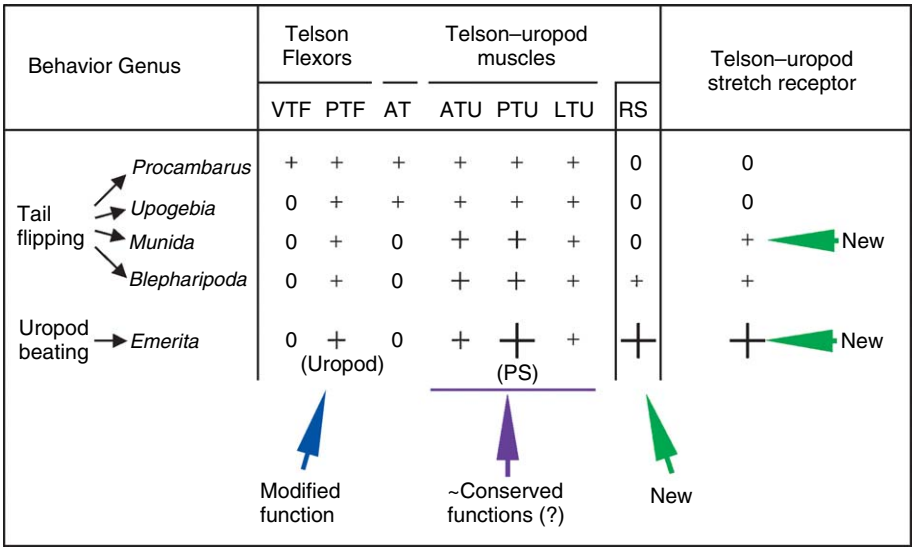

Figure 6 Summary of the muscles and stretch receptors in the telson of crayfish (*Procambarus*, Astacidea), mud shrimp (*Upogebia*, Thallasinidea), squat lobster (*Munida*, Galatheidae), albuneid sand crab (*Blepharipoda)*, and hippid sand crab (*Emerita*). In crayfish and mud shrimp, uropod movements independent of the telson are severely limited by the recessed joint, the linkages between the proximal uropod musculature and the musculature of the sixth abdominal segment and telson (see Figure 5b; Paul *et al.*, 1985; Paul, 1991), and the overlapping neural control of uropod and abdominal–telson movements (Larimer and Kennedy, 1969; Dumont and Wine, 1987). The sequential changes in the muscles that preceded the advent of hippid sand crabs' new mode of swimming with the uropods began with the shift of their combined action from axial flexion to uropod control when the AT muscle was lost and a new proprioceptor, which senses elevation of the uropod, appeared; these changes continued with the evolution of a muscle that inserts dorsally on the uropod (RS) in albuneid sand crabs, in which RS is too small to have any biomechanical action on the uropod. Finally, the shift in the insertion of the remaining axial flexor muscle (PTF) to the uropod and hypertrophy of the pair of antagonists, PTU and RS (see Figure 5), completely freed the hippid uropods from the axial neuromusculature. At the same time, a different telson–uropod stretch receptor replaced the original telson–uropod stretch receptor present in tailflipping anomalans. Muscles: AT, anterior telson; ATU, anterior telson–uropod; LTU, lateral telson–uropod; PTF, posterior telson flexor; PTU, posterior telson–uropod (PS, uropod power-stroke muscle in Hippidae); RS, uropod return stroke; VTF, ventral telson flexor. Data from Dumont and Wine (1987), Paul (1972, 1981b), Maitland *et al.* (1982), Paul *et al.* (1985), and Paul and Wilson (1994).

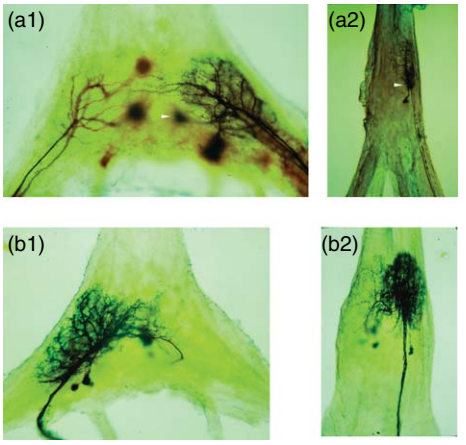

Figure 7 Similar central position and morphology of motoneurons provide evidence for muscle homologies. Photomicrographs of motoneurons in the sixth abdominal ganglion revealed in silver-intensified backfills from their axons. a1 (right side) The three uropod RS motoneurons in *E. analoga* (left side, the two uropod PS (PTU) motoneurons); a2, the RS motoneurons in *B. occidentalis* (white arrow to small medial soma in comparable relative position to its larger homologue in *E. analoga*). The intermingling of RS and uropod rotator motoneurons in sand crabs' sixth abdominal ganglion (not shown) and the similar positions and morphologies of the rotator motoneurons in sand crabs and crayfish may be evidence of the derivation of the RS neuromusculature from the sixth segment's uropod rotator neuromusculature (Paul, 1981b; Vidal Gadea *et al.*, 2003). b, The PTF motoneurons in A6 backfilled from the left side in *E. analoga* (b1) and the right side in *B. occidentalis* (b2); see Dumont and Wine, 1987 for their homologues in crayfish. Maximum width of ganglia: *E. analoga*, 1 mm; *B. occidentalis*, 0.35 mm.

muscle; Paul *et al.*, 1985) inserts directly opposite (ventral to the pivot point) the insertion of the RS muscle, whereas its smaller synergist (posterior telson flexor (PTF) homologue) inserts more medially. These muscles have homologues with different mechanical actions in crayfish (Figures 5 and 6, and Section 1.07.3.2.2 below).

Did the unique joint and constellation of muscular elements needed to swim with the uropods evolve together as a massive developmental transformation in the tailfan? Tracing backward the evolutionary history of hippids (Figure 2), starting with relatives most closely resembling hippids and moving to those more similar to crayfish in morphology and behavior, uncovers in reverse order the major evolutionary transitions on the way to the appearance of uropod beating. Comparative analysis of anomalan species that display less modified locomotion and tailfans than those of hippid crabs revealed that the uropod RS muscle is a new muscle that appeared prior to the split of the two sand crab families. Transformation of ancestral decapod neuromusculature began even earlier. I will briefly describe these events in reverse order of their occurrence.

1.07.3.2.1 New muscle, new motoneurons The RS muscle homologue in spiny sand crabs, which tailflip and retain telson flexor and telson–uropod muscles in their ancestral positions (see below), is so small and inconspicuous that it was overlooked until an anomalous tiny branch from the nerve innervating the uropod remoter muscles in the sixth abdominal segment was traced (Paul, 1981b). This tiny branch turns posterior into the telson to end on what appeared to be folds of arthrodial membrane in the anterolateral corner of the telson. These folds turned out to be a few short muscle fibers converging to an attachment on the dorsal arthrodial membrane of the uropod joint, a position corresponding precisely to that of the massive RS muscle of hippid crabs; this muscle has no counterpart in crayfish or other decapods (Figure 5). The hypothesis of homology of these vastly different-sized muscles is predicated on the assumption that a spiny sand crab's homologue of the RS muscle is innervated by three motoneurons having central positions and morphologies similar to those of the three RS motoneurons of mole crabs, and this proves to be the case. Although the RS motoneurons are vastly different in size, reflecting the size difference of the muscles innervated, their positions in the ganglion and the branching patterns of their neurites are very similar, despite the different overall shapes of this ganglion in the two sand crabs (Figure 7). The few, short muscle fibers of the albuneid RS muscle could have no mechanical action on the uropod, nor do they appear capable of even stiffening the joint. Their appearance in sand crabs that tailflip was presumably the result of an ontogenetic error in the common ancestor of hippids and albuneids that, while serving no function, had no detrimental effect and became fixed in the lineage and a preadaptation for uropod swimming.

1.07.3.2.2 Altered (and conserved) functions of conserved neuromusculature While the relative sizes of homologous muscles in mole crabs and spiny sand crabs differ, there is only one qualitative (functional) difference that distinguishes their tailfan neuromusculature. The insertion of the PTF muscle in hippids has been switched to the ventral–medial propodite, making it a limb muscle rather than the terminal member of the axial flexor musculature (Paul, 1981b; Paul *et al.*, 1985; Dumont and Wine, 1987). Thus, in contrast to the recent origin of the uropod RS muscle, the PS musculature turns out to have heterogeneous derivations from

separate components of ancestral tailfan muscles. Interestingly, the latter muscles assist in the PS phase (flexion) of tailflipping (Paul, 1981a, 1981b). That is, retained muscles that are PS synergists in tailflipping species became the PS muscles for uropod beating in Hippidae. This discovery was important, because it implies that qualitative changes in the central neural circuitry for swimming need not have been substantial in the transition from tailflipping to swimming with the uropods. In hippids, changes in the development of the tailfan brought together ancestrally disparate components that serve different mechanical actions in other species, but retained the ancestral association of PS (and recovery stroke, i.e., extension) neuromusculature. The conversion of decapod PTF muscle to a uropod PS synergist (Figures 5 and 6) would have removed the last constraint against uropod movement independent of the body axis, an essential step for evolution of hippids' mode of swimming with the uropods. Although recent molecular data have been interpreted to suggest a basal position of hippids within the Anomala (Haye *et al.*, 2002), the combined morphological, physiological, behavioral, and ecological data support the contrary view that hippids are more derived than albuneids, as shown in Figure 2. The small size of the RS muscle in albuneids, therefore, is not the result of reduction of a larger muscle, as would be implied by placement of Hippidae at the base of the Anomala. A basal position of the Hippidae in the Anomala would mean that several reversals in transformation of neuromuscular elements would have had to occur. However, spiking telson–uropod stretch receptors (TUSR) (see Section 1.07.4) could have arisen once, even if sister group status of Galatheidae and Albuneidae were supported.

1.07.3.3 Ontogeny and Evolution of Neuromusculature

The development of neuromusculature in arthropods starts with the muscle fiber pioneers, which are mesodermal cells that extend between attachment points of future muscles and, hence, determine the orientations and biomechanical actions of muscles (Ho *et al.*, 1983; Steffens *et al.*, 1995; Halpern, 1997). Later, the growth cones of motor axons exiting the central nervous system seek out the correct target muscle head or heads to innervate. The conversion of the axial PTF muscle to a limb muscle (PTFh in Figure 5a) described above probably arose by the wayward positioning of one or more muscle pioneers.

Muscles with multiple heads and new muscles are thought to originate by division of ancestrally unitary muscles, presumably by duplication of muscle pioneers (Ho *et al.* 1983; Halpern, 1997). One head can continue to serve the basic function, while the other(s) are less constrained functionally and may acquire new biomechanical and/or behavioral roles in response to selective pressure (Friel and Wainwright, 1997; Antonsen and Paul, 2000; Paul *et al.*, 2002). This process may have given rise to the uropod RS muscle in sand crabs: its dorsal position in the telson and insertion adjacent to the complex tendon of the uropod rotator musculature in abdominal segment 6 (Figure 5) suggest that the RS muscle might have evolved by the wayward positioning of a rotator muscle fiber pioneer to place its axial attachment within the telson. Originally a triply innervated single head, as in albuneid sand crabs, this new muscle would have subsequently divided (or duplicated) to produce the four heads of the large uropod RS muscle in hippid sand crabs (Figures 5 and 7).

1.07.3.4 Intermediates between Sand Crab and Crayfish Tailfans

Stepping further down the hippid phylogenetic tree brings us to the Galatheidae (squat lobsters) (Figure 2). Apart from the absence of the uropod RS muscle from galatheids, the tailfan neuromusculature of squat lobsters and albuneid sand crabs is very similar (Figures 5 and 6). Squat lobsters, however, walk on the surface rather than locomote through substrate. (The major neurobehavioral adaptations in control of thoracic appendages and intersegmental coordination that enable sand crabs to dig through sand are described in Section 1.07.2.3.) Did loss of an ancient tailfan muscle precipitate alterations in the decapod tailfan and evolution of Anomala?

Preceding the transformations described above (and others) was the demise of the only telson muscle that has no anterior segmental homologue in decapods or any malacostracan, the AT muscle described above (Dumont and Wine, 1987; Paul and Macmillan, 1997). The large area of the inner telson surface vacated was then filled by the spread of the ATU muscle fibers, which in species with the AT muscle attach to the dorsal telson via a slender tendon (Figure 5). The discovery that the similarly positioned and oriented anomalan ATU muscle and crayfish's AT muscle are not homologues (Figure 5) opened the way to solving the puzzle of how the telson musculatures in these two groups were related (Paul *et al.*, 1985). None of the anatomical

literature on hermit crabs (Paguroidea) illustrates a muscle in the appropriate position (although a modified AT muscle could perhaps have been useful in gripping the inner whorl of gastropod shells with the uropod). If the AT muscle is indeed absent from hermit crabs, then its loss might have triggered the evolutionary modifications of the tail for which the Anomala are famous.

1.07.4 Did Loss of an Ancient Tailfan Muscle Precipitate Alterations in the Decapod Tailfan and Origin of the Anomala?

1.07.4.1 Identifying Neural Homologies in Divergent Species

Neuroblasts generally do not migrate from their birthplace during the embryonic development of the central nervous system in arthropods (Harzsch, 2003). Neurons with somata in similar positions relative to other landmarks in different species are, therefore, likely to be homologous (Figure 5). Motoneurons are more easily identified functionally and described morphologically than interneurons, because of the ease of applying the backfilling technique. Homologous motoneurons in turn serve as landmarks for comparing other neurons, as well as architectural features such as axonal tracts, commissures, and neuropil areas (Figure 8; Paul, 1989, 1991; Strausfeld, 1998; Loesel *et al.*, 2002; Mulloney *et al.*, 2003). Conserved central neural architecture of the tailfan ganglion provides the backdrop against which the new RS motoneurons were discovered in hippids, and from which corresponding (homologous) motoneurons were postulated and found in albuneids (above). Their possible derivation is addressed in Section 1.07.3.3. The RS motoneurons were not the only new neurons essential for swimming with the uropods.

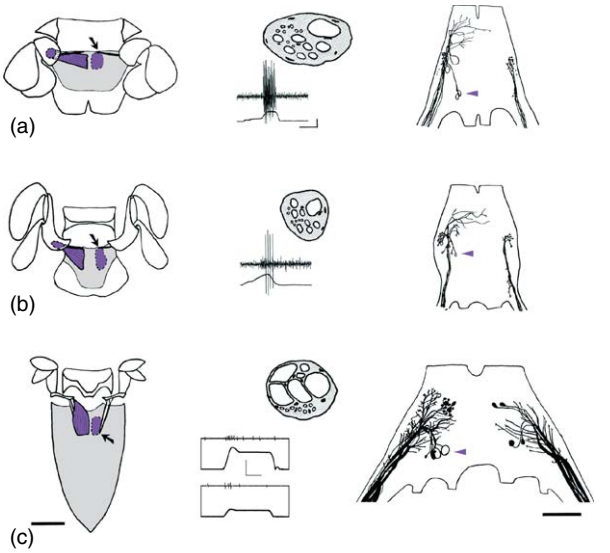

Figure 8 The spiking (a, b) and nonspiking (c) telson–uropod stretch receptors compared. Left column, ventral views of dissected tailfans to show position of the ATU muscle and the receptors in: a, *M. quadrispina* (Galatheidae); b, *B. occidentalis* (Albuneidae); and c, *E. analoga* (Hippidae). On the right side, the area of dorsal telson from which the ATU muscle fibers arise is shown; compare to position of AT muscle in crayfish (Figure 5b). Arrows point to dorsal attachment of receptor strand on inner surface of telson. Middle column, transverse sections of the receptor nerve midway between the receptor strand and the mixed nerve from the lateral telson which it joins (for nerve homologies, see Paul *et al.*, 1985). Sample recordings of the response to stretch of the receptor strand: extracellular receptor nerve recordings from *M. quadrispina* and *B. occidentalis*; intracellular recording from one of the four nonspiking sensory neurons (NSRs) of *E. analoga* (lower trace: stretch stimulus). Right column, camera lucida drawings of abdominal ganglion 6 with silver-intensified, CoCl-backfilled neurons from sensory nerves in the three species. On the right side, fills from close to the receptor strand show only the sensory neurons filled; on the left side, backfilled farther from the receptor, show a few other neurons, including the pair of ATU motoneurons with caudal somata easily identified in all species (purple arrow) also filled. Adapted from Paul, D. H. and Wilson, L. J. 1994. Replacement of an inherited stretch receptor by a newly evolved stretch receptor in hippid sand crabs. *J. Comp. Neurol.* 350, 150–160.

1.07.4.2 Joints with Wide Freedom of Movement Require Proprioceptors to Keep Them in Line

Sensory feedback from stretch receptors and other internal proprioceptors, which detect changed position or tension between body parts, is used to sustain strong adaptive movements during locomotion (Pearson and Ramirez, 1997; Hooper and DiCaprio, 2004). On basic principles, one would predict that any joint as flexible as that of hippids' uropod articulation with abdominal segment 6 would display one or more stretch receptors, and this is the case. Hippids posses a TUSR that is exquisitely sensitive to remotion and rotation movements of the uropod with respect to the body axis, i.e., the movement produced by the new RS muscle. Its sensory neurons are unusual both morphologically in having centrally located cell bodies and physiologically in that they are nonspiking (NSR, nonspiking stretch receptor). Nonspiking neurons transmit only graded potentials and are incapable of generating action potentials (Figure 8c; Paul and Bruner, 1999).

1.07.4.2.1 TUSRs evolved twice The discovery of TUSRs in albuneid sand crabs and squat lobsters in a roughly similar position as the nonspiking TUSR in hippids should not have been a surprise, because,

although these animals retain the ancestral behavior of tailflipping, their uropod articulation allows greater freedom of movement than that in crayfish (Paul *et al.*, 1985). Like the hippid TUSR, the albuneid and galatheid TUSRs arise from the dorsal telson and insert on the ventral–medial rim of the uropod propodite. In each of the three groups, the receptor flanks the major uropod depressor muscles, the anterior telson–uropodalis muscle (ATU), which is considerably larger in these Anomala than its homologue in crayfish (Figures 5 and 8). The hippid receptor strand, however, arises on the lateral side of the ATU, whereas the receptor strand in the two tailflipping anomalans arises adjacent to the medial–anterior face of this muscle. Also, the sensory neurons of the latter are spiking (generate action potentials).

No comparable proprioceptor monitoring movements of the basal joint of the uropod has been found in any macruran or paguroid. This suggests that the greater freedom of movement of the uropods relative to the rest of the tail in sand crabs and galatheids was not only accompanied by alterations in neuromusculature, as discussed above, but made desirable the evolution of a proprioceptor that could monitor whole limb movement and, therefore, mediate stabilizing reflexes in uropod motoneurons. In fact, all three TUSRs are ideally positioned to sense elevation of the propodite, and *in vitro* experiments show that they mediate similar stabilizing (negative feedback) reflexes (Paul and Wilson, 1994); *in vivo*, however, the roles of the TUSRs are more complex (see Section 1.07.5.2).

The most unusual features of all three TUSRs are the morphology of their sensory neurons (they are monopolar) and the central location of the sensory somata (within the sixth abdominal ganglion) (Figure 8; Maitland *et al.*, 1982; Paul and Wilson, 1994). This contrasts with typical arthropod mechanosensory neurons, including most proprioceptors, which are bipolar or multipolar and have peripherally located cell bodies. The TUSR sensory neurons are similar to those of stretch receptors associated with crayfish swimmerets and crayfish and brachyuran crab walking legs and all resemble motoneurons in general structure (Bush, 1976).

Given the phyletic relationships of these animals (Figure 2) and the comparative data on their neuromusculature (Figure 5; Paul *et al.*, 1985), it was reasonable to have assumed that spiking TUSRs were the precursor of the NSR in hippids. Why, then, are the hippid and the albuneid/galatheid receptors not homologues?

The clues are the different positions in the sixth abdominal ganglion of the sensory neuron somata and the orientations of the primary neurites relative to common landmarks, such as conserved (homologous) motoneurons and axon tracts in A6. The central features of the galatheid and albuneid spiking sensory neurons are almost identical, more similar even than their peripheral morphologies, but they are strikingly different from the central positions and morphology of the nonspiking sensory neurons, NSRs, in hippids (Figure 8, right column). These central differences stand out against the background of conserved ganglionic structure and lead to the conclusion that TUSRs evolved (at least) twice, once in the common ancestor of squat lobsters and sand crabs and once in hippids (Figures 2 and 6).

1.07.4.2.2 The extraordinary nonspiking TUSR of hippid sand crabs Why replace the spiking telson–uropod receptor that appeared in the common ancestor of sand crabs and squat lobsters with a nonspiking receptor across the same joint that mediates similar resistance reflexes (Figures 2 and 8)? The answer may be that the demise of the spiking receptor was a byproduct of the elongation of the telson in the hippid lineage (Figure 5). This shifted to longitudinal the orientation of the muscles, thereby giving them the mechanical leverage to execute large excursions of the uropods during swimming and digging, when both the load on the uropods and the variability of that load are likely to be greater. A more interesting question is why are the neurons of the hippid receptor nonspiking? What is the adaptive value of graded rather than action potential signaling in proprioceptors? Nonspiking cells are common in places where conduction distances are short; also, the speed of information transfer is higher than for action potential transmission (as in visual and auditory centers), but the former is untrue for the NSRs and the latter is an unlikely requirement for stretch receptors responding to whole limb movement. Combining neurobehavioral data on the motor system and what is known about how the NSRs mediate their diverse functions (next section) with data from the much better understood sensory and motor systems of crayfish suggests that these new neurons were wired into ancestral circuitry serving the tailfan. Nonspiking neurons are prominent in this and other neural networks (references in Paul, 2004). The evolution of the NSRs' physiological properties might have been determined by their being interconnected with nonspiking neurons in the central pattern generator (CPG) circuitry for the uropods; that is, proprioceptive feedback transmitted by graded-potential neurons could have been favored

for smoother integration by a network using graded potentials to perform its neurobehavioral functions (Paul and Bruner, 1999; Paul, 2004; see Section 1.07.5.2). Whatever its evolutionary cause, having nonspiking membranes may have been influential in the evolution of these sensory neurons' extraordinary peripheral morphology (Figure 9), which is thought to explain the unusual combination in single neurons of high sensitivity and ability to respond over the entire range of tension experienced by the receptor strand (Figure 9; Wilson and Paul, 1990; Paul and Wilson, 1994).

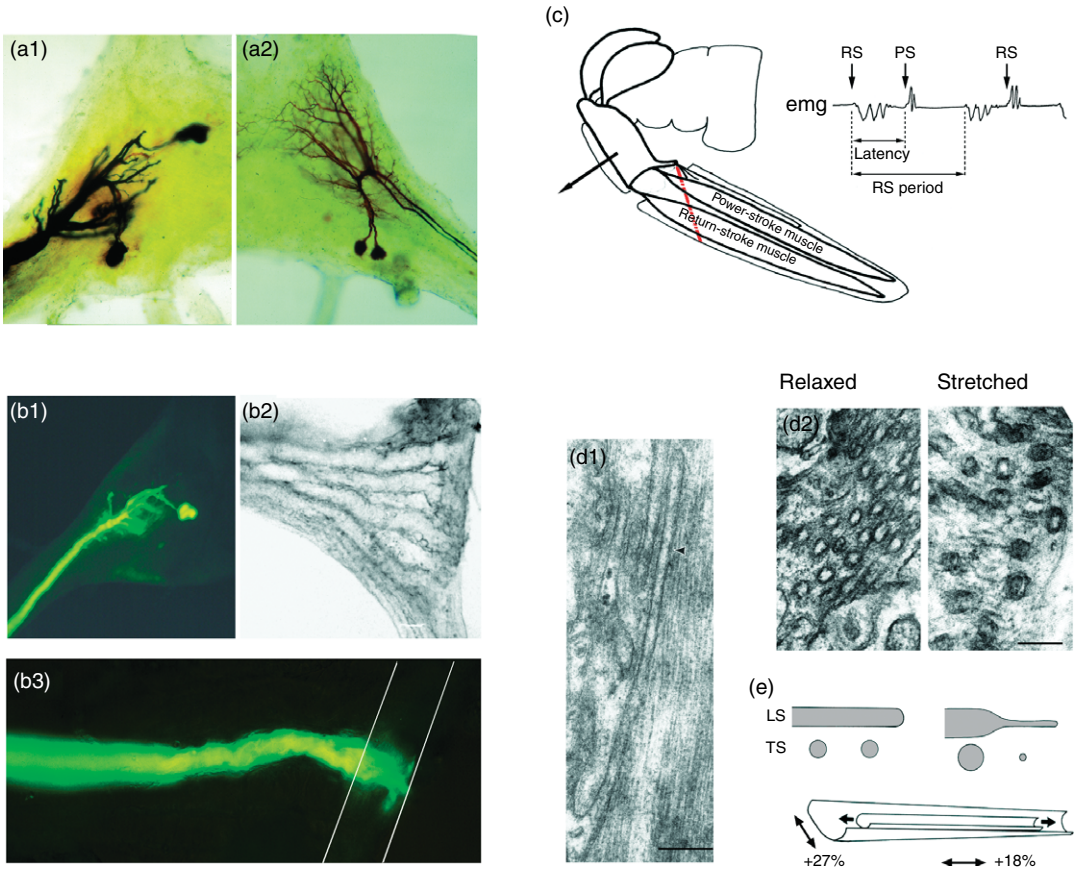

Figure 9 The NSRs of *E. analoga* (Hippidae) have distinctive central (a1, b1) and peripheral (b2, b3, d) morphology. a1 and a2, Silver-intensified backfills showing two of the four sensory neurons (a1) and the two relatively large ATU motoneurons (a2; see Figure 8c), each in one hemiganglion; note the general similarity between the sensory and motor neuron structures. b1, An NSR filled with Lucifer yellow by microelectrode injection. b2, The peripheral dendrites of the NSRs terminate linearly along the elastic receptor strand (uropod end toward top of page) in which their dendritic tips extend longitudinally for a short distance. b3, A peripheral end of a Lucifer yellow-filled NSR dendrite penetrating the elastic receptor strand (edges of strand marked by white lines). c, Cartoon cut-away view from the right side of the tailfan with uropod in rest position (see Figure 1), showing the receptor strand (red line) to which the NSRs attach, which is stretched during the uropod return stroke (arrow). The inset shows a sample electromyogram pattern recorded in freely swimming *E. analoga*. The motor patterns during swimming and treading water differ; the treading water motor pattern is dependent on the presence of intact NSRs, whereas the swimming pattern is unchanged after bilateral receptor ablation (Paul, 1976). d, The ultrastructure of the NSR dendrites within the elastic strand may explain these sensory neurons' unusual combination of features: (1) high sensitivity to stretch of the receptor strand and (2) ability to respond over the full range of tensions experienced by the whole stretch receptor. d1, The dendrite of each NSR gives rise to approximately 21 000, 0.1 μm-diameter tips oriented longitudinally within the receptor strand. d2, Cross-sectional profiles of these tips are larger and less numerous in stretched than relaxed receptors. e, Model to illustrate how differential compressibility of the extracellular matrix surrounding the dendritic tips could account for the change in tip profiles shown in (d2) by producing hydrostatic forces that would cause concomitant constriction of the distal portions and expansion of the proximal portions of the tips (and open stretch-activated ion channels); LS, longitudinal section; TS, transverse section profiles of tips. The number of tips contributing to the receptor potential recorded outside of the strand would increase with increasing stretch amplitude (full range fractionation by each sensory neuron). Below, diagram showing the difference in shape and dimensions of the elastic receptor strand between the relaxed and fully stretched states. d, Adapted from Wilson, L. J. and Paul, D. H. 1990. Functional morphology of the telson-uropod stretch receptor in the sand crab *Emerita analoga*. *J. Comp. Neurol.* 296, 343–358.

1.07.5 Life in the Swash Zone of Sandy Beaches: New Behaviors ↔ New Niche → New Sensory World

1.07.5.1 New Niche Means Altered Sensory Environment

Hippid sand crabs spend their lives alternating between two environments, above and in the sand, on beaches exposed to wave action (Figure 3; Dugan *et al.*, 2000), in which tailfan mechanosensory setae would not be expected to provide much meaningful information. The unpredictable turbulence in the swash zone alone would limit the kinds of information hydrodynamic sensors on the tailfan could provide, in contrast to the importance of this modality in crayfish (Paul, 2004). In addition, hippid uropods beat continuously both for swimming and treading water when the animals are up in the swash zone and while digging into sand, when they are coordinated with the second through fourth thoracic legs (Section 1.07.2.3). Not surprisingly, differences between the mechanosensory integrating network of neurons in the sixth abdominal ganglion of hippids and crayfish are evident. They suggest that alterations in integration of sensory input from the tailfan were important components in the neural evolution that made hippids' new modes of locomotion fully adaptive for their active life on wave-swept beaches. As I treated this subject at length elsewhere (Paul, 2004), I will not discuss it further here.

1.07.5.2 Roles of Hippid Sand Crabs Nonspiking Proprioceptor

Two functions have been ascribed to the NSRs and a third appears likely. They provide clues about these new neurons' connections with ancestral decapod sensorimotor networks serving the tailfan. These clues suggest several potential explanations for the selective advantage of the use of graded potentials in neurons that send signals from the periphery to the central nervous system.

1.07.5.2.1 Suppress reafference during uropod beating The integration of the phylogenetically new NSR input with the sensorimotor network for the tailfan is such that exteroceptive input from the tailfan is depressed whenever the uropods are beating, i.e., continues through the RS and PS phase. The long-lasting nature of the inhibition mediated by the NSR may stem from the combination of the sustained (graded) input and long-lasting inhibitory synapses downstream (Paul, 2004). The ability of hippids to move seamlessly across the water–sand interface, for which continuous uropod beating is required (see above), may have been contingent upon the evolution of proprioceptors that could suppress tailfan mechanosensory input whenever the uropods are beating.

1.07.5.2.2 Switch between motor patterns for swimming and treading water Whenever hippids are above the sand, they are either swimming, i.e., moving with respect to the substrate, or treading water, i.e., keeping themselves suspended vertically (Figure 3) – except when being swept by surging currents! In the electromyogram pattern for swimming (Figure 9c), the phase of the power stroke with respect to RS period is relatively constant (around 0.55), whereas in the electromyogram pattern for treading water, PS muscle bursts occur at relatively constant latency with respect to return strokes. Switching between motor patterns often occurs in the course of one swim sequence. The treading water motor pattern is dependent on intact NSRs, since bilateral ablation of the receptor strands deletes this motor pattern from these animals' behavioral repertoire without diminishing their ability to swim (Paul, 1976).

1.07.5.2.3 Coordinate fourth legs and tail? The NSR input reinforces power strokes during high-frequency uropod beating (Paul, 1976), which occurs during digging as well as in spurts during swimming. When digging, the fourth legs cycle at the same frequency as the uropods (Figure 4), but never in unison with them. The power strokes of the left and right fourth legs are evenly spaced between the uropod return strokes, which means that the phase relationship between the fourth legs shifts from bilateral synchrony to one-third of a cycle out of phase as uropod frequency declines during a dig (Figure 4). The behavioral significance of this complex motor pattern was explained above (Section 1.07.2.1.2). In other species, including crayfish, leech, and lamprey, sensory feedback is incorporated into the intersegmental coupling signals between CPGs located in different parts of the body (Friesen and Chang, 2001), and the NSRs may do the same (they influence the activity of several candidate neurons for this coordination; Paul, 2004). Evolutionarily speaking, the interest of this is that it would mean the superposition of the new afferent signals onto an inherited set of coordinating neurons between dissimilar CPGs, paired for the fourth legs and unpaired (most likely) for the uropods – assuming the uropod and nongiant tailflipping circuitries are homologues (see Section 1.07.2.2).

Paul and Bruner (1999) discuss the possibility that the NSRs are ontogenetically related to motoneurons (see also Bush, 1976; Wilson and Paul, 1990).

1.07.6 Discussion: Lessons from Reconstructing Neurobehavioral Pedigrees

Tracing the evolutionary history of an individual neurobehavioral circuit backward through time uncovers sequential convergences with other behavioral lineages (these are more commonly described in the reverse order, i.e., as major branch points) in its lineage, and should ultimately lead to its origin. This approach is akin to the one used by Dawkins to arrive at his series of concestors in his pilgrimage to the dawn of life (Dawkins, 2004). Some discoveries made along the way, at confluences with variants of the circuit whose lineage is being traced, may seem counterintuitive and offer wonderfully assorted jumping off points for new lines of neurobehavioral investigation. Because nervous systems are heterarchically rather than hierarchically organized (Cohen, 1992), each change, whether peripheral or central, in the ancestry of sand crabs, or any other species, would have presented a new challenge to inherited central circuitry.

Ontogenetic variation within populations arises by chance and then evolves to make species anatomically and behaviorally distinct (True and Haag, 2001; Shubin and Dahn, 2004). The initial mutations may affect peripheral or central structure or function. The neurobehavioral lineage of sand crabs presents examples of both (Figures 2 and 10). The pedigrees of individual species' neurobehavioral mechanisms offer more than descriptions of nervous system evolution. The comparative data generate functional hypotheses for individual species that are testable when the species have identifiable neurons that can be studied physiologically.

1.07.6.1 Peripheral Is Important

Patterns of efferent activity produced by the central nervous system are transformed into sequences of movements by the neuromusculature that operates the skeleton (whether internal, external, or hydrostatic). The association of peripheral morphological changes with major evolutionary events, such as vertebrates' movement onto land and the repeated evolution of flight (in insects, reptiles, and mammals), is well known. However, ignoring the contributions that peripheral differences in musculature make to species-specific behaviors risks misinterpreting the roles of central mechanisms in producing those behaviors and may obscure the very features sought in comparative neuroethological studies (Friel and Wainwright, 1997, 1999; Antonsen and Paul, 2000; Hooper and Weaver, 2000). In addition to illustrating well the interplay of peripheral and central factors during the evolution of adaptive behavior in new species, reconstructing the neural pedigrees of sand crab swimming and digging behaviors has opened new avenues for investigating function and evolution of nervous systems in other species that otherwise would have gone unnoticed.

1.07.6.2 Spin-Offs from Reconstructing the Neurobehavioral Pedigrees of Hippid Sand Crabs

The species encountered at convergence points along the backward evolutionary chronology of neurobehavioral circuitry raise new questions, provide new experimental animals, and suggest mechanisms for neurobehavioral evolution that could not be evident from research on a few model systems or *in vitro* experimentation, useful as the latter are for elucidating potential morphogenetic mechanisms that could lead to similarity by either common descent or convergence (Gerhart and Kirschner, 1997; Hall, 1999; True and Haag, 2001; Antonsen and Paul, 2001, 2002; Eisthen and Nishikawa, 2002; Wainwright, 2002; Wray, 2002).

1.07.6.2.1 Testable hypotheses from comparative studies Because neuronal positions and basic morphologies are conserved between species, comparison of the central positions and morphologies of motoneurons innervating individual heads of complex musculature both within and between species assists in identifying homologous muscles. The power of comparative morphological analysis is beautifully exemplified by the results of its application to the innervation of electric organs in fish (electrocytes are modified muscle fibers), which illustrate well the greater evolutionary plasticity of the physiology than of the morphology of neurons (Labas *et al.*, 2000).

Similar central morphologies of motoneurons in derived and ancestral species suggest that similarly organized central motor networks control them, the root of my argument for homology of swimming by uropod beating and tailflipping. Conserved network structure does not, of course, imply identical function or behavior, as neurons and synapses may evolve individually and be correlated with behavioral differences among species (Shaw and

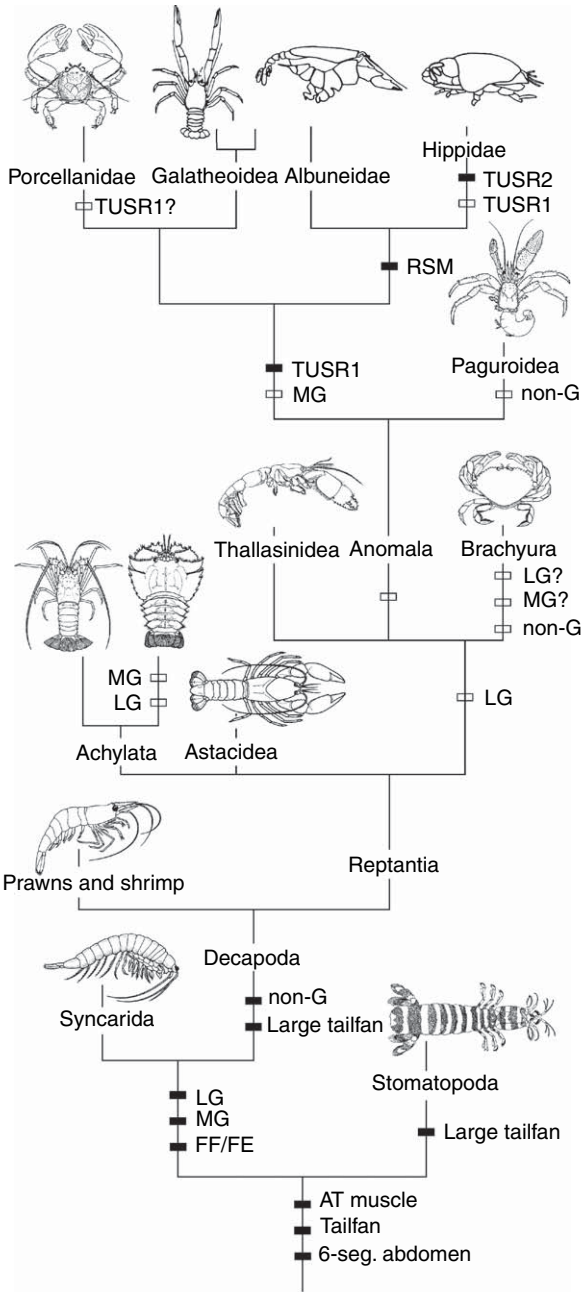

Figure 10 Partial phylogeny of the class Malacostraca with traits associated with the tailfan and tailflipping behaviors mapped. The tree includes the orders (except Euphasiacea, kril, for which neurobiological date are lacking) that retain the elongate abdomen and tailfan that distinguishes the class. Most Decapoda (e.g., shrimp, lobsters, crayfish; see Figure 2) share with the ancient orders Stomatopoda (mantis shrimp) and Syncarida (represented by *Anaspides tasmaniae*) all muscular components of the tailfan. *A. tasmaniae* share with Decapoda the characters of fast abdominal flexor and extensor muscles (FF/FE), the MG and LG interneurons, and the ability to perform rapid tailfips. Non-G (the CPG for tailflipping, character 7 in Figure 2) is shown as appearing with the Decapoda, although it may have evolved later (see Figure 2). The association of abdominal musculature for performing tailflips (not present in stomatopods) and the two sets of giant command interneurons for rapid ventral flexion of the body appeared together in syncarids, which retain many primitive features (Silvey and Wilson, 1979). Note the evolution of the machinery for tailflipping (FF/FE in the abdomen, giant interneurons (MG, LG)) after the divergence of Stomatopoda and the greater antiquity of the tailfan muscles (their number is the same and their organizations are very similar in these three orders, Paul and Macmillan, 1997). Clearly, the enlargement of the tailfan in Decapoda and Stomatopoda, which do not tailfip, evolved independently. N.B. the loss of the AT muscle at the base of the Anomala (absent also in Brachyura, whose relationship with the Anomala are poorly understood). The subsequent derivation of the uropod PS musculature in Hippidae from heterogeneous components of ancient malacostracan tailfan muscles that flex the telson or help cup the tailfan is summarized in Figure 2 and illustrated in Figures 5 and 6.

Moore, 1989; Fetcho, 1992; Katz and Harris-Warrick, 1999; Katz *et al.*, 2001; Remmers *et al.*, 2001). Differences between homologous motoneurons in squat lobsters and crayfish, for example whether neurites cross or do not cross the midline, may indicate differences in central circuitry correlated with the degree of bilateral interactions between motor centers or reflex pathways (Antonsen and Paul, 2000). Neural differences without apparent behavioral differences occur between two species of squat lobsters (Wilson and Paul, 1987). Future comparative neuroethologists may identify, with hindsight, this difference as incipient behavioral evolution! Behavioral evolution without altering central circuitry may occur by altering neuromodulatory control, as when key neurons loose, gain, or change their ability to synthesize or release a neurotransmitter or modulator (Harris-Warrick *et al.*, 1992; Katz *et al.*, 2001). An example of altered neuromodulatory control of a complex behavior is the ability of artificially elevated blood serotonin levels to transform normally gregarious squat lobsters (*Munida quadrispina*) into aggressive and vicious combatants (Antonsen and Paul, 1997, 2002). The details of this uncharacteristic behavior, which is displayed only under the influence of serotonin, strongly resemble the aggressive behavioral repertoire displayed by dominant crayfish, and some other crustaceans, to maintain their position in the social hierarchy (Edwards *et al.*, 1999). We think that fighting is the ancestral condition and its expression was lost in *M. quadrispina*'s lineage while the fight circuitry was retained, at least in part (Antonsen and Paul, 1997). Detailed comparisons of immunocytochemical maps of the nerve cords of crayfish and *M. quadrispina* could not provide suggestions for which neurons might be involved with fight initiation, but did provide suggestions about possible loci of some other functional differences between these species, along with insights into the evolution of aminergic systems (Antonsen and Paul, 2001).

1.07.6.2.2 Mosaic neurobehavioral evolution from modularity of neural mechanisms and ontogeny Some themes recur frequently, and similarities by homoplasy are possible. Grillner and Dickinson (2002) caution against associating common themes in motor mechanisms and homology between motor elements, especially when the phylogenetic relatedness of the species is not close. Recognition of convergence requires well-understood functional demands. These are more easily specified for sensory than motor systems, which explains in part the larger number of examples from sensory systems (Eisthen and Nishiikawa, 2002). The requirement for controlled movements for survival appears to constrain rapid evolutionary change in core elements of motor systems (e.g., CPGs) but allows changes in their coupling and control by other neural centers (Figure 4; Cohen, 1992; Fetcho, 1992; Nishikawa *et al.*, 1992; Remmers *et al.*, 2001; Paul *et al.*, 2002; Vasilakos *et al.*, 2003). Recent advances in our understanding of the ontogenetic processes that can give rise to convergent similarities provide insight into how morphological novelty and selection bring about evolution (Gerhart and Kirschner, 1997; Hall, 1999; True and Haag, 2001; Wray, 2002).

1.07.6.3 The Emerging Picture

1.07.6.3.1 Understanding the present by reconstructing the past A brief look further back into the ancestry of hippid sand crabs' mode of swimming with the uropods, stepping back into the phylogeny of Malacostraca (Figure 10), provides greater depth to the timescale and gives a sense of relatively rapid and recent upheavals in an ancient neurobehavioral network in anomalen decapods. The decapods share with some other, including the most basal, malacostracan orders: (1) organization (but not size) of tailfan musculature, (2) the MG and LG interneurons, and (3) the ability to tailflip. Most interesting is the appearance of these features in the syncarid *Anaspides tasmaniae* (Silvey and Wilson, 1979; Paul and Macmillan, 1997), which is one of the two most ancient orders of Malacostraca (Figure 10). From the distribution of neurobehavioral traits in Figure 10, a picture of sets of nested changes seems to emerge. The number and organization of tailfan muscles are essentially the same in stomatopods, syncarids, and decapods, although the tailfans differ substantially in size, shape, and behavioral use (Paul and Macmillan, 1997). Not until the loss of the only axial muscle without serial homologues in anterior abdominal segments (the AT muscle) in reptantian decapods was there any change in this ancestral plan.

The well-developed abdominal flexor and extensor muscles and the MG and LG interneurons preceded evolution of the stiff decapod tailfan, with its recessed uropod joint, which increased the power of tailflips. The tailfan (telson and uropods) of crayfish is often referred to as an appendage so tightly integrated are its parts. It is hard to escape the impression that this complex structure of tightly integrated parts with the MG and LG neurons as centerpiece restrained evolution of new morphologies and uses of the malacostracan tail until one

or both of these interneurons were lost, as happened apparently several times (Figures 3 and 10). The more ancient AT muscle was apparently an even greater restraint on tinkering with the premiere malacostracan invention, the tailfan, because only following its demise did evolution produce a delightful assortment of anomalan tails, some of which are described in this article. Evolutionary neuroethology, by its comparative analytical approach to understanding motor systems, and other neural networks in general, is becoming increasingly fruitful. This is because the rapid progress in understanding the mechanisms that work at different levels of organization to foster and limit morphological and functional evolution provides plausible explanations for how the neural evolutionary events, charted by comparative anatomy and physiology of extant species, may have come about (Gerhart and Kirschner, 1997; Hall, 1999; True and Haag, 2001; Shubin and Dahn, 2004). These hypotheses are testable if the species in question are amenable to genetic and developmental analysis. Stated in reverse: evolutionary neuroethology tests the plausibility and sufficiency of mechanisms described in research on *in vitro* systems and model organisms (which model only themselves) to explain how nervous systems really evolve.

1.07.6.3.2 Predicting the future The ability to predict the course of future neurobehavioral evolution in any lineage would be proof that neurobiologists have achieved their goal of understanding the rationale behind the organizations of neurobehavioral networks (Section 1.07.1.1). The degree of understanding that will be required may not be achievable, given the dynamic interactions among the numerous systems at play, many of which are themselves already quite complex (Barrow, 1998). What can be predicted, however, is the course evolutionary neurobiological research will take in the near future to bring us closer to this goal. The combined use of diverse new research tools and methods of computational analysis now available for research at single levels of organization, from synapses and single neurons to large networks and whole nervous systems, will be applied comparatively to carefully chosen species, and the results should greatly deepen our understanding of how nervous systems evolve as conditions change.

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1.08 The Evolution of Encephalization

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Glossary

allometry The scaling relationship between two characters A and B (e.g., brain mass and body mass), where changes in A and B are not proportional. (Proportional change in A and B is termed isometry.) Allometry is thought to reflect design constraints, so predictions on the effects of directional selection are usually conducted on residual trait variance after removal of allometric scaling.

altricial A relatively slow mode of juvenile development, where offspring are usually not mobile and are strongly dependent on parental care. The term can be opposed to 'precocial.'

behavioral drive hypothesis The idea that generalism, exploration, opportunism, social learning, and behavioral flexibility can have accelerating effects on evolutionary rate, by increasing the range and frequency of selective contexts in which randomly occurring mutations can confer higher fitness.

behavioral flexibility A general term describing the capacity to modify behavior. Often used as blanket term that encompasses learning (a change in behavior contingent on an association with a stimulus and/or a reward), opportunism (exploitation of a temporarily abundant resource not part of the usual diet of a species), or innovation (see definition below).

ecological intelligence Cognitive complexity selected in the context of interactions with the physical environment. Usually contrasted with Machiavellian (or social) intelligence, which is selected in the context of social interactions.

encephalization In comparative biology, the term describes the difference between animals in the amount of neurons available beyond the average determined by allometric body design. In paleoanthropology, it designates the observed increase over evolutionary time in the absolute and relative size of the brain in hominids. In neuroanatomy, it describes the increased importance that higher brain structures play over lower ones in birds and mammals compared to other vertebrates and to invertebrates.

generalism	One extreme of a continuum where the other extreme is specialization, generalism refers to the use of a relatively broad range of foods or habitats by a taxonomic group or an individual.
independent contrasts	The most widely used procedure for removing the effects of common ancestry on taxonomic similarities or differences between traits. The technique measures 'differences' between related taxonomic groups in the values of biological traits, rather than actual trait values on extant taxa. Relatedness between groups is usually measured through mitochondrial or nuclear DNA data.
innovation	A behavior pattern performed for the first time by an animal and that is not the result of a genetic change or a pathology. The novel behavior is an attempt to solve a problem (feeding, social) that the standard repertoire cannot resolve. Also defined as a new or modified learned behavior not previously found in the population (Reader and Laland, 2003, p. 14).
specialized cognitive skills	Cognitive abilities that are specific to a restricted selective context (e.g., spatial memory, learned song) and whose neural substrate is often a restricted brain area (e.g., hippocampus, nucleus HVC). The terms 'adaptive specialization' (implying adaptation to a particular selective context, e.g., food caching, brood parasitism) or 'module' (implying that information relevant to one specialized context or domain is not available in other contexts) embody related ideas. The term can be opposed to general process skills, where cognitive differences between taxonomic groups are thought to reflect broad, unspecialized abilities based on large and/or diffuse neural substrates.

1.08.1 Introduction

The term 'encephalization' expresses different ideas in different scientific disciplines. In comparative biology, it describes the difference between animals in the amount of neurons available beyond the average determined by allometric body design (e.g., Jerison, 1991; Schoenemann, 2004). Porpoises, for example, are said to be more encephalized than tenrecs because they are far above the regression line of log brain size plotted against log body size for all mammals, while tenrecs are far below. In paleoanthropology, encephalization designates the observed increase over evolutionary time in the absolute and relative size of the brain in hominids (e.g., McHenry, 1994; Bruner *et al.*, 2003; Rightmire, 2004; Stedman *et al.*, 2004). The brain of *Homo erectus*, at *c.* 1000 g, is thus considered to be more encephalized than the brain of *Australopithecus*, at *c.* 500 g. In neuroanatomy, encephalization describes the increased importance that higher brain structures play over lower ones in birds and mammals compared to other vertebrates and to invertebrates (Reiner *et al.*, 2004). In this view, the average mammal brain is more encephalized than the average fish brain.

Despite their differences, all three usages share one common assumption: the information-processing advantage provided by extra neurons, increased size, and increased forebrain involvement should normally be the major evolutionary driving force behind encephalization. Extra neurons in the forebrain, whether they evolve in a hominid, a cuttlefish, a capuchin monkey, or a crow, should provide faster and/or more complex and/or a greater amount of information processing and information storage about changing environmental conditions. Natural selection can lead to efficient genetically biased responses to conditions that are stable over long periods of time. But when relationships between events change rapidly, neuronal storage allows animals to respond faster than information stored only in the genome. The main functional and evolutionary hypothesis on encephalization is thus that something about extra neurons, increased size, and increased forebrain involvement is associated with the speed, complexity, and amount of information processing in these structures.

It is useful to envision variation between animals in information-processing capacity as a cognitive continuum. A corvid, for example, seems to learn more items faster (Wilson *et al.*, 1985) and with more complex processes (e.g., episodic memory, prospection; Emery and Clayton, 2004) than a columbiforme does. Its higher brain centers (the meso- and nidopallium) are eight times larger than that of a columbiforme of the equivalent body size (Rehkämper *et al.*, 1991a; Boire, 1989). The whole brain of a corvid is more than 1.5 standard deviations above that of the average bird, while a columbiform brain is 1.5 standard deviations below. This kind of organ–function correlation is not very controversial when it involves wings and flight or beaks and feeding. When the correlation involves brains and cognition, however, this is often a different story. Critics plead that cognitive complexity is difficult to assess in a way that is

fair to all species (Macphail, 1982). They say that brains have many contradictory functions unlikely to lead to directional selection for an overall size increase (Shettleworth, 1998). Others claim that a correlation between morphology and function does not necessarily imply adaptation (Gould and Lewontin, 1979) and point out that adaptationist accounts of brain–cognition co-evolution in humans have often been politically tainted (Gould, 1981; see Human Cognitive Specializations).

Such qualms are legitimate and must be addressed, but they do not invalidate a critical empirical examination of brain–cognition questions. How is brain size variation distributed among taxa? Is the distribution continuous or patchy? Is it whole brain variation that we should be concerned with or variation in some specific structures? Is it relative rather than absolute size of neural structures that matters and if the former, relative to what? Can we reliably show that brain size variation is associated with variation in cognition? How did the variation evolve? What are the costs and benefits of larger versus smaller brains, and in what ecological contexts do these costs and benefits apply? These are the who, what, and why questions we will examine in this article.

1.08.2 How Is Encephalization Distributed Among Taxa?

1.08.2.1 Variation Between Classes

Size and structure of the central part of the nervous system differ clearly between the major animal taxa. From the nerve ring of nematodes to the cerebral ganglion of insects to the brains of cephalopods, birds and mammals, there are major discontinuities in the size and organization of the central organs. If we were to statistically partition the variance in nervous system characteristics over all animals, we would likely find that most of it lies at very high taxonomic levels. Neurons themselves are highly conserved in all animals, as are synaptic processes. For example, memory appears to be based on similar rules of long-term potentiation via glutaminergic synapses from Aplysia (Bailey *et al.*, 2000; Pittinger and Kandel, 2003) and cephalopods (Hochner *et al.*, 2003) to humans. The basic building blocks of learning and cognition might thus show a strong constancy throughout evolution.

The way these neurons are organized in the brain and how many of them are available for more complex information processing also appear to be conserved within major taxa. A mammalian brain is, on average, larger than an avian brain and features a laminar cortex, while avian brains are organized in discrete nuclei (Karten, 1997). In turn, bird brains are larger than cephalopod brains, which are organized in supra- and subesophagal lobes. These average differences have sometimes led to a *scala naturae* vision of brain evolution, where more recent lineages are seen as more encephalized on average than older ones. At least two hypothetical mechanisms could produce such a trend. The first is the possibility of an evolutionary arms race. The oldest animals on earth had no nervous system at all. These were followed by animals with neurons that are linked by a central chain, then by animals with neurons linked to a central organ. Odontocetes and apes have the largest brains and are relatively recent (23 and 34 My respectively; Marino, 2002; Marino *et al.*, 2004). The assumption here is that the bigger the brain, the more information it can store, the faster it can change behavior in response to environmental contingencies (Sol, 2003), and the more complex a behavioral repertoire it can program (Changizi, 2003). Animals that can do more of all this are assumed to have an advantage over those that can do less, once the costs of an enlarged brain are taken into account. A further positive feedback effect of the behavioral flexibility associated with larger brains may add extra pressures for encephalization via social channels. The more flexible the behavior, the trickier it is for other animals to predict and the more useful is a large brain to make such predictions and change behavior quickly in response to the rapid change of others (Byrne and Whiten, 1988). The consequence of these mechanisms is that a competitive arms race might then follow, as it does for sexual selection, limited by the costs of increasing the size of the organ.

The second evolutionary phenomenon that would lead to encephalization over time is behavioral drive (Wyles *et al.*, 1983; Wilson, 1985). All other things being equal, animals that come into more frequent contact with environmental conditions likely to provide a selective context for randomly occurring mutations should be characterized by faster evolutionary rates. Opportunism, generalism, and invasiveness are three traits that will increase the rate of contact with new selective pressures. If these traits are associated with larger brains (see below), then encephalization should also correlate positively with rate of evolution. Because generalism and invasiveness also make animals more likely to range farther, they should also increase the probability of allopatric speciation. If larger-brained taxa beget more descendant species than smaller brained ones, then the average size of the brain

should increase over time. The fact that individuals from larger-brained species tend to have fewer descendants per unit time than those from smaller-brained taxa will, to a certain degree, counteract the positive effects of an arms race and behavioral drive.

Discontinuities in encephalization over major clades prompted comparative psychologists in the 1960s to ask whether certain learning differences paralleled neuroanatomical trends. Overall, the results of these programs tended to show that there are quantitative differences in learning performance in the direction predicted by encephalization differences. Some researchers however, have questioned the heuristic value of these findings. One comparative learning researcher, Riddell (1979, p. 95), ironically summarized his experience:

> The comparative psychologist often appears to know little more than a grade school child who would rather have a pet dog than bird, or bird than fish, or fish than worm, simply because they make better friends, as they can be taught more.

Beyond these problems, there are two other limits to comparisons between classes: small sample sizes and the overlap between the encephalization distributions of the taxa. Because each class is $n = 1$, comparative statistics cannot be used to test predictions about the costs, benefits, evolutionary history, ecological associations, and behavioral correlates of encephalization. Comparing the average fish to the average bird to the average mammal has a sample size of 3. Some birds are as or more encephalized than some mammals. If a crow has a larger brain and more complex cognition than does a tenrec, is it useful to think of an average mammal versus an average bird? Intraclass variance might be biologically as important as interclass variance and the question of whether similar patterns govern intraclass or order variation in different taxa might be the more useful one to ask.

1.08.2.2 Variation Within Classes

The study of intraclass variation solves the statistical problem mentioned above (classes Aves vs. Mammalia: $n = 2$; variation between avian species: $n = 10000$). It also increases the validity of cognitive comparisons by measuring animals with more similar sensorimotor worlds. Comparing the results of several within-class or within-order analyses might thus be a good way of finding general patterns in encephalization.

The taxonomic distribution of a trait as well as its co-occurrence with other traits can be due to two types of processes: ancestral descent and repeated independent evolution. Ancestral descent may represent simple inertia or it may be the source of an important adaptive radiation. To separate ancestral descent and repeated independent evolution, we must know something about the phylogeny of the taxon and control for its effects on the distribution of apparently co-evolved traits. Most phylogenies today are based on differences in molecular sequences of either nucleic or mitochondrial DNA. When a well-resolved (a complete, well-differentiated tree at all levels) and robust (different parts of the genome lead to similar phyletic conclusions) molecular phylogeny is not available for a given taxon, a classical taxonomy, based either on Linnean characters or cladistics is still useful, but some degree of resolution will be sacrificed, usually yielding nonbranching elements and/or equal branch lengths (i.e., with no known genetic distance or estimated time of divergence).

Evolutionary biologists have long been concerned that interpreting correlations between traits in extant species as adaptive consequences of co-evolution might be biased by two sources of type 1 error (Felsenstein, 1985; Harvey and Pagel, 1991). First, two species might show similar values on two traits because they are closely related, not because of independent evolutionary events. This violates the assumption of data point independence for correlations and inflates the sample size via pseudoreplication. The similar values might thus be the result of inertia from an ancestral state, and cannot be considered the result of adaptive co-evolution. Techniques such as independent contrasts have since been routinely applied to trait correlations to deal with such problems. Contrasts are nodal differences between estimated ancestral values of the traits we are interested in. The nodes represent hypothetical ancestors, whose values are assumed to be averages of the trait values for the two branches descending from the node, often weighted for genetic distance. While the trait values of a given pair of taxa may not be independent, the difference between them can be assumed to represent independent evolution. Imagine that we have data for relative brain size and for diet breadth on 100 species and that the independent contrasts yield a nonsignificant correlation between the two traits. Comparative biologists will usually conclude that the null hypothesis for adaptive co-evolution has not been rejected. Imagine now that you examine the taxonomic distribution of your two traits and find that of the two subgroups in your taxon, one contains 85 large-brained species whose diet varies from three to ten food types, and a second one contains 15 small-brained species whose diet varies from one to four food types. What can we conclude?

That there has been no repeated co-evolution of generalist diets and large brains in this order? In all likelihood, yes. That diet and brain size cannot be proven to have had a selective effect on each other in this group? Yes. However, if we run a normal regression (nonphyletically corrected) on the 100 extant species and find a highly significant brain–diet correlation, we would run the risk of type 2 error if the nonsignificant contrast analysis leads us to conclude that the observed pattern contains nothing of evolutionary interest. Clearly, species that combine large brains and a generalist diet are or have been in the recent past quite successful. How they got the combination of the two traits is what a nonsignificant regression on the independent contrasts tells you: they inherited it from their ancestors and the combination did not appear through repeated independent evolution.

Despite its difficulties, the phylogenetic approach has two advantages: the occurrence of repeated independent events is a much more stringent test for adaptative co-evolution than is a single ancestral event. Phyletic trees, combined with molecular clocks, can also generate hypotheses on evolutionary sequences and timescales. If the large-brained generalist combination occurs in six widely separated clades and there is more brain size variance at older phyletic levels, (corresponding, say, to 100 My BP) and more diet breadth variance at more recent levels (say, 20 My BP), then we can hypothesize that large brains in general allow the evolution of broader diets, because variation in brain size precedes variation in diet. For example, most of the variance in avian brain size is at high phyletic levels like the parvorder. The molecular data of Hedges *et al.* (1996) suggests that divergence of extant birds at this level is 100–125 My old and may coincide with episodes of continental splitting. In contrast, the variance in avian innovative feeding (see below) is highest at much more recent levels of divergence, e.g., the species. The hypothesis that brain size divergence preceded feeding divergence thus follows and can be tested with statistical techniques such as path analysis.

1.08.3 What Is Encephalized?

1.08.3.1 Relative Brain Size

As recently as the 1960s, some researchers had a logic based on uncorrected absolute size for their evolutionary and/or ecological hypotheses on encephalization. Most researchers today (although see Byrne and Corp, 2004, for discussion) assume that encephalization should be studied after some kind of complete or partial control for allometry, often assessed by body size (see Scaling the Brain and Its Connections, Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals, Principles of Brain Scaling).

Usually, body size allometry is considered a confounding variable and is removed from most analyses of relative brain size. The assumption here is that as a body gets bigger, it takes more brain cells to analyze the information coming from more skin, a bigger retina, larger ears, a bigger nose, as well as to program more motoneurons for bigger and more numerous muscles. It also takes more interneurons to mediate all this added sensory and motor machinery. The brain–body relationship is not 1 to 1. As bodies get bigger, the increase in brain size follows at a slower pace. Not all organs follow this trend; the heart/body relationship, for example, is linear even when the data are not log transformed. The brain is thus a peculiar organ and its relationship with body size may differ from that of other organs. The decreasing slope of the brain–body relationship might mean that ever-larger bodies require proportionally fewer and fewer extra neurons in the brain or that the cost of enlarging the brain increases faster than the cost of enlarging the body. However, metabolic costs, one of the best known costs of encephalization (and the interpretation often cited for the slope of the log-log brain–body line; Martin, 1981; see however, Symonds and Elgar, 2002), decrease with body size. As animals get bigger, there is less surface-to-volume heat loss, body temperatures decrease, and it takes proportionally less energy to fuel a large body compared to a small one.

Another problem with using body size as an allometric control is that selection, both natural and sexual, operates on it and that a seemingly small brain relative to a large body may simply mean that there has been stronger selection on an enlarged body than on an enlarged brain. When sexual selection for enlarged bodies leads to gender dimorphism, this presents a further problem, though one possible solution is to take only the brain and body measurements of the gender under the lowest sexual selection pressure. Selection for specific organs that make up a large proportion of the body could also affect total size estimates. Herbivores and folivores have a large digestive system because the low digestibility and nutrient quality of their food requires larger amounts of food and longer digestion. Gorillas and ruminants may thus have a spuriously small relative brain size if allometry controlled via body size is biased by selection on a large digestive system. Some estimates

of size (e.g., body length) may be less sensitive to this problem than others (e.g., mass), but the general problem remains. An alternative explanation for small brains in herbivores and folivores would argue that the demands of eating leaves and grass do not select for a large brain relative to body size because these foods are abundant and predictable, but the point is that both explanations are logical.

A third problem with body size is that large bodies are associated with longer generation times. When environments change, there are two ways an animal can modify its response. First, natural selection can increase the frequency over successive generations of alleles (mutated or already appearing in low frequencies) coding for traits that lead to higher fitness in the changed conditions. Alternatively, phenotypic plasticity such as innovation, individual learning, or social learning may allow animals to track the changed conditions. If large brains favor behavioral flexibility and large bodies (and brains; see below) decrease the rate of natural selection via long generation times, then bodies again will not have a neutral effect on brain size. Encephalization might thus follow evolution of enlarged body size, as Nealen and Ricklefs's (2001) analysis of birds suggests (but see Deaner and Nunn, 1999 on primates).

One proposed solution to the problems posed by body size allometry is to use a part of the nervous system itself as a control. This solution also reduces the measurement error inherent to estimates such as body mass, which can change rapidly as a result of food conditions. Harvey and Krebs (1990), Barton (1999), and Deaner *et al.* (2003) have pointed out that such measurement errors can create spurious positive correlations between relative brain size and other allometrically corrected variables such as life history traits. For example, correcting absolute brain size and longevity by the same erroneous body mass estimate will create a similarly high residual of the two traits in a species whose correct mass is underestimated by the erroneous estimate, and a similarly low residual for the species whose mass is overestimated. These correlated errors may create artificially correlated traits.

When a part of the nervous system is used to remove allometry, we need to specify the higher level centers that are assumed to be more closely involved with cognitively driven encephalization and the lower brain areas that can be used as the control. For this, we depend on neuroanatomy and neuropsychology. The encephalized areas can be very broad, such as the telencephalon in birds and mammals or the supraesophagal lobes in cephalopods. The areas chosen for the allometric control could, for example, be the brainstem in birds and mammals and the subesophagal lobes in cephalopods. The lower brain structure could be either that of the species itself or of a primitive evolutionary baseline. Portmann (1946, 1947a, 1947b) pioneered the use of these methods, which were later applied to mammals and cephalopods by Wirz (1950, 1959) and primates, bats, and insectivores by Stephan and collaborators (Stephan *et al.*, 1988, 1991; Baron *et al.*, 1996). In birds, the primitive reference group is usually galliformes, while in mammals, it is insectivores. For this method of removing allometry, there are thus three assumptions: the upper brain structure is the one most closely involved in encephalization, the lower brain structure has been subject only to the allometrically driven selection, and encephalization can best be understood by comparing primitive taxa to more recently encephalized ones. All these assumptions can be questioned.

Whether one uses whole bodies or lower brain structures as controls, there are in essence two statistical approaches to the removal of allometry: residuals and ratios. Residuals use the deviation from the best fit log-log regression as the measure of relative size, often transformed to a standardized scale so that all distributions are comparable from one analysis to another and normalized for parametric statistics. A problem with residuals is that they all change when you add only one new species. If this species has unusual weighting in the data point cloud, this will have a strong effect on all residuals. For example, if you add Rehkämper *et al.*'s (1991b) 23 hummingbird species to Portmann's (1947a) 140-species database, you tip the best-fit line counterclockwise due to the small body and brain size of hummingbirds. This might introduce an artifact due to the particular flight mode of hummingbirds, which might constrain both brain and body size evolution. You would thus be allowing a taxon that is a special case to influence every single residual.

In analyses that use ratios, the numerator is the brain part predicted to be most closely involved in cognitively driven encephalization (e.g., the neocortex of mammals, the mesopallium–nidopallium complex of birds, the vertical lobe system of cephalopods, and the mushroom bodies of insects; see below). The denominator is either a structure that encompasses the one in the numerator (e.g., whole brain or telencephalon or supraesophagal lobes or cerebral ganglia) or the lower brain structure not thought to control cognition (e.g., the brainstem, the subesophagal lobes, and the spinal ganglia). Allometric effects are assumed to be (wholly or partly; see below) controlled in ratios, because they apply to both the numerator and denominator.

One problem with ratios is that they are not normally distributed and thus present a statistical problem for parametric statistics. Large ratios tend to get larger faster than do small ratios. For example, parrots and corvids may easily reach values of 20 in a Portmann ratio, while ducks vary only around 1.6. Log transformations of the ratios can solve the problem by compressing the skewed high values (Lefebvre *et al.*, 1997). A second, more important, problem is that ratios may not entirely remove the confounding effect of body mass (Deacon, 1993). If we conclude, for example, that carnivory is associated with large brains and our estimate of relative brain size is confounded with body mass, there is a risk of type 1 error if carnivores also have larger bodies. In this case, the apparent brain–diet relationship could be a spurious effect of the brain–body and diet–body associations.

A third problem is that ratios of variables whose relationship is not 1 to 1 will overestimate one end of the continuum and underestimate the other. The lower the slope is below 1, the more neural structure size (normally plotted on the y axis) of animals that are at low values of the x axis will be overestimated. When the slope is above 1, the reverse will hold, with larger x values being overestimated. It is well known, for example, that expressing relative brain size as the proportion of total body mass represented by the brain will result in higher ratios in chickadees than parrots simply because chickadees are much smaller (Packard and Boardman, 1999). The brain-to-body-size ratio is often used in human paleoanthropology. The same problem may occur if the telencephalon is expressed as a proportion of the whole brain or the neocortex as a proportion of either the brain or the telencephalon (Clark *et al.*, 2001; Burish *et al.*, 2004). If the structures are thought to be progressive, the slopes of the y–x relationship are likely to be higher than 1. This will overestimate the larger-brained species (Barton, 2002), potentially favoring type 1 error of any prediction associating relative brain structure size and cognition.

It may be noted that one quantitative expression of encephalization, Jerison's (1973) encephalization quotient (EQ), combines the advantages and disadvantages of residuals and ratios. EQ expresses relative brain size as the ratio of the observed (unlogged) y value of a given species on a log-log body–brain graph, divided by the unlogged y value of the best fit regression for the x value of the species. If a species has a brain size of 20 g and the y value of the brain–body regression for an equivalently sized animal is 5, then EQ = 4. If the brain mass of a small-brained species of equivalent body size is 2.5, then EQ = 0.5. Given that EQ is based on a log-log regression, it is statistically better to calculate standardized residuals from this regression, which by definition will be normally distributed, instead of using ratios, which are not. If EQ was intended as a reference to IQ, it is puzzling that Jerison did not express his results as standardized residuals fitted to a mean of 100 and a standard deviation of 16. On this scale, parrots would score around 130, while quail would score around 75. Another problem with EQ is that values calculated from a regression line at one taxonomic level may be biased when they are used to test a hypothesis at another taxonomic level. For example, the EQ values of cetaceans (see Cetacean Brain Evolution) are routinely calculated with respect to the log-log regression line for all mammals. If one then tests a hypothesis on variation within cetaceans only, EQ may hide a confounding negative correlation with body size; small-bodied cetaceans tend to have larger EQs than large-bodied ones (Marino *et al.*, 2006). Allometry can thus still be present in EQ, even if the calculation was initially designed to remove it.

1.08.3.2 Whole Brains or Parts Thereof?

Are larger whole brains the consequence of selection for increased size of some of its components only or is enlargement of the whole brain the means by which larger specific structures evolve? Do these components vary independently of others or are there functional links between anatomically distant areas that cause change in them to occur together? The answer to these questions will depend in part on how much room the components occupy in the brain. The higher the proportion of the whole brain a component structure occupies, the more its enlargement will have a consequence for the size of the whole brain. For example, the mesopallium–nidopallium complex of a crow represents 72% of its telencephalon, which represents 78% of its whole brain. When we say a crow has a large brain, we might really only be saying that it has a large mesopallium. Selection for an enlarged high vocal center (HVC) or hippocampus in a chickadee will not have that effect, because these are small structures compared to the whole brain.

1.08.4 Why Be Encephalized?

1.08.4.1 Costs

Encephalization should normally only occur if the benefits of enlarged brains exceed its costs. These benefits and costs will operate in specific lifestyles,

which need to be specified in any evolutionary account (Johnston, 1982). The two major costs to encephalization appear to be developmental and metabolic (Bennett and Harvey, 1985b). All other factors being equal, bigger brains require a longer time to develop and are energetically more expensive to maintain. The metabolic cost of brains is particularly high in humans and other primates (Aiello and Wheeler, 1995; Aiello and Wells, 2002; Fish and Lockwood, 2003), but less clear in other taxa (e.g., bats; Jones and MacLarnon, 2004). It is important to note that both metabolism and development are related to body size and diet and that their relationship to brain size is thus likely to be complicated by these interactions. If slower development means fewer offspring per unit time and is an allometric correlate of large body size, then the relative importance of natural selection and behavioral flexibility as alternative mechanisms to track environmental change will be affected. If high metabolic rate is associated with the higher surface-to-volume mechanism of heat loss in small-bodied animals, then this might affect the amount of energy available for encephalization. If herbivores are on average characterized by precocial development, low metabolic rates due to low nutrient quality, large ranges required to collect large amounts of low-quality food and large body size due to selection for an enlarged digestive system as well as defense against predators, then all these factors are also likely to affect brain size.

The brain develops slowly and some researchers have proposed that it is the major developmental constraint on time to reproduction (Sacher, 1978). Apart from parental behavior, four life history traits will affect the number of descendants per unit time, which affects the selection probability of a mutation favoring adaptation to environmental change: length of the reproductive period (reproductive longevity, time to sexual maturity), number of offspring per reproductive event, time to sexual maturity of the offspring, and time intervals between successive reproductive events. An animal that lives for 25 years, takes 5 years to mature sexually, and has one offspring every 2 years will have far fewer descendants at the end of 100 years than an animal that has three offspring per yearly reproductive event, lives for 5 years and takes 1 year to mature. Changes due to selection will occur much more slowly in the first species because fewer generations per century mean both fewer mutations and less differential reproduction, thereby increasing the value of behavioral flexibility as a mechanism for change.

1.08.4.2 Benefits

The major hypothesis for the explanation of encephalization is that bigger brains allow enhanced cognitive abilities, abilities useful in certain lifestyles. The problem then becomes the definition of enhanced cognitive abilities and the lifestyles they could be useful in. This hypothesis can be tested by specifying the lifestyles that could benefit from more complex cognition, operationalizing the complexity of cognition, then looking for a statistical association between lifestyle, brain size, and cognitive complexity. General cognitive complexity can be opposed to specialized cognitive skills associated with specific lifestyles in a restricted set of taxa. The spatial memory associated with food caching in corvids and parids or brood parasitism in cowbirds is thought to be one example of the latter, as is the acoustic memory associated with song repertoire size in oscines (DeVoogd *et al.*, 1993). In these cases, specialized neural structures are studied and the cognitive variation is relatively easy to operationalize: memory for more cache locations or acoustic memory for more songs. The small size of the structures (HVC, RA, hippocampus) implies that they are unlikely to form the basis of encephalization. What we are looking for instead is variation in unspecialized cognition over potentially all species, based on the involvement of a large enough part of the brain that can address the issue of whole brain encephalization.

The difficulty is operationalizing unspecialized cognition in all species. Researchers usually look to abilities associated with complex cognition in humans. If we want to include as many taxa as possible in our tests, we have to look outside of tests that only a few nonhuman species can solve, such as learned sign language, episodic memory, fast-mapping, or understanding of the mental states of others (theory of mind). Associative learning is one obvious possibility. If we define the continuum of cognitive complexity as the latency of or the number of errors in learning, however, we face the problem of confounding variables and ecological validity. If a crow solves a learning test in the lab faster than does a kiwi, this might be because that the crow is tamer and less neophobic in the lab, the task favors visual rather than olfactory cues, or the task resembles situations crows encounter often in the field but kiwis do not. What we need is cognition that occurs spontaneously in the field, without the confounding variables of tests in captivity and in a situation that is natural to each animal. Tool use, play, and presumed deception and social learning are possible choices. We can also look to innovative behavior.

We then plot the taxonomic distribution of our cognitive measure from the field and see if, as predicted by encephalization theory, the distributions correlate positively with relative brain size, taking out as many confounding variables as possible. If the results of field and laboratory tests are positively correlated, this would support the assumption that they are both valid estimates of cognition. Research on birds and primates, based on re-analyses of published data as well as new tests (Webster and Lefebvre, 2001; Reader and MacDonald, 2003; Lefebvre *et al.*, 2004), suggests that tests in captivity are indeed positively correlated with field measures. We can further test our assumption that unspecialized cognition exists by predicting positive correlations between the distribution of the different cognitive measures. All of these relationships have been tested in birds and primates (see below). Overall, the analyses conducted up to now on the two taxa suggest convergent evolution of interspecific variation in cognitive abilities (see also Emery and Clayton, 2004). Similar positive correlations between innovation rate, tool use rate, and reversal learning performance have been found in birds and primates, perhaps suggesting that these cognitive abilities are nonmodular (Lefebvre *et al.*, 2004). The only exception for the moment seems to be the relationship between food storing and innovation in 22 species of birds; a negative relationship is found in New World corvids and Old World parids, suggesting a (possibly modular; Lefebvre and Bolhuis, 2003) trade-off between storing and innovativeness.

It is important to note that correlations between brain size and cognitive variables do not demonstrate the survival value of having a large brain, nor are they evidence for natural selection on enlarged brains. The correlations suggest that large brains are on average present in tool using, innovative, playful, social taxa that develop slowly, but they also suggest that small-brained taxa can do well, provided their lifestyles do not include these attributes. A survey of long-term population trends (1968–95) in 40 British bird species provides evidence for selection on large over small brains, with larger declines observed in small-brained species than in large-brained ones (Schultz *et al.*, 2005). Sol *et al.* (2002, 2005) examined colonization success of introduced birds in different parts of the world; some species succeed almost everywhere (e.g., sparrows and blackbirds), while others are extinct after only a few years. Relative brain size (and innovation rate in the zone of origin) significantly predicts variance in colonization success. Contrary to natural invasions, where unsuccessful cases are seldom documented, introductions allow good coverage of the entire spectrum of responses. If introductions are an unbiased estimate of all invasions, then establishment in new areas might be one of the key selective forces that affect encephalization trends (Sol *et al.*, 2005) and the allopatric divergence that often follows invasion, a key mechanism in the association between speciosity and relative brain size in birds (Nicolakakis *et al.*, 2003).

1.08.4.3 Ecology and Lifestyles

To express ecological theory in a very simplified way, the distribution of abiotic factors drives the distribution of vegetation, which in turn drives the distribution of animals. Lifestyles (diet, sociality, and sexual selection) are then driven by the distribution of animals and plants. If biotic and abiotic resources are spatially and temporally predictable and in relatively low-density clumps, a specialized, conservative, territorial polygynist with monoparental care may do better than a generalist, opportunistic, invasive, gregarious monogamist with biparental care. The reverse would apply to spatially and temporally unpredictable resources found in abundant patches. We would then expect selection to act on cognition to provide the information-processing capacity that best suits each lifestyle, with accompanying selection on encephalization (Bennett and Harvey, 1985a). Testing the idea that omnivory should be associated with brain size is thus not an ecological prediction on cognition, but a dietary prediction on encephalization with two missing links: how does resource distribution favor omnivory and how does omnivory require more complex cognition or lift dietary constraints on brain size? The use of an ecological framework is all the more important because the same resource distribution may lead to similar predictions on lifestyle differences that are sometimes viewed as independent pressures for complex cognition and encephalization. For example, social and diet breadth pressures on the evolution of cognition and brains are often seen as alternatives (see Forebrain Size and Social Intelligence in Birds). If, however, one type of resource distribution favors gregarious generalists and another favors territorial specialists, then the two pressures go in the same direction. Whether or not the lifestyle differences are independent is a matter of empirical test (with multivariate techniques, for instance), not a logical *a priori*.

Diet (Eisenberg and Wilson, 1978), sociality (Dunbar, 1992; Dunbar and Bever, 1998), sexual selection (Madden, 2001), and parental care (Gittleman, 1994) have all been shown to be associated with encephalization. In some of these tests, we do not know to what extent the apparent

co-evolution of the traits is due to common ancestry or repeated independent events, given that independent contrasts have not been conducted. Larger brains have been found in omnivorous and frugivorous groups (Allman *et al.*, 1993) compared to folivorous or herbivorous animals. For sexual selection, there is interspecific evidence for an association between brain size and bower building (Madden, 2001) and intraspecific evidence for an association between telencephalon size and song repertoire size in zebra finches (Airey and DeVoogd, 2000). Monoparental versus biparental care, which is a consequence of sexually selected mating systems, has also been implicated in brain size differences (Gittleman, 1994).

1.08.5 Mammals

Mammalian encephalization has received considerable scientific attention, probably because the class contains two of the most encephalized orders, primates and cetaceans, and because we humans number among the 5000 or so mammal species (see Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals). Mammals thus provide a valuable case study for understanding the selection pressures favoring evolutionary changes in brain size, with many hypotheses regarding brain evolution originally applied to mammals and relatively large databases of whole brain and brain component volumes available (for extensive discussion, see the articles on brain evolution in mammals, various mammalian orders, and humans, this volume, e.g., Primate Brain Evolution in Phylogenetic Context, The Evolution of Hemispheric Specializations of the Human Brain, The Evolution of Human Brain and Body Growth Patterns, Mosaic Evolution of Brain Structure in Mammals, Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals, Evolution of the Cerebellum, Evolution of the Hippocampus). This has made possible large-scale comparative studies. Moreover, experimental data have been combined with comparative studies of brain evolution, and breeding experiments have also addressed the evolution of larger brains.

Mammalian encephalization, like encephalization in all animals, is generally assumed to result from the selective benefits of enhanced cognitive, perceptual, or motor abilities, with most research focusing on cognition (Macphail, 1982; Barton, 1999). Evidence regarding the assumed link between brain volume and cognitive capacity has come from two sources: correlations of behavioral demonstrations of cognitive ability and brain size, and from associations between enlarged brains and lifestyles thought to require increased cognitive demands. The behavioral data provide a more direct test of the assumption that cognitive capacity and brain size are linked, and so are key in this respect (Macphail, 1982; Deaner *et al.*, 2000). Several lines of evidence in mammals have pointed to a correlation between a species' relative brain volume and its cognitive capacity. First, laboratory learning data collated from a variety of sources has been shown to correlate with relative whole brain size, although the number of species tested is small (Riddell and Corl, 1977). Second, various measures assumed to indicate general cognitive capacities correlate with relative neocortex size: the frequency of reports of tactical deception, innovation, social learning, and tool use all correlate with relative neocortex volume in primates (Reader and Laland, 2002; Byrne and Corp, 2004). These latter measures have the advantage of covering a larger number of species. Third, it has been proposed that the ability to perform apparently complex cognitive acts such as imitation and understanding the intentions of others are associated with brain enlargement (Byrne, 1992). For example, among mammals, the consensus view is that true imitation has only been experimentally demonstrated in large-brained apes and dolphins, while the smaller-brained mammals tested present equivocal evidence of imitation (Mitchell *et al.*, 1999; Caldwell and Whiten, 2002). However, tests of complex cognition have been the subject of much controversy (e.g., Tomasello and Call, 1997). Moreover, because research has tended to focus on only a few, typically large-brained mammal species, it is difficult to know the true phylogenetic distribution of such traits and to conduct proper comparative analyses.

Problems with comparative studies can be solved by experimental studies of evolution. Studies on rhesus macaques and mice have demonstrated that brain size is heritable (Jensen, 1979; Atchley *et al.*, 1984; Cheverud *et al.*, 1990; Markina *et al.*, 2001), which indicates that it is open to modification by selection. Moreover, selection experiments in mice have bred large- and small-brained lines and have found differences between the lines in performance on learning tasks (Markina *et al.*, 2001). Interpretation of these findings is controversial, not least because learning ability is inferred from performance and a number of other behavioral changes are observed in the selection lines, such as changes in anxiety or exploratory behavior (Jensen, 1979; Johnston, 1982; Markina *et al.*, 2001). A further problem is that selection on learning performance has tended to result in selection for task-specific abilities (Jensen, 1979). Moreover, the critical test of whether selection on learning capacities can lead to evolutionary changes in brain size would be to

select for cognitive ability and to examine the effect on brain size (Johnston, 1982). As far as we are aware, such experiments have not been done, although one study reports a decrease in size of a hippocampus area in mice lines exposed to environmental stressors (such as natural predators; Poletaeva *et al.*, 2001).

The vast majority of work on encephalization has focused on identifying the selection pressures associated with brain enlargement. Hypotheses have tended to fall into two camps, social and ecological explanations for brain enlargement. Social (or Machiavellian) intelligence hypotheses argue that enlarged brains evolved as an adaptation to living in large, complex social groups (Jolly, 1966; Humphrey, 1976; Byrne and Whiten, 1988; Flinn, 1997; Whiten and Byrne, 1997). Byrne and Whiten (1997) distinguish the term "narrow Machiavellianism," the idea that it is selection for strategies of social manipulation or deception that has driven brain evolution, from their own broader use of the term "Machiavellian intelligence," which includes all forms of social intelligence such as social learning. Ecological explanations for the evolution of large brains are also common and include the extractive foraging (Parker and Gibson, 1977) and cognitive/spatiotemporal mapping hypotheses (Milton, 1988; Deaner *et al.*, 2000). Unpredictability and patchiness of resources are often cited as key ecological factors favoring brain enlargement (Eisenberg and Wilson, 1978). For example, fruit is likely to be distributed more patchily in space and time than are leaves, and so a fruiteating diet may be expected to have more cognitive demands than a leaf-eating one. Technical intelligence hypotheses that argue that technology or technical skills drove brain evolution can be considered with the ecological intelligence hypotheses, because they tend to focus on foraging or antipredator tool use (Passingham, 1982; Byrne, 1997a, 1997b).

What evidence is there in support of these hypotheses? Relative neocortex or brain size is positively correlated with social group size in nonhuman primates, carnivores, cetaceans, bats, and some insectivores, consistent with social intelligence hypotheses (Worthy and Hickie, 1986; Sawaguchi and Kudo, 1990; Dunbar, 1992; Marino, 1996; Barton, 1999; but see Connor *et al.*, 1998; van Schaik and Deaner, 2003 on cetaceans). In ungulates, social species tend to have larger relative brain and neocortex sizes than do nonsocial species, (Schultz and Dunbar, 2006). Barton (1993) finds a correlation between group size and neocortex size in haplorhine primates, but not strepsirhines, which may indicate that group living favored brain size evolution among haplorhines only. Adding support to Machiavellian intelligence hypotheses, the frequency of reports of deceptive behavior has been found to correlate with relative neocortex size (Byrne and Corp, 2004). The findings that human and other primates tend to have superior abilities in problems involving social knowledge versus those involving nonsocial knowledge has also been taken as evidence that cognitive abilities developed as a response to social pressures (Cheney and Seyfarth, 1988; see also Baron-Cohen *et al.*, 1999).

In support of ecological intelligence ideas, diets presumed to require increased cognitive demands have been shown to correlate with relative brain volume in several mammalian groups. For example, primate relative whole brain size and neocortex size correlate with frugivory (Barton, 1999) and diet breadth and relative neocortex volume are correlated in African anthropoid primates (Reader and MacDonald, 2003). Similar associations with diet, albeit not based on independent contrasts, have been found in bats (aerial insectivores have smaller brains for their body size than frugivores and nectarivores, with piscivores, foliage gleaners, carnivores, and sanguivores falling between the two extremes; Eisenberg and Wilson, 1978), small mammals (in rodents and lagomorphs, folivores and insectivores have small brains relative to their body mass and body length compared with those of other dietary categories; Harvey *et al.*, 1980), and the Carnivora (carnivorous and omnivorous species have larger relative brains, though not significantly so, than insectivorous carnivora; Gittleman, 1986). In a phylogenetically controlled study of 59 bat species, Ratcliffe *et al.* (2006) have recently reported a larger relative brain size in species that use a flexible combination of gleaning and hawking techniques, compared to those that are specialized on either of the hunting modes. Home range correlates with relative brain size in primates, in support of spatiotemporal mapping ideas (Clutton-Brock and Harvey, 1980; Deaner *et al.*, 2000). Consistent with technical intelligence hypotheses, tool use frequencies correlate with relative neocortex volume in primates (Reader and Laland, 2002; Lefebvre *et al.*, 2004). However, the fact that few primates appear to make regular use of tools in the wild questions the idea that technical intelligence was a driving force for primate brain enlargement (van Schaik *et al.*, 1999).

A number of other lifestyle correlates of relative brain size have been described. For example, in didelphid marsupials, preference for arboreal activity is associated with relative brain volume (Eisenberg and Wilson, 1981). Cerebellum volume

has also been linked to locomotion mode in primates, bats, and cetaceans (Stephan and Pirlot, 1970; Rilling and Insel, 1998; Marino *et al.*, 2000). Females of carnivore species where the females provide the sole parental care have larger brains than those of biparental or communal species (Gittleman, 1994). Neocortex volume has also been linked to sexual selection, being correlated with mating competition in frugivorous primates (Sawaguchi, 1997).

Potential costs and constraints of mammalian encephalization have received less attention, though there is support for the idea that brain enlargement carries metabolic and developmental costs, with brain size negatively correlated with litter size in marsupials (Eisenberg and Wilson, 1981), and positively correlated with the age of sexual maturity and life span, but not gestation length, in primates (Barton, 1999; Allman *et al.*, 1993; Kaplan and Robson, 2002; van Schaik and Deaner, 2003). In odontocetes, relative brain size is also associated with relative time to sexual maturity and life span. Within life span, however, length of the adult period is more closely correlated with relative brain size than is length of the juvenile period, suggesting that the temporal costs of delayed maturity might be compensated by a longer time as a reproducing adult (Lefebvre *et al.*, 2006). Primate brain size is not correlated with basal metabolic rate, and comparative analysis indicates that improved diet quality, by allowing reduction in gut mass relative to body size, is one possible mechanism allowing the energetic constraints on the evolution of the metabolically expensive large brain to be lifted (Aiello and Wheeler, 1995; Barton, 1999; Fish and Lockwood, 2003).

Given that there is support for a number of hypotheses regarding mammalian encephalization, can any consensus be formed? The findings described above are consistent with the idea that several selective pressures are responsible for the evolution of encephalization. An alternative view is that one factor is driving brain evolution, but that the cognitive abilities afforded by a large brain are applied to other domains. Determining causation is difficult since most comparative studies are based on correlational evidence. Moreover, the divisions between social and ecological intelligence may be fuzzy and social and ecological demands on cognition may evolve together, making it difficult to consider social and ecological intelligence hypotheses as alternatives (Barton, 1999; Deaner *et al.*, 2000; Reader and Laland, 2002; Seyfarth and Cheney, 2002). It is also difficult to separate perception from cognitive demands. For example, primate brain size variation is associated with visual specialization. Visual processing may be critically involved in the treatment of both social and ecological information; relative expansion of parts of the visual system is correlated with both frugivory and group size (Barton, 1999). What is clear, however, is that evolution has shaped mammalian brains in response to the demands of their lifestyles, with convergent evolution of brain structures in several groups (De Winter and Oxnard, 2001; Kaas, 2002).

1.08.6 Birds

It was long believed that the avian forebrain was composed of hypertrophied basal ganglia with only meager pallial derivatives, whereas in mammals, the pallium had grown into a highly parcellated laminar neocortex (Ariens-Kappers *et al.*, 1936). The recent revised nomenclature of the avian telencephalon (Reiner *et al.*, 2004) recognizes many anatomical and functional similarities between the avian and mammalian forebrains. In particular, the newly named avian nidopallium, mesopallium, and arcopallium are considered homologous to mammalian pallial derivatives, the neocortex, claustrum, and pallial amygdala (Karten, 1969, 1991; Güntürkün, 1991; Wild *et al.*, 1993; Butler, 1994; Veenman *et al.*, 1995; Striedter, 1997; Reiner *et al.*, 1998; Smith-Fernandez *et al.*, 1998; Medina and Reiner, 2000; Puelles *et al.*, 2000).

A large amount of variance in the size of both adult and hatchling avian brains can be explained by the altricial versus precocial dichotomy in development mode. Birds that develop slowly and require extensive parental care are born with relatively smaller brains than birds that are mobile only a few minutes or hours after birth. The reverse applies to adult brain size, where altricial birds have larger brains than do precocial ones (Portmann, 1946; Bennett and Harvey, 1985a, 1985b). In Bennett and Harvey's study, most of the ecological variables (e.g., diet, habitat) that showed a relationship with relative forebrain size in univariate analyses became nonsignificant when development mode was included in multivariate statistics. Only mating system (monogamous → polygynous) and mode of prey capture (moving from a perch vs. other categories) remained significant predictors of relative size of the brain and forebrain. All large-brained avian clades develop slowly, but the reverse is not true. The altricial Columbiformes (pigeons and doves) and Caprimulgi (nightjars) are, in relative terms, not much more encephalized that the precocial Galliformes and ratites. On average, growing a large brain may impose some limits on incubation

energetics, as well as the length of intrauterine growth. It might not be possible for birds to stock sufficient energy in the egg for extensive brain growth. Stark (1993) offers some interesting observations of the comparison of brain growth in altricial and precocial birds. He found that in the freshly hatched buttonquail, a precocial species, all telencephalic areas and fiber pathways have undergone differentiation and started myelinization. There are no more areas of cell proliferation in the hatchling, indicating that the number of neurons is definite and that postnatal volume increase can be exclusively attributed to growth. Similarly, the optic tectum of the hatchling muscovy duck has almost reached adult size and differentiation level. In contrast, the three altricial species that Stark (1993) studied have a significant posthatch cell proliferation in the periventricular zone. In the budgerigar and Java sparrow, cell proliferation continues until the 10th postnatal day. Stark suggests that a large postnatal increase in the volume of the brain depends on a persistence of this large periventricular proliferation zone, which can be maintained as long as there are no functional demands on the developing systems. This is possible in altricial hatchlings freed from the need for a functional forebrain by extended parental care. Stark (1993) proposed that to arrive at a larger brain volume, more cells have to divide during the proliferation phase. Theoretically, the increase in cell numbers can be achieved in three ways: increasing the rate of periventricular cell division, increasing the area of cell proliferation, or lengthening the proliferation period. There is no empirical support for the first two options in birds; the third option is possible only in altricial species where parental care compensates for the lack of functional independence by the chicks. Stark (1993) also suggests that nutritional constraints on the hatchlings affect the options: precocial species tend to eat foods that can be easily obtained, while altricial ones eat foods that are widely dispersed or difficult to find. In a recent study, Iwaniuk and Nelson (2003) corroborate the conclusions of Stark (1993) using continuous development time measures in addition to the dichotomous precocial/altricial classification. They divide development into four periods: incubation, fledging, postfledging parental care, and total period of parental care. All developmental periods except time to fledging are significantly correlated with brain size, once common allometric correlates are removed. The relationships vary with development mode, and Iwaniuk and Nelson (2003) suggest that factors such as diet and foraging techniques interact with development in determining brain size.

If the relationship between ecology and encephalization appears to be confounded by developmental constraints, this is not the case for more direct measures of cognition. Lefebvre and collaborators have quantified avian cognition in the field by measuring the frequency of novel, unusual, or rare feeding behavior in over 800 species in five areas of the world (see Lefebvre *et al.*, 1997, 1998 for examples). They have collated over 2300 cases of innovative feeding and 130 cases of tool use and shown that both measures of cognition show taxonomic distributions that are positively correlated with relative size of the brain and forebrain (Lefebvre *et al.*, 1997, 1998, 2001, 2002, 2004; Nicolakakis and Lefebvre, 2000). Nine potential confounding variables have been included in these analyses, to ensure that biases inherent to the quantification of anecdotal judgments on novelty and cognitive complexity do not affect the biological trends. One of these confounding variables was development mode, which does not account for the correlation between encephalization and either innovation rate or tool use frequency.

Using the detailed brain data on 32 species from 17 parvorders gathered by Boire (1989) and Rehkämper *et al.* (1991a), and the innovation and tool use rates from previous papers, Timmermans *et al.* (2000) and Lefebvre *et al.* (2002) were able to pinpoint the avian telencephalic areas most closely associated with cognition. Rehkämper and Zilles (1991) and Boire and Baron (1994) have suggested that it is the disproportionate increase of the size of the nidopallium and mesopallium that drove the enlargement of the avian telencephalon. Consistent with these predictions, Timmermans *et al.* (2000) showed that it is the relative size of the mesopallium that correlates most closely with innovation rate. In simple regressions, the nidopallium, hyperpallium (Wulst), and components of the striatopallidal complex were also all correlated with innovation rate, with or without phylogenetic corrections. In multiple regressions, however, these structures dropped out of the model because of their strong correlations with the size of the mesopallium, which explains a larger proportion of the common variance. Lefebvre *et al.* (2002) repeated a similar analysis with two types of tool use, true tools and proto-tools, which are described in over 100 avian species (see also Boswall, 1977, 1978, 1983a, 1983b for comprehensive reviews). Proto-tools involve the use of objects that are part of a substrate, e.g., anvils on which prey are battered or dropped, or wedges and thorns with which food is held. True tools are detached from the substrate, e.g., hammers, probes, scoops, sponges, and levers held directly in the beak or foot;

their use is presumed to require more complex cognition that that of proto-tools. Lefebvre *et al.* (2002) confirmed that true tool users have larger brains than do proto-tools users and that, within the forebrain, relative size of the nidopallium and mesopallium are the best predictors of avian tool use frequency. The mesopallium comprises higher-order, multimodal processing areas. The nidopallium features tertiary areas of this type, but also includes primary projection fields from both somatosensory and visual pathways, as well as secondary areas that receive input from these primary fields (Rehkämper *et al.*, 1985). The nidopallium thus has the necessary features for both the cognitive and sensorimotor aspects of tool use, in particular the integration of visual and somatosensory information involved in the fine manipulation of objects.

Beyond these comparative studies of the whole avian spectrum, a few authors have concentrated on encephalization patterns within particular orders such as Anseriformes, Trochiliformes, and Psittaciformes. Iwaniuk and Nelson (2001) recently examined a large number of waterfowl. This group is of particular interest because it is precocial, keeping constant the main confounding variable identified by Bennett and Harvey (1985a). Iwaniuk and Nelson worked from endocasts of museum specimens of 354 individuals representing 55 species. Their analysis did not show any significant relationship between foraging mode or diet and relative brain size in Anseriformes, which does not preclude that further analyses on finer brain structures might not reveal clearer trends. One interesting species in their sample is the musk duck, *Biziura lobata*. It has a large brain compared to its sister species and also shows a much more altricial mode of development than do other Anseriformes, raising only one or two offspring that do not feed themselves right after hatching, but rely instead on the mother and slowly become independent. Trochiliformes (hummingbirds) were studied by Rehkämper *et al.*, (1991b); they show a level of encephalization intermediate between that of Galliformes and Passeriformes. It is not clear if hummingbirds' encephalization level is a product of relative brain enlargement or selection for small bodies. Boire (1989) and Boire and Baron (1994) suggest that it is cerebellum size that might be the main component of brain enlargement in this order, in line with the complex motor control required for hovering. Terns and swifts, which have more complex flight behavior than other birds, also show an enlarged cerebellum (Boire, 1989; Boire and Baron, 1994). Psittaciformes have recently been examined by Iwaniuk *et al.* (2005), who measured whole brain size in 180 species, as well as the size of brain regions in 19 species. Their study confirms previous work (Portmann, 1946, 1947a; Boire, 1989, Boire and Baron, 1994) showing that this order, which shows complex cognitive abilities (Pepperberg, 1999, 2002; Borsari and Ottoni, 2005), has a larger telencephalon than other nonpasserine birds, while subtelencephalic brain components show a much smaller range of variation. Psittaciformes are among the birds showing transactional social behavior in the classification proposed by Burish *et al.* (2004). This complex form of sociality is associated with larger ratios of telencephalon to total brain size.

Besides the avian equivalents of the mammalian neocortex, the mesopallium and nidopallium, areas such as the olfactory bulb and the hippocampus have also been subject to comparative studies. In general, birds are considered microsmatic, but there is increasing evidence that many of them use smell in foraging, orientation, and homing, as well as site and individual recognition (see references in Healy and Guilford, 1990). There is a large database on the size of avian olfactory bulbs (Bang and Cobb, 1968) showing considerable taxonomic variation; unfortunately, these data are not actual volumes, but the ratio between the largest diameter of the olfactory bulb and that of the longest length of the cerebral hemispheres. This measure is not independent of the size and shape of the cerebral hemispheres, and the data should therefore be interpreted with care. Initial interpretations of these data led to the conclusion that large olfactory bulbs are associated with aquatic habitats (Bang and Cobb, 1968; Bang, 1971). A more careful statistical analysis suggested that nocturnal birds have larger olfactory bulbs (Healy and Guilford, 1990). The hypothesis was that olfaction might be useful for birds in low-light conditions for tasks such as site recognition and location of predators and slow-moving or stationary prey. For the moment, this proposed association between activity pattern and olfactory bulb size is interesting, but awaits a more reliable database. It should be noted that large olfactory bulbs in birds are not generally associated with the enlargement of the telencephalon. However, it has been suggested that in Anseriformes, the increased telencephalization is in part correlated with enlarged olfactory structures (see Rehkämper *et al.*, 2001). This is shown by the considerable expansion of telencephalic targets of olfactory projections (Ebinger *et al.*, 1992). For example, the olfactory structures in Anseriformes are twice the size of those in the pigeon (Ebinger *et al.*, 1992).

In absolute size, the avian hippocampus is quite small compared to that of mammals, but several

studies suggest a correlation between the size of this structure and lifestyles implying more spatial cognition. The hippocampus is larger in food-storing birds than in nonstorers (Krebs *et al.*, 1989; Sherry *et al.*, 1989; Healy and Krebs, 1992, 1996; Healy *et al.*, 1994; Hampton *et al.*, 1995; Basil *et al.*, 1996; Volman *et al.*, 1997). Spatial cognition is not only relevant to food gathering, but also to homing abilities (Rehkämper *et al.*, 1988) and spatial abilities in finding host nests in brood-parasitic cowbirds (Sherry *et al.*, 1993; Reboreda *et al.*, 1996). Some authors (e.g., Bolhuis and Macphail, 2001) have criticized this literature, but Lucas *et al.* (2004) have recently shown that despite differences between species from North America and Europe (Brodin and Lundborg, 2003), there is a clear correlation between the degree of food-caching specialization and hippocampus size in Corvidae and Paridae. In pigeons, breeds that were artificially selected for homing have a larger telencephalon than nonhoming breeds, and this seems to be the result of an enlarged hippocampus (Rehkämper *et al.*, 1988). In food-caching birds, most studies conducted at the species level report no correlation between the size of the hippocampus and that of the telencephalon (Healy and Krebs, 1992, 1996; Healy *et al.*, 1994; Hampton *et al.*, 1995; Basil *et al.*, 1996), but others have found a correlation at the level of the subfamily and family within Passerines (Sherry *et al.*, 1989). It is interesting to note that food-caching experience leads to neurogenesis both in the hippocampus of young marsh tits and in the mesopallium (Patel *et al.*, 1997). This could mean that the more specialized, hippocampal, component of spatial memory may be linked to more generalized problem-solving processes in the mesopallium. This might explain the fact that species differences in food caching in the field are often stronger than those seen in spatial memory tests in captivity. Lefebvre and Bolhuis (2003) report a negative or zero correlation between innovation rate and reliance on food caching in corvids and parids. If the captive tests solicit both specialized spatial memory and more general problem-solving ability, interspecifc differences would be magnified by a positive correlation between the two processes, but dampened by a negative or zero correlation. More research is clearly needed on this point. In the other intensively studied avian specialization, imitated song (Jarvis *et al.*, 2000), correlated evolution of small, specialized nuclei and larger telencephalic structures has been suggested by DeVoogd and coworkers. Airey *et al.* (2000) have shown that zebra finches have heritable variation in both the size of their song repertoire and the size of nucleus HVC, the control center for syllable organization (Yu and Margoliash, 1996). HVC size is positively correlated with whole telencephalon size in zebra finches, leading DeVoogd (2004) to suggest that song repertoire might be an honest signal for general cognitive ability.

1.08.7 Invertebrates

Encephalization has not been as well studied in invertebrates as in vertebrates. Some structures have, however, been thought to play equivalent roles to the ones that the forebrain plays in mammals and birds. Invertebrates often cited for their cognitive skills are the hymenoptera on the one hand and the octopus and cuttlefish on the other (see Cognition in Invertebrates). Hymenoptera have the most complex social behaviors of all insects. Octopus and cuttlefish are at the extremes of the habitat complexity distribution proposed by Hanlon and Messenger (1996, figure 3.9). The same intraclass logic we have applied earlier to birds and mammals can thus be applied to the groups that hymenoptera and octopus belong to, insects and cephalopods. In these classes, the mushroom bodies and vertical lobes, respectively, are the brain structures most often mentioned in studies of encephalization.

1.08.7.1 Insects

In insects, the mushroom bodies have long been seen as the higher centers that might be the substrate of cognition (see Strausfeld *et al.*, 1998, and Farris, 2005, for reviews). They control sensory integration, learning, and memory, and, according to Farris (2005), are convergent equivalents of the mammalian cortex. Their crucial role in memory is evidenced by *Drosophila* mutants that lack both the vertical lobes of the mushroom bodies and long-term (but not short-term) memory (Pascual and Préat, 2001). The insect taxa (ants, honeybees, and wasps) that have evolved complex societies with division of labor, as well as altruistic reproduction and nest defense, have enlarged mushroom bodies (Howse, 1974; Gronenberg *et al.*, 1996; Ehmer and Hoy, 2000). Diet might be as important as social life in determining insect mushroom body size. Mares *et al.* (2005) found that honeybees (*Apis mellifera*) do not have larger mushroom bodies than does the bumblebee *Bombus impatiens*, as one would have predicted from the much more complex social life of honeybees. *B. impatiens* is a dietary generalist, however, which raises the intriguing possibility that specialized species of *Bombus* might have smaller

mushroom bodies than either *B. impatiens* or *A. mellifera*. Farris and Roberts (2005) compared 11 generalist and specialist scarab beetle species and found sharp differences in mushroom body size and structure associated with dietary differences. Generalist (e.g., phytophagous) beetles have larger and more convoluted mushroom bodies featuring double calyces, whereas specialist species (e.g., dung beetles) have smaller mushroom bodies with single calyces. Ontogenetic changes, both natural and experimentally manipulated, that make honeybees switch from larval care to the much more complex task (e.g., learning and dance communication of flower patches, swarming; Seeley and Burhman, 1999) of foraging outside the hive are accompanied by an increase in the Kenyon cells of the mushroom bodies (Withers *et al.*, 1993). Similar results have also been reported for carpenter ants (Gronenberg *et al.*, 1996)

1.08.7.2 Cephalopods

Compared to other classes, the relative brain size of cephalopods is between that of fish and reptiles on the one hand and birds and mammals on the other (Packard, 1972). Within the 800 or so cephalopod species, there is a large degree of variation in learning performance, brain size, and vertical lobe size. The vertical lobe is the area of the cephalopod brain that Nixon and Young (2003) describe as the modulator for the systems that guide visual and tactile responses. Nixon and Young (2003) list the relative size of 14 brain areas in 63 species (see also Wirz, 1959). The data in their table 2.6 are expressed as fractions of total brain size. As Hanlon and Messenger (1996) point out, the two genera that are most often mentioned as intelligent, octopus and cuttlefish (*Sepia*), do not have the largest vertical lobes according to this fraction estimate. However, when we use the more usual technique of regressing either whole brain size or vertical lobe size against body size (in this case, mantle length, given for 49 of the species), the two species are 1.5 (octopus) and 2.5 (cuttlefish) standard deviations above the mean cephalopod regression line. The third cephalopod whose nervous system has been intensively studied, the squid *Loligo*, places around 2 standard deviations above the line.

Many studies have been conducted on associative learning in octopus by Young and his colleagues (Wells, 1966). Three features of avian and primate cognition, innovation, social learning, and improvement over successive learning reversals, have been described in the field by Norman (1999) and in the lab by Fiorito and Scotto (1992) and Mackintosh and Mackintosh (1964). Octopus in Indonesia forage for complimentary fragments of coconut shells thrown by humans in shallow water, using them as portable dens (Norman, 1999). Octopus (Fiorito *et al.*, 1998) can also solve the kind of innovative food-finding problem that passerines, but not doves, readily succeed at (Webster and Lefebvre, 2001; see however, Bouchard, 2002 for pigeons). Finally, octopus that observe a trained conspecific attack a white stimulus (instead of the normally preferred dark stimulus), will also attack the white stimulus when tested alone after the observation sessions (Fiorito and Scotto, 1992). Lesions of the vertical lobe, which are known to affect associative learning in octopus, also affect observational learning, but only over short time intervals (Fiorito and Chichery, 1995). The octopus vertical lobe seems to show evidence of convergent evolution with vertebrate learning mechanisms, with long-term potentiation of glutaminergic synaptic field potentials (Hochner *et al.*, 2003). In cuttlefish, the vertical lobe also appears to be involved in learning. In particular, Dickel *et al.* (2001) show a striking similarity between ontogenetic increases in the relative size of the vertical lobe (but not of other areas) and improvements in learning.

1.08.8 Conclusion

Studies of encephalization often focus on taxonomic differences in the size of whole brains or of forebrain areas. This does not necessarily mean that size is the key causal variable behind differences in cognitive performance. Many other features of nervous systems, e.g., synaptic networks, neuronal density, neurotransmitter facilitation, might be equally or more important. Comparative studies on brain size have often been justified by the ease with which broad data bases could be gathered on preserved brains (Portmann, 1946, 1947a, 1947b), endocasts (Mlikovski, 1989a, 1989b, 1989c, 1990; Iwaniuk and Nelson, 2002) and fossils (Burish *et al.*, 2004; see The Hominin Fossil Record and the Emergence of the Modern Human Central Nervous System). However, recent molecular work suggests that size may indeed be one of the crucial aspects of adaptive evolution of brains. Dorus *et al.* (2004) examined 214 genes in humans, macaques, mice, and rats and found that those with the highest rates of evolution in primates determine brain size. Genes not involved with the nervous system or involved in physiological rather than developmental aspects of the brain showed similar evolutionary rates in rodents and primates. This suggests that size differences may be more than easy proxies for subtler differences in anatomy and function. Disregarding for the

moment the major interclass transitions in the way the nervous system is organized, comparisons within taxa suggest that differences in cognitive performance show convergent co-evolution with differences in the size of association areas and of whole brains. Within the constraints of a highly conserved neuronal and synaptic machinery, building an intelligence might be similar to moving an animal through the air. In the same manner that locusts, bats, and albatrosses have evolved convergent, independent solutions to similar problems posed by gravity and lift, honeybees, cuttlefish, crows, and chimpanzees might also have evolved convergent solutions to the common problem of flexible processing and storage of information.

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1.09 Epigenetic Responses to a Changing Periphery – Wagging the Dog

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Glossary

cortical barrel A circular region of the neocortex visible in various histological stains of the somatosensory area in rodents where touch information from a single whisker projects. First recognized by Woolsey and Van der Loos (1970) in mice.

cortical magnification The relative size of a representation, or processing area for a sensory input, in the cortical map. This generally refers to the larger representations of behaviorally important sensory inputs as compared to less important inputs. A common example in humans is the large area of cortex devoted to processing touch information from the hand relative to other, larger body parts (such as the leg or back) that have a proportionally much smaller representation in the cortex.

cytochrome oxidase A mitochondrial enzyme. Processing brain tissue to reveal the distribution of this enzyme often reveals different subdivisions, particularly in the neocortex. Cortical barrels can be seen in the distribution pattern of this enzyme (Figure 1).

Eimer's organ A small (40–80 μm) swelling in the nasal epidermis of talpid moles that contains an orderly array of mechanoreceptors used for tactile discriminations. Similar to a push-rod in monotremes.

epigenetic Generally refers to changes to the phenotype that are not the direct result of alterations to the DNA sequence. In the more restricted case of this review, changes to the phenotype of the brain are considered that may result from alterations to the body, rather than from changes in gene expression in the central nervous system.

glabrous Hairless.

mystacial vibrissae The large, mobile whiskers on the face of a rodent.

neocortex The outer six-layered sheet of brain tissue in mammals where much of the information from sensory receptors projects. Often shortened to 'cortex' in discussions of the mammalian brain. Many investigators prefer the term 'isocortex' to avoid the implication of an invalid phylogenetic sequence suggested by the term 'neo'.

ocular dominance column Stripes of cortical tissue in layer IV of primary visual cortex that receive input from the lateral geniculate nucleus projecting information from primarily only one eye. Each stripe is generally bound by similar stripes representing the opposite, contralateral eye.

saccadic In a manner similar to a saccade. A saccade is a sudden, jerky movement. The term 'saccade' is most frequently used in reference to an eye movement. In the visual system a saccade is the characteristic sudden movement of the eye that positions different parts of a visual scene on the retinal fovea.

sensory representation Generally refers to a topographic map of primary afferent inputs to the central nervous system. In the case of the somatosensory system, the sensory representations reflect the distribution of mechanoreceptors in the skin, and as such they form a map of the body surface which can be identified in neocortex by recording the activity of nerve cells in response to stimulating the skin.

somatosensory cortex The area of neocortex that receives and processes touch information from mechanoreceptors on the body.

tactile fovea The descriptor draws an analogy between the high-resolution retinal fovea in the visual system and the high-resolution part of the star-nosed mole's nose used for detailed, tactile investigations of objects of interest. A similar analogy with the visual system has been made in the auditory system of bats, where an auditory fovea is said to represent the most important echolocation frequencies.

1.09.1 Introduction

Recent advances in the ability to investigate gene expression during brain development have revealed a number of ways in which the neocortex is patterned during development and suggested how these patterns may have been altered in the course of mammalian evolution. Accumulating evidence indicates that gradients of proteins produced from signaling centers provide a coordinate system for the position of cortical fields and incoming thalamocortical afferents during development, and manipulation of the expression patterns of these molecules results in predictable alterations in the positions of entire cortical fields (Nakagawa *et al.*, 1999; Bishop *et al.*, 2000; O'Leary and Nakagawa, 2002; Fukuchi-Shimogori and Grove, 2003; Shimogori *et al.*, 2004).

In a recent landmark study, Fukuchi-Shimogori and Grove (2001) were able to induce the development of a second, mirror-image representation of the mystacial vibrissae in mouse somatosensory cortex by generating an extra, caudally located signaling center producing the fibroblast growth factor FGF8. This result is of particular relevance for theories of brain evolution in mammals because the addition of a mirror-image cortical area, or map, adjacent to an existing sensory representation has clearly occurred repeatedly in the course of mammalian evolution as brains have become larger and more complex (Kaas, 1987, 1993; Krubitzer, 1995). Thus, Fukuchi-Shimogori and Grove have been able to alter gene expression to produce a configuration of cortex that mimics a commonly observed product of brain evolution. Such studies suggest the genetic mechanisms by which relatively large-scale changes to sensory representations may have occurred.

However, my purpose is to emphasize a more gradual process of brain evolution that may often be a precursor to larger-scale changes requiring altered gene expression in the central nervous system (CNS). Here I suggest that many important evolutionary changes in brain organization occur simply by altering the development of the body. The well-documented, plastic nature of the developing nervous system could then accommodate new configurations of the sensory periphery through cascades of inductive events, largely independent of altered expression of patterning genes in the CNS. This idea is not new, or necessarily surprising. However, much attention is currently being focused on gene expression in the CNS and it seems important to consider simultaneously the sensory periphery – the interface between an animal and its environment.

My goal is briefly to summarize evidence from studies that support the instructive relationship between the sensory periphery and the CNS and to extend the discussion in some small but important ways. In particular, I will present evidence for variations in the sensory periphery that are paralleled in the brains of star-nosed moles. The mechanosensory star in this species and corresponding modular representation in cortex are the result of relatively recent selective pressure for elaboration of the somatosensory system, and the variations reported here were found in adult animals captured in the wild. These variations therefore represent the raw materials upon which natural selection is currently acting in these populations, and as such they are particularly relevant examples for the topic of brain evolution. I will also discuss evidence for another mechanism by which evolution may alter brain organization by acting in the periphery. Evidence from star-nosed moles suggests that the timing of developmental events in sensory sheets may have an important effect on the corresponding magnification of these areas in developing cortical maps. To lay the groundwork for this discussion, I first describe the whisker-barrel system in cortex of rodents and some of the variations observed in their sensory systems (see Mosaic Evolution of Brain Structure in Mammals, The Evolution of Neuron Classes in the Neocortex of Mammals, Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications, Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals, The Evolution of Crossed and Uncrossed Retinal Pathways in Mammals, Do All Mammals Have a Prefrontal Cortex?, The Evolution of the Basal Ganglia in Mammals and Other Vertebrates, The Evolution of the Dorsal Thalamus in Mammals).

1.09.2 Supernumerary Whiskers Revisited

Van der Loos and Dorfl (1978) published a short paper posing the provocative question: does the skin tell the somatosensory cortex how to construct a map of the sensory periphery? The focus of their investigation was the relationship between the whisker pattern on the face of mice and the visible, isomorphic reflection of this pattern in the primary somatosensory face region by a series of cortical barrels (Figure 1). The authors suggested three alternative possibilities for how this relationship becomes established: (1) the brain imposes a map on the periphery, (2) the periphery imposes its spatial organization on the brain, and (3) brain maps and the periphery develop independently.

Their paper revolved around the observation that some of the mice in their colonies were born with extra whiskers, and in these cases there were invariably corresponding extra barrels in the cortical representation in the correct spatial location. The central question was how the cortex became matched to the altered sensory periphery (Figure 2). The possibility that the brain imposes a pattern on the periphery was ruled out on the basis of clear developmental evidence showing that the sensory periphery develops well before the map in the somatosensory cortex and thus the sensory representation does not have an opportunity to direct the development of whiskers (for review, see Killackey *et al.*, 1995). At the same time, the peripheral-to-central developmental sequence provides at least the opportunity for the sensory periphery to guide the formation of somatosensory cortex through cascades of inductive events that are communicated from the skin, to the brainstem nuclei, to the thalamus, and finally to the developing neocortex.

The last possibility, that the brain and periphery make their debut independently, was considered unlikely. Van der Loos and Dorfl (1978) argued that it would be an extraordinary task for the genome to code for these changes independently, and concluded that the answer to the question in their title was yes – the skin tells the somatosensory cortex how to construct a map of the periphery.

Yet despite the well-reasoned argument from supernumerary barrels, it was not possible to rule out completely a genetic code for their representation in the brain. A recent investigation by Ohsaki *et al.* (2002) provides new evidence for the role of the skin surface in guiding the developing barrel cortex by selectively altering early gene expression specifically at the whiskerpad to induce the development of extra whiskers. By transfecting the follicular skin surface of early mouse embryos (E9.5–E11.5) with an adenovirus containing the Sonic hedgehog gene from chicken, various configurations of supernumerary whiskers were induced. The supernumerary whiskers were represented in the contralateral somatosensory cortex by extra barrels in the correct topographic position. This elegant separation of gene expression in the skin surface and CNS seems to provide conclusive support for the extrinsic nature of the signal to cortex for producing an extra barrel, as originally suggested by Van der Loos and Dorfl (1978).

The whisker-barrel system in rodents has thus been a critical model system for investigating the instructive relationship between the sensory periphery and the brain. This was possible because of the clear reflection of peripheral receptor topography in the architectural organization of rodent somatosensory cortex (Woolsey and Van der Loos, 1970). But how general is this kind of relationship between sensory organs and modules in the brain, and what kinds of variations of receptors arrays are found outside laboratory rodents?

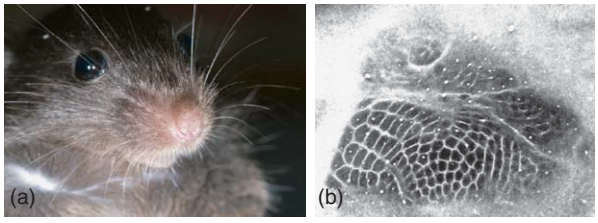

Figure 1 Cortical barrels in the mouse as revealed by cytochrome oxidase histochemistry. In many rodents, the cortical representation, or map, of various body parts in primary somatosensory cortex (S1) can be made visible with a range of cellular stains when the cortex is flattened out and sectioned from the top down to reveal layer IV. a, The face of a mouse showing the prominent facial vibrissae. b, A section of flattened right cortex processed for the metabolic enzyme cytochrome oxidase to reveal the prominent cortical barrels in layer IV. Rostral is to the right and medial is up. The large facial vibrissae correspond to the barrels visible in the lower left quadrant, whereas the smaller facial whiskers are represented by the smaller, more rostral barrels.

1.09.3 Stars and Stripes in the Cortex

Star-nosed moles have an exceptionally well-developed somatosensory system allowing them to identify and consume small prey items faster than any other mammal (Catania and Remple, 2005). This ability stems from the densely innervated mechanosensory star covered by tens of thousands of tactile Eimer's organs (Catania, 1995). As occurs for whiskers in rodents, the specialized glabrous

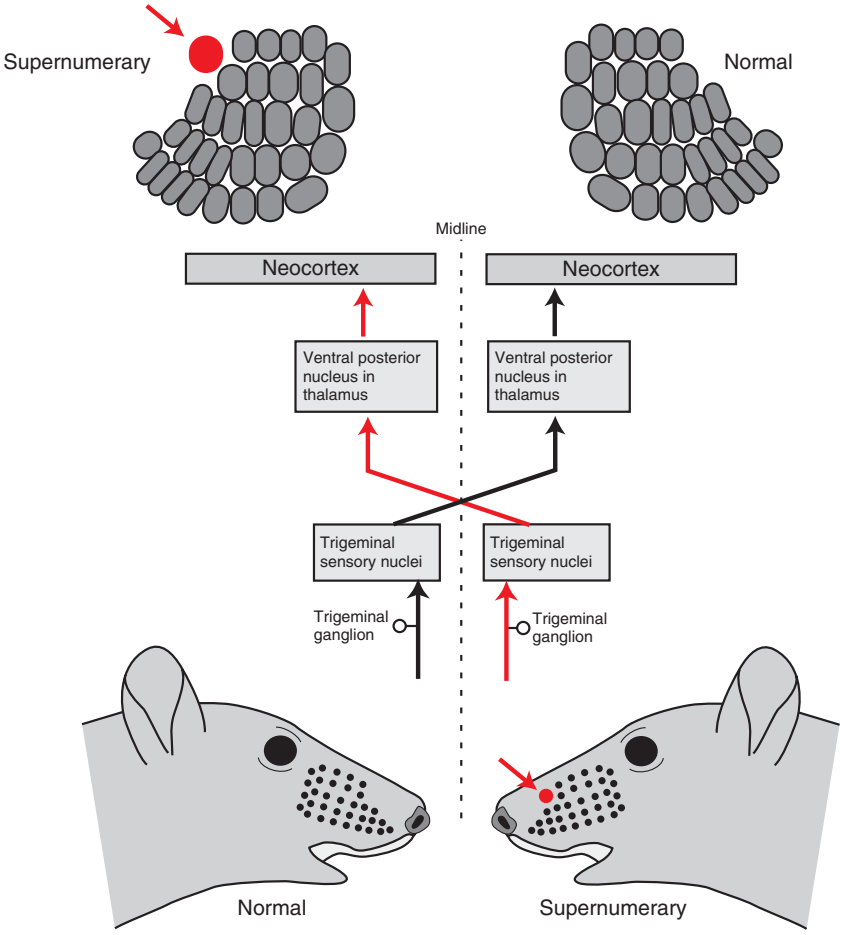

Figure 2 The relationship and pathways between the sensory vibrissae on the rodent snout and their representation in cortex by a series of barrels. Note the extra whisker illustrated on the left face of the mouse (right bottom side of figure) and the corresponding extra barrel in the contralateral somatosensory cortex. This observation inspired Van der Loos and Dorfl (1978) to suggest that the skin surface tells the somatosensory cortex how to construct a map of the sensory periphery.

skin surface of the mechanosensory star is represented in somatosensory cortex by a series of modules that reflect the topographic arrangement of mechanosensors in the periphery. The modules appear as a series of stripes visible in flattened brain sections processed for the metabolic enzyme cytochrome oxidase (Figure 3; Catania *et al.*, 1993). In star-nosed moles, histologically visible representations of the mechanosensory appendages are found in three separate cortical areas (S1, S2, and a third area we have called S3). Similar modular representations of skin surfaces (including the primate hand) have since been identified in other species as well (for review, see Catania, 2002).

The elaborate mechanosensory star and its multiple, modular representations in cortex are the result of comparatively recent selective pressure for the expansion of the somatosensory system (Catania, 2000; Catania and Remple, 2005). As a result this is a particularly useful species to examine when searching for clues to how peripheral receptor arrays and central brain organization have coevolved to produce complex sensory systems that are functionally integrated.

Star-nosed moles usually have a complement of 22 appendages ringing the snout. These occur as 11 symmetric pairs that are numbered from 1 to 11 on each side, starting with the dorsal-most appendage (Figure 3). As is the case for laboratory rodents, star-nosed moles are sometimes born with an abnormal configuration of the snout. This often includes the loss or addition of an appendage to one side of the nose and, when this occurs, the contralateral somatosensory cortex (Figure 4) invariably reflects the topography of the abnormal star (Catania and Kaas, 1997a).

There are a number of interesting implications of this result. Most obviously, this finding parallels the results of Van der Loos and Dorfl (1978) supporting the contention that skin surfaces play an instructive

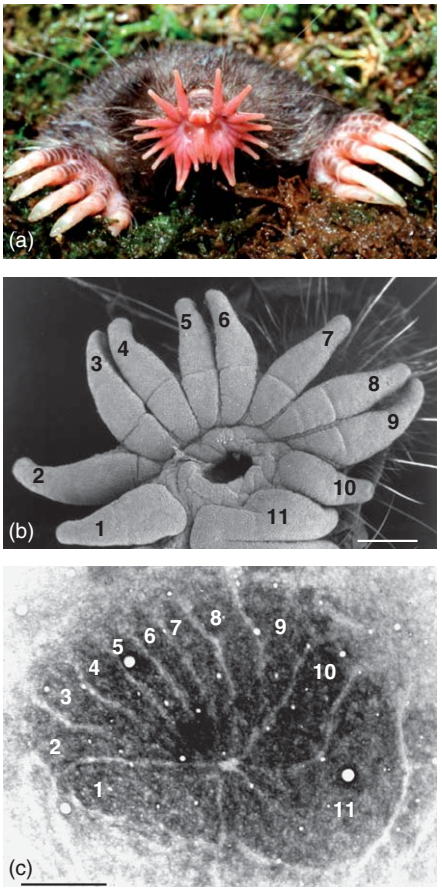

Figure 3 The sensory system of the star-nose mole. As occurs for the whiskers of rodents, the cortical representation of the mechanosensory appendages of the star-nosed mole is visible in various histological stains of the cortex. a, A front view of the star-nosed mole emerging from its underground burrow. Note the 22 appendages ringing the nostrils. b, A scanning electron micrograph of half of the star, rotated to match the representation in cortex in plate (c) below. The dark ring in the middle is the nostril. Dorsal (appendage 1) is to the left. c, A section of the flattened somatosensory cortex processed for the metabolic enzyme cytochrome oxidase to show the 11 modules where information from each of the 11 nasal appendages projects in primary somatosensory cortex (S1). Note the greatly overrepresented (enlarged) representation of the 11th appendage. The 11th appendage is the somatosensory fovea of the star (see text). Scale bars: b, 1 mm; c, 500 μm. b and c, Reproduced from Catania, K. C. 2001. Early development of a somatosensory fovea: A head start in the cortical space race? *Nat. Neurosci.* 4, 353–354, with permission.

role in guiding the formation of topographic maps in the CNS, and demonstrating the generality of this instructive relationship beyond the whisker-barrel system of rodents. It also shows that these variations are occurring in wild populations of mammals upon which natural selection is currently acting to shape the sensory periphery, and therefore the brains, of natural populations. Finally, the relatively frequent occurrence of these abnormalities is interesting. We have found as much as 5% of the population may have an abnormal number of appendages. This can be contrasted with the much lower rate of abnormalities for other appendages (Castilla *et al.*, 1996; Zguricus *et al.*, 1998). Darwin (1859) suggested an explanation for this kind of observation in *On the Origin of Species*, stating that "in those cases in which the modification has been comparatively recent and extraordinarily great ... we ought to find the *generative variability* still present to a high degree." The argument is that selection has had less time to fix characteristics of recently evolved complex structures which are the product of selection for variations. This is exactly what we find in wild populations of star-nosed moles and this is not surprising given that the star, and its modular reflection in cortex, are "comparatively recent and extraordinarily great" modifications of the mole's snout.

The conclusion that can be drawn from the observations in laboratory rodents and star-nosed moles is that the sensory periphery and corresponding neocortical areas are developmentally linked in such a manner that variations in the periphery, which may be the result of small, local changes in the expression patterns of morphogenetic genes, are communicated to the brain through inductive cascades. As a consequence minor (and perhaps major) alterations to receptor arrays result in functional representations in the CNS upon which natural selection can act to modify sensory systems efficiently. For instance, it is hard to examine variations of the nose and cortex in star-nosed moles (Figure 4) without imagining ancestral stars with fewer and perhaps shorter appendages similarly reflected in the somatosensory cortex, or even a potential future mole with 24 or more appendages ringing the snout.

So far I have emphasized evidence that sudden changes to discrete structures in the sensory periphery can be communicated to the CNS during development to produce matching cortical representations. However, we have also found intriguing evidence that the sizes of cortical sensory representations may be influenced by differential timing of development in the sensory periphery. This may be a convenient mechanism by which behaviorally important sensory surfaces capture the most cortical territory.

1.09.4 Cortical Magnification in Star-Nosed Moles

In a striking example of convergent evolution, star-nosed moles have developed a mechanosensory system that functions like an eye. The star has a

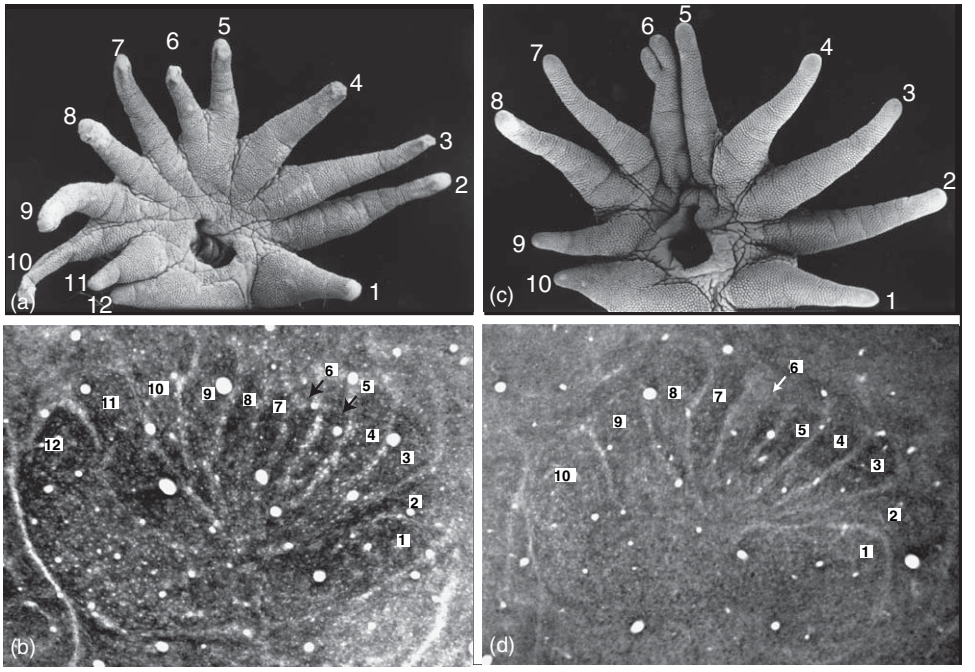

Figure 4 The nasal appendages and cortical representations of the half-nose for two abnormal star-nosed moles. a, Scanning electron micrograph of 12 appendages of an abnormal star. b, Section of flattened cortex processed for cytochrome oxidase to reveal the corresponding 12 modules in the primary somatosensory area. c, Scanning electron micrograph of 10 appendages of an abnormal star. d, Section of flattened cortex processed for cytochrome oxidase to reveal the corresponding 10 modules in the primary somatosensory area.

central tactile fovea (the 11th appendage) used for detailed exploration of objects of interest, whereas the larger peripheral appendages (1 through 10) are used to scan for potentially interesting objects or food items (Catania and Kaas, 1997b). This functional division is apparent in star-nosed mole behavior, which includes frequent saccadic movements of the star that bring the high-resolution tactile fovea into contact with objects of interest. In addition, the preferential use of the fovea is reflected in the organization of somatosensory cortex (Figure 3). The 11th foveal appendage is greatly overrepresented, occupying approximately 25% of the star representation in primary somatosensory cortex (S1). The enlargement of the 11th appendage is not simply a reflection of a higher innervation density (Catania and Kaas, 1997b), as suggested by previous investigations in rodents (Welker and Van der Loos, 1986), but rather is an overrepresentation of the primary afferent input – much like the retinal fovea is overrepresented in cortex relative to the number of ganglion cells concentrated in the fovea (Perry and Cowey, 1985; Silveira *et al.*, 1989; Azzopardi and Cowey, 1993; Quevedo *et al.*, 1996).

An obvious question is how afferents capture more than their share of somatosensory cortex relative to behaviorally less important inputs. The observation that star-nosed moles preferentially use the 11th appendage for tactile investigations, coupled with previous studies that indicate cortical representational areas may expand as a result of preferential stimulation and use (Recanzone *et al.*, 1992a, 1992b; Xerri *et al.*, 1999), suggest that behavior patterns may play an important role in establishing this relationship. However, our developmental studies suggest an alternative and perhaps surprising explanation for how the periphery may drive the expansion of the fovea representation in cortex.

In the course of investigating how the appendages of the star develop (Catania *et al.*, 1999) an unusual discrepancy in the relative sizes of embryonic and adult appendages on the star was noticed. In contrast to the small size of the 11th appendage in adult star-nosed moles, this appendage was comparatively large in embryos (Figure 5). In fact, the relative sizes of the star appendages in embryos reflect the relative sizes of the cortical representations seen later in adults (Figure 6a). Over the course of subsequent development, the proportion of the star taken up by the fovea gradually changes until, in adults, the 11th appendage takes up only a small fraction of the star (and contains only a relatively small proportion of the mechanosensory Eimer's organs), and thus the adult star proportions no longer reflect the magnification factors seen in cortex.

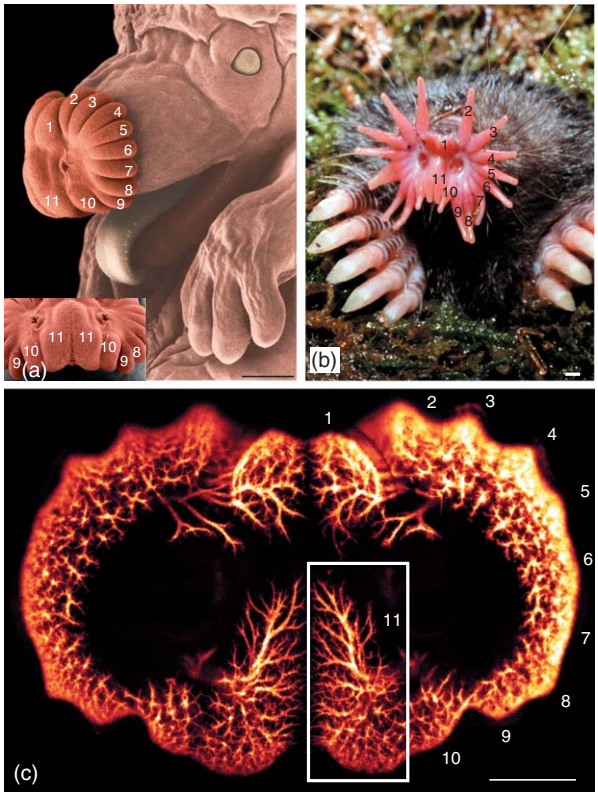

Figure 5 Embryonic star-nosed mole compared to an adult, showing the relatively large 11th appendage early in development. a, An embryonic mole showing the developing nasal appendages numbered 1–11. In early embryos the 11th appendage of the nose (the tactile fovea) is the largest and has the greatest innervated surface area. Inset shows a front view of the ventral star from the same specimen to illustrate the large 11th appendage. b, An adult star-nosed mole emerges from its tunnel. In adults the 11th appendage takes up only a relatively small proportion (7%) of the star, despite its important role as the tactile fovea. c, Scanning confocal microscopy of DiI-labeled fibers innervating the developing star. The 11th appendage (boxed area) takes up the largest area of the star and has the largest innervated surface area. The more lateral appendages have only begun to form at this stage. Scale bars: a and c, 500 μm; b, 1 mm. Reproduced from Catania, K. C. 2001. Early development of a somatosensory fovea: A head start in the cortical space race? *Nat. Neurosci.* 4, 353–354, with permission.

A more detailed examination of the developing appendages, associated sensory organs, and their innervation suggests the explanation for the changing relative proportions of the different appendages during development – the 11th, foveal appendage leads the development of the star. It is not only large earliest, but also has the largest innervated surface area in embryos and develops mature nerve terminals and associated sensory organs first in newborn moles (Catania, 2001). Finally, these patterns of development, with the 11th appendage in the lead, are reflected in the cortex by the early emergence of a cytochrome oxidase-rich zone representing the 11th appendage (Catania, 2001).

This developmental sequence provides the fovea with a strong basis for competitive advantages during development and suggests that foveal afferents may outcompete their neighbors in an activity-dependent race for cortical territory. The mechanism for this outcome is suggested by previous investigations in the visual and somatosensory systems. For example, when activity from one eye is reduced by suturing the lid shut during critical periods, ocular dominance columns devoted to the open eye develop expanded representations at the expense of the closed eye (Hubel *et al.*, 1977). Activity-dependent expansions have also been documented for the barrel system in rodents (Schlaggar *et al.*, 1993).

The suggestion is that the skin surface can provide not only instructions to the cortex regarding the arrangement and number of sensory surfaces but also information about the optimal size for these representations in later adults. Presumably the larger area for foveal representation allows for more efficient and perhaps faster sensory processing for these important inputs. The developmental sequence observed for the star suggests that evolutionary processes can change the size of representations in the brain by selectively altering the relative timing of developmental events in the periphery. It is significant in this regard that the fovea leads development in the primate retina (Rapaport and Stone, 1984) and is also overrepresented in cortex relative to the peripheral part of the retina.

1.09.5 Conclusions

In this article I have discussed some of the evidence from star-nosed moles and rodents for how changes in the details of neocortical maps may be effected through alterations of sensory surfaces in the periphery. The title of this article was inspired by the conclusion that many of these peripheral influences on the organization of the brain may not require a change in the expression of patterning genes in the CNS during the course of evolution. Rather, relatively small changes in gene expression at the level of sensory sheets may be communicated centrally by a cascade of inductive events during the course of development. The information communicated from the periphery includes not only details of how to construct a map of the topographic distribution and number of receptors in the periphery, but may also include important information about how much processing space should be devoted to the different afferent inputs. For example, the

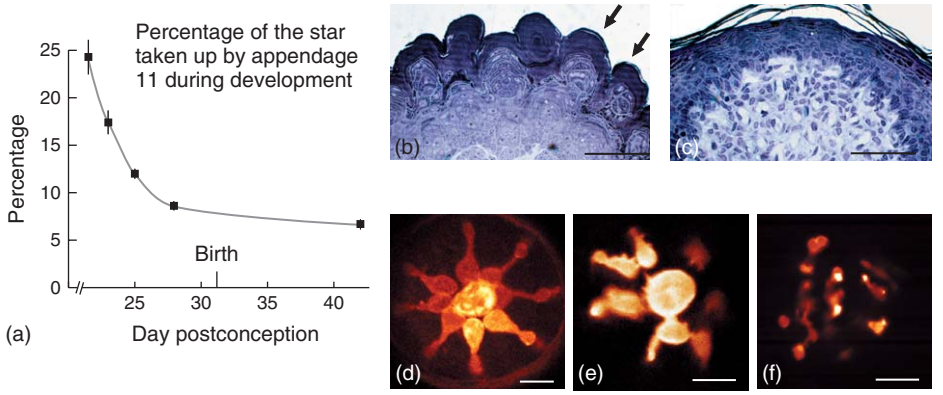

Figure 6 Differential maturation of the appendages, epidermis, and free nerve endings of the star at different postnatal stages. a, The proportion of the star taken up by the tactile fovea (appendage 11) over the course of development. b, Thin section of the epidermis of appendage 11 on postnatal day 1 showing the differentiated Eimer's organs consisting of keratinocytes in separate papillae (arrows). c, Thin section of appendage 6 at the same stage showing the undifferentiated epidermis. d, The adult configuration of free nerve endings in the epidermis at the apex of a mature Eimer's organ. The fibers form a remarkable hub-and-spoke configuration of nerve terminals just below the skin surface. e, On postnatal day 18, the fibers at the apex of each Eimer's organ on the 11th appendage have formed the hub-and-spoke configuration, though short extensions of the neurites remain. f, The fibers at the apex of each Eimer's organ on the lateral appendages remain disorganized, although they have reached the appropriate location. Thus, the 11th appendage leads the development of the star, not only in size, but also in the rate of sensory organ maturation. Scale bars: b and c, 50 μm; d–f, 5 μm. Reproduced from Catania, K. C. 2001. Early development of a somatosensory fovea: A head start in the cortical space race? *Nat. Neurosci.* 4, 353–354, with permission.

competitive nature of afferent distributions in cortical maps provides a developmental mechanism for skewing the allocation of cortical territory in favor of some inputs – namely those that lead the development of a sensory surface and are therefore most active, in the greatest numbers, at the earliest time.

It is clear that much larger-scale changes to brain organization have occurred in many mammalian lineages. Obvious examples include the Primate and Carnivore orders that have brains with more cortical subdivisions than smaller-brained rodents and insectivores. In this regard it is significant that star-nosed moles have more cortical subdivisions in their somatosensory system (S1, S2, and S3) than other moles and shrews, which have only two areas (S1 and S2). It seems likely that the additional area facilitates processing of the high volumes of complex sensory information from the star. Such changes in the number of cortical areas require corresponding changes in gene expression in the CNS, and recent studies suggest potential mechanisms by which these more global changes can occur (Fukuchi-Shimogori and Grove, 2001). However, it is possible that a modified sensory periphery often sets the stage for subsequent adaptive modifications to the CNS in the course of evolution (see Evolution of the Somatosensory System – Clues from Specialized Species). In support of this possibility, Bush *et al.* (2004) have found fossil evidence that suggests high-acuity vision preceded brain expansion in anthropoid evolution.

Acknowledgments

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1.10 Compensatory Innervation in Development and Evolution

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Glossary

altricial	The state of being born at an early stage of development. Opposite of precocial.
callosal connectivity	Connections between cerebral hemispheres made through the corpus callosum.
cortical areal specification	The developmental or evolutionary process of subdividing cerebral cortex into functional units.
cortical parcellation	The evolutionary process of subdividing cerebral cortex into functional units.
cross-modal plasticity	Plasticity induced in the brain that results in a sensory area of one modality coming to represent sensory information of a different modality.
fossorial	Animals that burrow underground.
Hebbian learning	As defined by Donald O. Hebb in 1949, synaptic changes that occur as a result of use-dependent plasticity. Inputs that succeed in activating a postsynaptic neuron become more efficacious and are stabilized.
horizontal connectivity	The lateral connections made between excitatory neurons within one cerebral cortical area.
inhibitory interneurons	Nonspiny, nonpyramidal neurons making local, suppressive connections with other neurons.
map compression	Loss of sensory target area can result in the forcing of a topographic sensory representation within the remaining target area.
orientation tuning	Neurons in visual cortex prefer elongated bars of light that have a particular orientation.
sensory substitution	After loss or deficit in a sensory organ, the cortical region that once represented the missing sensation becomes activatable by a different modality of input.
topographic map	Orderly representation of the sensory epithelium or some other type of information within a brain region.

1.10.1 Introduction

In January 2004, the State Superintendent of Schools in my adopted state of Georgia, USA, eliminated evolution from the public school science curriculum. Fortunately, a small group of dedicated activists, combined with worldwide media attention, blocked the attempt to turn back the scientific clock, for now. Nonetheless, the teaching of evolution remains under threat in many other locations around the USA and the world (Forrest and Gross, 2004; Scott, 2006). We evolutionary biologists must do something about the fact that, 150 years after Darwin's famous book, the lay public has an appallingly poor understanding of the foundational theory in biology. The production of this book series will hopefully promote not only a greater understanding of brain evolution by scientists, but also kindle a desire to provide more and better instruction in evolutionary biology for tomorrow's citizens of the world.

A major problem in obtaining public support for quality evolution education has been that the average citizen sees no need for learning evolutionary biology. The key is to illustrate for students the

connection between their everyday lives and evolutionary principles. In the following, I will discuss the largely unrecognized importance of evolutionary theory in guiding medical research toward a better understanding of brain development and pathology, and in guiding clinical interventions today and in the future (see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates, Primate Brain Evolution in Phylogenetic Context, Organization and Correspondence of the Auditory Cortex of Humans and Nonhuman Primates, The Evolution of Neuron Types and Cortical Histology in Apes and Humans, The Origin of Neocortex: Lessons from Comparative Embryology, Cortical Evolution as the Expression of a Program for Disproportionate Growth and the Proliferation of Areas, Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications, Captured in the Net of Space and Time: Understanding Cortical Field Evolution, The Evolution of the Dorsal Thalamus in Mammals, Epigenetic Responses to a Changing Periphery – Wagging the Dog).

1.10.2 Evolution Exploits Developmental Events

The relatively new discipline of evo-devo relies on the fact that, because early developmental events determine the ground plan for further development, small alterations in the genetic programs underlying early development can lead to drastic changes in phenotype. Developmental neuroscience and evolutionary biology are thus complementary approaches. By studying normal development and the plastic mechanisms used by the brain to respond to injury, we can determine the constraints under which brain evolution operates when faced with alterations in peripheral innervation.

1.10.2.1 Brain Evolution Results from Minor Variations on a Basic Vertebrate Plan

Just as constructing a building requires first laying the foundation, constructing any vertebrate requires a foundation or Bauplan, incorporating bilateral symmetry, paired appendages, a dorsal nerve cord encased in a spinal column, and so forth. This requirement ensures that, at early stages, vertebrate embryos of any type will have common features. The prediction, and the finding, is that, the more common the feature, the earlier it appeared in evolution. The morphological and resulting functional differences that distinguish one vertebrate from another arise later in development, and represent fairly small deviations from the pre-existing plan. For example, a two-chambered heart becomes a three- and then a four-chambered heart over evolutionary time, but the heart itself is universal. Similarly, the vertebrate brain consists of hindbrain, midbrain, and forebrain, but the relative size and organization of each brain division and the functional regions within them can be modified during development according to the evolutionary history of each species (Pallas, 2001b; Krubitzer and Kaas, 2005).

What sorts of beneficial evolutionary modifications have been made to developmental programs? It is difficult to imagine how changes could even be tolerated, much less produce adaptive circuits, in a highly interconnected network such as the nervous system. It would seem that a change in one brain region or a change in one morphogenetic signal would either be ignored at the next level or would create a mismatch in the network and severely disrupt function. As we know from studies of recovery from brain trauma, however, the brain has a remarkable capacity to rewire itself in an adaptive fashion.

1.10.2.2 Developmental Mechanisms can Accommodate New Neurons and Trigger Matching Changes in Connected Populations

Both neurons and synapses are massively overproduced during development, and the final circuitry is pruned by processes such as programmed cell death and collateral elimination (see Finlay and Pallas, 1989, for a review of earlier literature). This pruning is directed in large part by activity-dependent processes (Hebb, 1949; Schneider, 1973; Innocenti *et al.*, 1977; Pilar *et al.*, 1980; O'Leary and Stanfield, 1986), ensuring that the final wiring pattern is appropriate to the environment and experiences of each individual. Extra neurons and connections produced during development can be stabilized by providing additional target space (Hollyday and Hamburger, 1976). Thus the natural plasticity built into the nervous system ensures that developmental (or evolutionary) errors can be absorbed, and even exploited. Any such acquired connections gained within an animal's lifetime would of course not be heritable, but relative excesses of target neurons produced through gene duplication, modifications of the cell cycle, or cell death of input neurons would automatically integrate into a circuit. Throughout vertebrate evolution there has been a consistent trend toward increasing the number of neurons, producing local variation in size of nuclei or layers and an increasing modularization (Finlay and Slattery, 1983; Caviness *et al.*, 1995; Kornack and Rakic, 1998; Kornack,

2000; Ohki *et al.*, 2005). Because of inherent plasticity in the inputs, matching changes in the pathway are not required. Environmental change is the primary source of selection pressure over generations, and a nervous system that responds on a developmental timescale to the environment provides a huge selective advantage, if the cost in terms of unintended connections is low. More importantly to this discussion, exuberant connections and extra neurons at any level of a sensory pathway are possible sources of variation in neural circuits between individuals (Sperry, 1963), and individual variations in connectivity could be selected for if they provide a survival advantage. Thus a mutation-induced increase or decrease in a neuronal pool could influence both upstream and downstream neuronal survival and collateral elimination. In this way, a change in one component of the pathway need not require a simultaneous and matching change in all components. Hebbian mechanisms would instruct the pathway to process the new inputs sensibly. In the following, I will illustrate how this can occur in developmental time, and propose how it might have occurred over evolutionary time.

1.10.3 Target Specificity and Its Role in Development and Evolution

The task facing the nervous system during development is largely one of population matching: neuronal input populations must find the correct target populations, and form the correct number of synapses, in the correct position, within each target (Katz and Lasek, 1978). Molecular guidance cues are responsible for many aspects of target specificity, but, given the impossibility of prespecifying every connection genetically, some general rules are enforced instead. Target choice occurs in a hierarchical fashion and is not absolute. Targets apparently possess labels that are recognized by their usual input population, but if that target population is missing, other target choices are possible, with some preferred over others.

1.10.3.1 Population Matching in the Retinotectal System

Like many central sensory brain structures, the optic tectum (also known as the superior colliculus (SC), in mammals) contains a map of visual space conferred on it by the orderly projection of retinal ganglion cells. In amphibians, if an eye is surgically rotated and the optic nerve allowed to regenerate, the retinotectal projections grow to their previous termination sites, suggesting that the regenerating retinal axons follow pre-established chemoaffinity markers in the tectum (Sperry, 1963). This suggestion was supported by observations that, if half of the tectum is removed, it is initially innervated only by the corresponding half of the retina (Attardi and Sperry, 1963). With time, however, the map regulates such that the retinal axons fill the remaining target space, creating a compressed retinotectal map of visual space (Schmidt, 1982). The same regulatory process occurs during development in rodents; removal of part of the SC in neonatal hamsters results in orderly compression of the retinotopic map on to the SC fragment (Finlay *et al.*, 1979; see Goodhill and Richards, 1999, for review).

1.10.3.1.1 Role of ephrins in retinotectal map formation A large body of research points to the ephrins and the Eph tyrosine kinase receptors as the molecules responsible for chemoaffinity in the retinotectal projection (Drescher *et al.*, 1997; Flanagan and Vanderhaeghen, 1998; Frisén *et al.*, 1998; O'Leary and Wilkinson, 1999; Knoll and Drescher, 2002; McLaughlin *et al.*, 2003). EphA receptors and ephrin-A ligands are distributed in opposing gradients, with ephrin-A expression high in caudal tectum and EphAR expression high in nasal retina (Figure 1). Binding of receptor and ligand results in growth cone collapse, and thus the anteroposterior axis of the retinotectal map is built by repulsion of incorrect terminations. Knockout of ephrin-As leads to severe disruptions in map topography, in particular caudal overshoot of retinal axon targeting (Ciossek *et al.*,

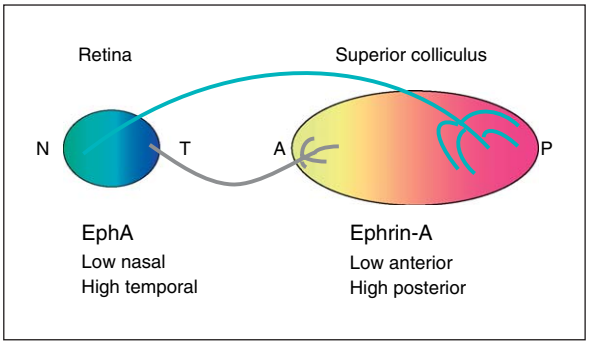

Figure 1 EphA tyrosine kinase receptors are expressed in a nasotemporally increasing gradient on retinal ganglion cells and ephrin-A receptors are expressed in a reverse gradient within the SC. Repulsive interactions between ligand and receptor-containing neurons cause temporal retinal axons to be repelled from posterior SC. The combined action across the gradient sets up the rostrocaudal topography of the retinocollicular map. Adapted from Cheng, H. J., Nakamoto, M., Bergemann, A. D., and Flanagan, J. G. 1995. Complementary gradients in expression and binding of ELF-1 and Mek 4 in developement of the topographic retinotectal projection map. *Cell* 82, 371–381.

1998; Frisén *et al.*, 1998; Yates *et al.*, 2001). This raises the question, if ephrin distributions in tectum define the termination sites of retinal axons, how can the retinocollicular map compress after caudal SC injury? It has been suggested that the ephrin gradients must be altered for compression to occur (Schmidt, 1982). Alternatively, retinal axons may be responding to relative rather than absolute expression levels of ephrins (Brown *et al.*, 2000). To investigate this question, we quantified ephrin-A expression levels in normal and compressed retino-SC maps in hamsters. We found that the reduction in target size causes a compensatory alteration in ephrin expression such that the pitch of the ephrin-A gradient is steeper in the smaller target (Tadesse *et al.*, 2004). Viewed from an evolutionary perspective, alterations in the size of a target area may produce changes in mapping cues that lead to compensatory alterations in topography, thus smoothly integrating the change in neuronal number.

1.10.3.1.2 Role of NMDA receptors in conservation of response properties It might be expected that compression of the map would result in compromised visual function. In a series of studies, we have shown that this is not the case. Instead, the circuitry within the SC compensates for the loss of target in a way that preserves the response properties of the component neurons. Rather than increasing the convergence ratio between ganglion cells and SC neurons, the retinal axon arbors are pruned in such a way that the receptive field size of individual SC neurons is conserved (Figure 2, top) (Pallas and Finlay, 1989, 1991; Xiong *et al.*, 1994). The mechanism underlying this conservation of receptive field size is the same as that operating during normal development of the map; through *N*-methyl-D-aspartate receptor (NMDAR)-dependent long term potentiation (LTP) (Constantine-Paton and Cline, 1998). Chronic blockade of NMDAR in normal SC prevents the activity-dependent refinement of retino-SC projections, resulting in stabilization of the initial, overlapped ganglion cell terminals. As a result, receptive fields of SC neurons remain larger than in normal adults (Huang and Pallas, 2001; see also Simon *et al.*, 1992). Combining NMDAR blockade with map compression results in further enlargement of receptive fields, because both the normal refinement process and the compensation process are blocked (Figure 2, bottom).

1.10.3.1.3 Role of lateral inhibition in conservation of response properties Curiously, we found that response properties such as stimulus size and velocity tuning were unaffected by either the map compression or by the NMDAR blockade and subsequent receptive field enlargement (Razak *et al.*, 2003; Figure 3), suggesting that an NMDAR-independent mechanism is responsible for conservation of stimulus tuning. In order to explore this possibility, we first had to understand the circuitry responsible for velocity and size tuning in normal animals. Previous models of velocity tuning in retinal and visual cortical neurons have direction tuning as a necessary component (Barlow and Levick, 1965; Borst and Egelhaaf, 1989; Ascher and Grzywacz, 2000), but the velocity-tuned neurons in hamster SC are not intrinsically directional. We found that temporally asymmetrical lateral inhibition within the receptive field is largely responsible for size tuning, and that surround suppression is a major component of velocity tuning (Razak and Pallas, 2005).

Given that inhibition underlies stimulus tuning, and blockade of NMDARs had no effect on tuning, we hypothesized that the reason stimulus tuning is preserved in compressed or NMDAR-blocked SC

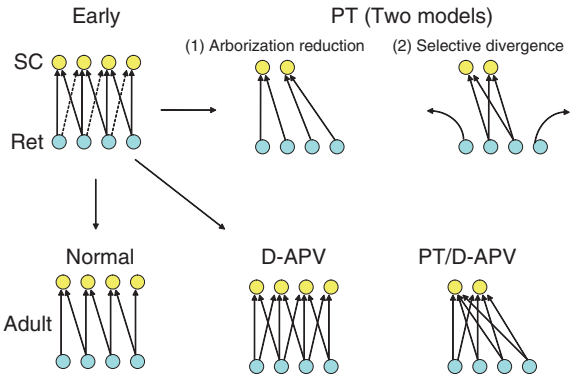

Figure 2 Left: Early in development, SC neurons (SC layer) have large and diffuse receptive fields, but the refinement of retinal ganglion cell (Ret layer) axon arbors results in smaller receptive fields by adulthood in normal animals. Top: If the caudal half of the SC is ablated at birth (PT), the retinal projection compresses on the remaining SC. The mechanisms underlying the compression include a reduction in retinal axon arbor size as well as selective collateral elimination from the SC. Note that the number of retinal inputs and the amount of visual space represented by each SC neuron is conserved, although the degree of overlap between adjacent receptive fields is reduced and multiunit receptive fields are larger. Bottom: The refinement process is dependent on NMDAR (Huang and Pallas, 2001). Chronic blockade of NMDAR with D-APV prevents the refinement process, leading to large receptive fields in adults. If the NMDAR blockade is combined with target loss (PT/D-APV), then, in addition to preventing the refinement, loss of NMDAR function prevents the compensation for target loss, leading to further receptive field enlargement. Adapted from Huang, L. and Pallas, S. L. 2001. NMDA receptor blockade in the superior colliculus prevents developmental plasticity without blocking visual transmission or map compression. *J. Neurophysiol.* 86, 1179–1194.

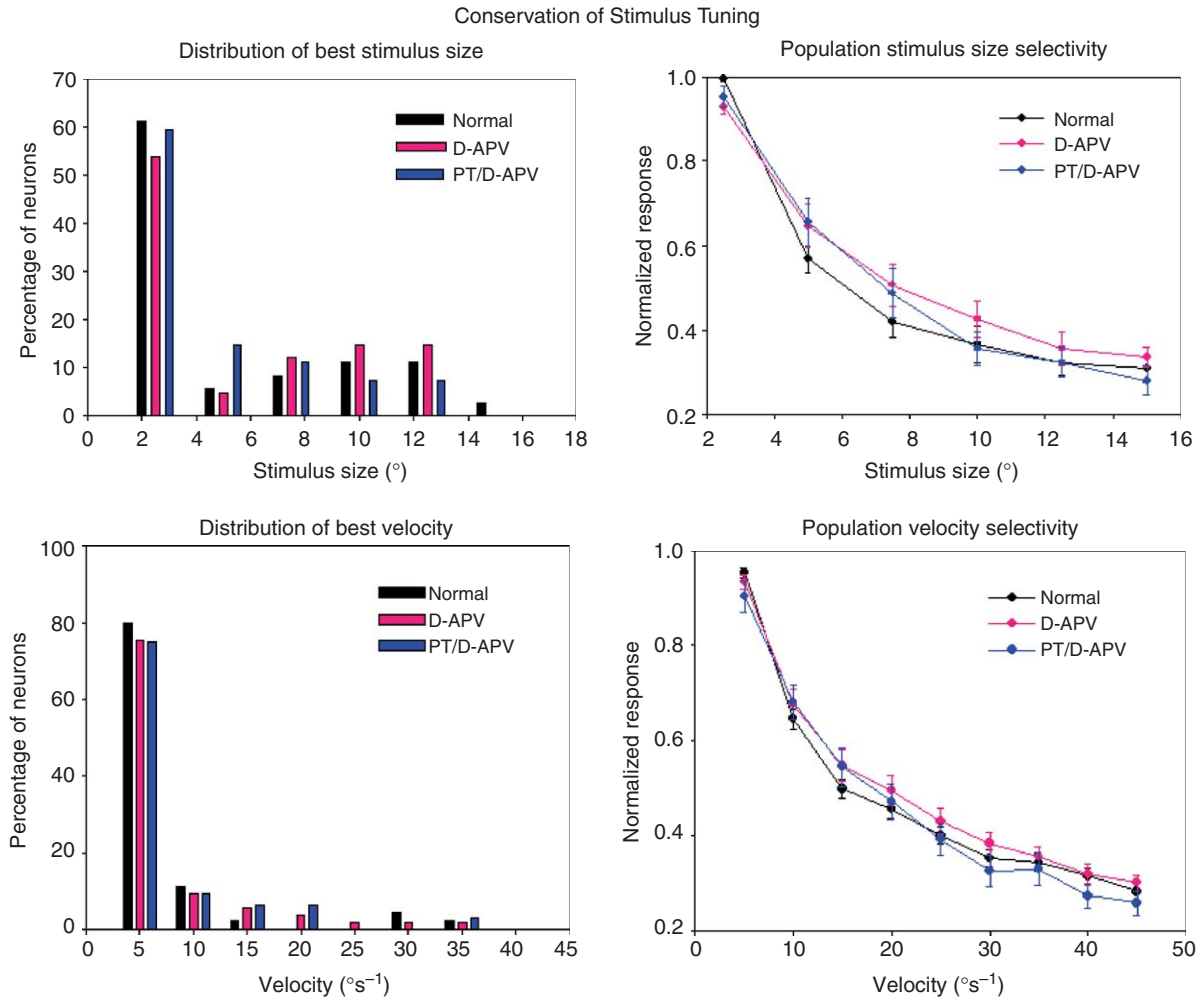

Figure 3 Although chronic NMDAR blockade in full-size or compressed retino-SC maps results in enlarged receptive fields, tuning of SC neurons to visual response properties such as stimulus size and velocity is unaffected. Measures of best stimulus size and velocity in individual SC neurons as well as normalized response levels across the population of neurons were not significantly different between the normal, chronic NMDAR blockade (D-APV), and NMDAR blockade combined with PT ablation (PT/D-APV) groups. Adapted from Razak, K. A., Huang, L., and Pallas, S. L. 2003. NMDA receptor blockade in the superior colliculus increases receptive field size without altering velocity and size tuning. *J. Neurophysiol.* 90, 110–119.

maps is that inhibitory circuits undergo compensatory modification of the enlarged receptive fields. To test this hypothesis, we used electrophysiological techniques to map out the contribution of inhibitory inputs to the receptive fields of SC neurons in partial tectum (PT) and 2-amino-5-phosphonovaleric acid (APV) cases. We found that the extent and the strength of inhibition were increased, maintaining a balance between excitatory and inhibitory subfields that compensated for the increased number of excitatory inputs in the APV group (Razak *et al.*, 2002, 2003).

These results together show that an increase in the number of afferents can be compensated for by homeostatic, matching alterations in both excitatory and inhibitory circuitry, maintaining the ability of the system to identify visual objects. This has obvious utility from an evolutionary perspective. Any individual variation producing a change in the number of inputs could not only be incorporated but could also produce an adaptive advantage.

1.10.3.2 Role of Sensory Deprivation in Specificity of Thalamocortical Pathways

Connections between sensory thalamus and sensory cortex are demonstrably quite specific from early in development (Crandall and Caviness, 1984; Miller *et al.*, 1991), and thus are a good experimental system in which to study target specificity. If sensory inputs can specify cortical identity through thalamocortical axon (TCA) pathfinding, then alteration of TCAs could change cortical identity. Multiple, large tracer injections in primary auditory cortex (A1) of

deafened ferrets revealed an anomalous thalamocortical pathway in the deaf ferret brains, connecting visual thalamus (lateral geniculate nucleus (LGN) and lateral posterior nucleus (LP)/pulvinar) with auditory cortex (Pallas *et al.*, 2002). This result is particularly surprising because previous thinking holds that thalamocortical targeting is hard-wired by molecular guidance factors and not affected by sensory experience. This LGN-A1 projection is not a normal developmental exuberance that was stabilized, rather it is novel and a response to the deafferentation occurring several synapses away. This finding is relevant clinically because it could provide a substrate for the recovery of function seen in deaf individuals (Neville, 1990). If a remodeling of the thalamocortical pathway could occur evolutionarily, it could provide a means of generating a new cortical area (Kaas, 1993). Our observation thus provides us with a model system for brain evolution. If a change in thalamocortical afferent source can direct cortical circuitry for processing to its own ends, then peripheral change could induce a matching central change without the need for genomic modification.

1.10.3.3 Cross-Modal Plasticity in the Retinogeniculocortical System

1.10.3.3.1 Parcellation of mammalian neocortex

The evolution of the mammalian neocortex has involved a spectacular amount of parcellation and diversification (Felleman and Van Essen, 1991; see Rosa and Krubitzer, 1999, for review). Several explanations for this evolutionary change seem possible. Perhaps the least likely is simultaneous, matching mutations that affect both afferent inputs and their cortical targets. Alternatively, intrinsic genetic information, either from afferent inputs or from cortex itself, could drive parcellation and circuit arrangement (Rakic, 1988). Third, unique spatiotemporal patterns of afferent activity reaching each presumptive cortical area could instruct parcellation and circuit organization (Hebb, 1949; O'Leary, 1989; Kaas, 1995; Northcutt and Kaas, 1995; Kaas and Reine, 1999). Finally, cortical circuitry could be sufficiently similar in different regions that changes would be unnecessary to accommodate adaptive changes in afferent inputs.

1.10.3.3.2 The cross-modal plasticity paradigm

Cross-modal plasticity provides a way of examining the mechanism behind cortical parcellation during development and evolution. The paradigm permits experimentally changing the modality and activity pattern within TCAs without changing their molecular identity. The hypothesis being tested with this approach is that the sensory information received by a region of cortex during development (or evolution) plays an essential role in organizing its functional identity. In contrast to specification according to intrinsic instructions, afferent instruction would allow animals to be responsive to a changing sensory environment or to alterations in peripheral sensory systems. This dependence on experience would have obvious adaptive significance. However, although some advances in understanding cortical parcellation have been made, it is not known which characteristics distinguishing different cortical areas are due to intrinsic, preprogrammed differences, and which are due to activity in the sensory inputs, and in the latter case, whether sensory activity is permissive or instructive (Levitt *et al.*, 1997; Crair, 1999).

In cross-modal plasticity experiments, afferents of one sensory modality are induced to innervate thalamus of a different modality, which then carries the cross-modal information to the sensory cortex (Figure 4). These experiments were first performed in hamsters (Schneider, 1973; Frost, 1981) and then in ferrets (Sur *et al.*, 1988), which are carnivores with sensory cortical physiology similar to that of the cat, except that they are born earlier in development. The manipulation is performed before sensory information has gained access to cortex (Luskin and Shatz, 1985; Jackson *et al.*, 1989). In ferrets, external visual and auditory cues are not available until after postnatal day 30 (Moore and Hine, 1992; Akerman *et al.*, 2002), in part because the ears and eyes open at this time. This protracted developmental period allows a more detailed examination of the process than is possible in rodents.

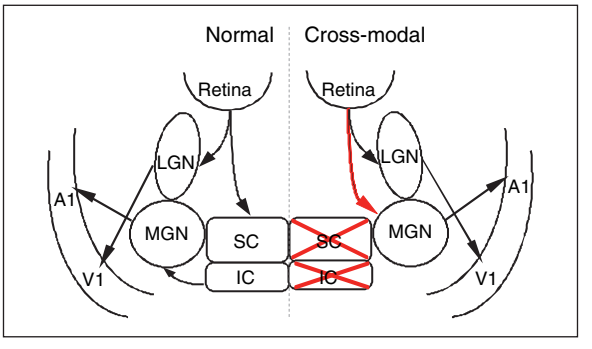

Figure 4 Procedure for inducing retinal projections into auditory thalamus (MGN). Midbrain lesions simultaneously deafferent MGN and eliminate retinal target space. As a result, retinal axons sprout into MGN. MGN in turn carries visual information into primary auditory cortex (A1).

1.10.3.3.3 Cross-modal A1 can process and map visual inputs Experiments performed in the Frost lab demonstrated that retinal axons lacking target space could invade either somatosensory or auditory thalamus, inducing visual responses and some retinotopic organization in the cortical area affected (Frost, 1981; Metin and Frost, 1989). Extending the cross-modal paradigm to ferrets (Sur *et al.*, 1988) showed that early visual activation of A1 causes auditory cortex to resemble visual cortex in both its topography and receptive field properties (Roe *et al.*, 1990, 1992; see Sur *et al.*, 1990, for review). Remarkably, cross-modal A1 in ferrets or hamsters can mediate rudimentary visual perception (Frost *et al.*, 2000; von Melchner *et al.*, 2000). Thus it appears that supplying A1 with early visual input transforms it in a functional sense into a visual cortical area. The remaining question is how this transformation occurs. Either visual and auditory cortex are so similar that they can process each other's inputs without modification, or the visual input has caused a modification in A1's circuitry that allows it to process visual information appropriately. Our goal has been to distinguish between these two possibilities.

1.10.3.3.4 Anatomical basis for visual responses and topography in cross-modal A1 Using an anatomical approach, we showed that the early anomalous visual inputs do in fact cause modifications to A1's circuitry on multiple levels, including intracortical (horizontal) and corticocortical (callosal) connectivity patterns and local inhibitory circuitry. In normal auditory cortex of cats and ferrets, callosal connections are organized into the so-called binaural bands along the tonotopic axis, uniting A1 neurons in both brain hemispheres that are excited by sound presented to either ear (EE cells: Imig and Brugge, 1978; Figure 5, normal). Another class of A1 neurons is inhibited by sound in one ear (EI cells) and does not project callosally. The EI cells form interdigitating bands with the callosally projecting EE cells (Imig and Brugge, 1978). The pattern of callosal bands arises during development from a more diffuse early pattern (Feng and Brugge, 1983), suggesting that auditory experience is involved in its refinement. Perpendicular to the tonotopic axis, intracortical, horizontal connections in A1 unite neurons that have similar frequency tuning (Matsubara and Phillips, 1988; Wallace and Bajwa, 1991; Gao and Pallas, 1999). In this case, the early projections are somewhat specific in their targeting, although there is some refinement occurring at about the time of hearing onset that is lost with early deafening (Figure 5, deaf; Gao *et al.*, 1999a; Moerschel and Pallas, 2001). Interestingly, it seems that there is more error correction occurring in visual cortex during development than in auditory cortex (Ruthazer and Stryker, 1996; White *et al.*, 2001), but in both cases neural activity is critical for normal development.

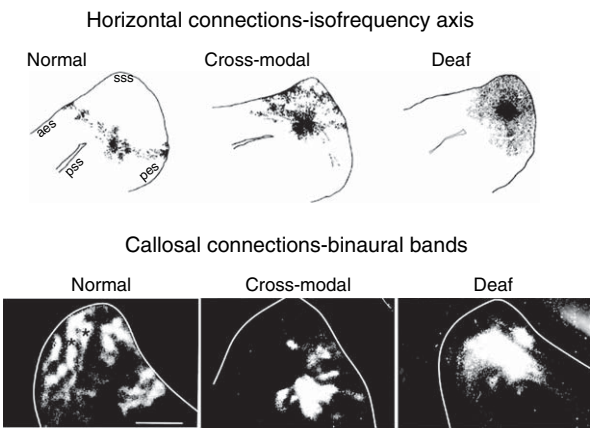

Figure 5 Injection of local or long-distance tracers reveals the connectivity of auditory cortex in normal, cross-modal, or deafened ferrets. Local horizontal projections within A1 interconnect neurons with similar sound frequency tuning. Callosal connections run along the binaural bands of neurons receiving excitatory inputs from both ears. Deafferentation of A1 results in loss of specificity in these connection systems, suggesting an activity-dependent basis. Visual input to A1 in the left hemisphere alters the connections with respect to both pattern and location. Adapted from Gao, W.-J. and Pallas, S. L. 1999. Cross-modal reorganization of horizontal connectivity in auditory cortex without altering thalamocortical projection. *J. Neurosci.* 19, 7940–7950; Pallas, S. L., Littmann, T., and Moore, D. R. 1999. Cross-modal reorganization of callosal connectivity in auditory cortex without altering thalamocortical projections. *Proc. Natl. Acad. Sci. USA* 96, 8751–8756.

We reasoned that, if the cortical connectivity pattern in A1 of adults is dependent on auditory experience, then supplying visual inputs to A1 in cross-modal ferrets should result in a pattern more closely resembling that in visual cortex. Horizontal connections in primary visual cortex (V1) are organized as a periodic, symmetrical array of clusters that interconnect neurons with similar visual orientation tuning (Callaway and Katz, 1990; Malach *et al.*, 1993). These clusters are refined under the influence of visual activity (Callaway and Katz, 1991; Ruthazer and Stryker, 1996). Callosal connections in visual cortex of adults are made between neurons at the border of visual cortical areas 17 and 18 (Lewis and Olavarria, 1995; Olavarria, 2001; Riederer *et al.*, 2004; see The Role of Transient, Exuberant Axonal Structures in the Evolution of Cerebral Cortex for review).

The rewiring manipulation is performed on one side of the brain only; thus the normal, contralateral

auditory cortex can communicate auditory information across the corpus callosum. This raises the interesting possibility that A1 may have both visual and auditory-responsive neurons. Our anatomical results were consistent with this possibility (Figure 5, cross-modal). Horizontal connections in cross-modal A1 were arranged in a radially symmetric array of clusters as in V1, and, unlike in normal A1, extended toward the medial part of cross-modal A1 (Gao and Pallas, 1999). Callosal connections, in contrast, were pushed laterally and were entirely absent from the medial part of cross-modal A1. The remaining callosally projecting neurons were organized in patches instead of the binaural bands seen in normal A1 (Pallas *et al.*, 1999).

Because we found that the callosal and horizontal connections in cross-modal A1 were arranged in a mutually exclusive pattern, we have proposed that A1 is split in two by the anomalous visual and normal auditory inputs (Pallas, 2002). Laterally, callosal connections would interconnect sound-responsive neurons in both hemispheres. Visually responsive neurons would be preferentially located in medial A1 of cross-modal ferrets, where they would be interconnected according to their orientation tuning. Evidence for iso-orientation connectivity in cross-modal A1 has been provided using optical imaging methods (Sharma *et al.*, 2000), supporting this idea. If it is the case that cross-modal A1 is subdivided by its bimodal inputs, then we can model the evolution of a new cortical area on a developmental timescale. This would provide an ideal system in which to study how evolutionary changes in sensory input could trigger development of a cortical area to process those inputs (Kaas, 1995). In either case, the results show that changing only the pattern of activity without changing the molecular identity of thalamic inputs can configure cortical circuits adaptively.

1.10.3.3.5 Role of inhibition in cross-modal plasticity We have also tested the hypothesis that rearrangements of inhibitory circuitry occur in cross-modal A1. The rationale is that the pathway from the auditory thalamus (medial geniculate nucleus (MGN)) to the auditory cortex projects as a one-dimensional sheet along the isofrequency axis (Pallas *et al.*, 1990), suggesting that in order to represent visual spatial topography in two dimensions, suppression of select subsets of these one-dimensional projections would be necessary. Using immunocytochemical methods to catalog subsets of GABAergic nonspiny, nonpyramidal interneurons, we found that there was an increase in number and a change in morphology of a calbindin-containing subset of inhibitory neurons in A1 as a result of early visual inputs (Gao *et al.*, 1999b, 2000; see Pallas, 2001a, for review). The next question that must be addressed is whether changes in inhibitory circuitry are necessary or sufficient to reconfigure auditory cortex for a visual processing role. We predict that local blockade of intracortical inhibition will eliminate response properties that depend on the two-dimensional nature of visual stimuli, as opposed to the one-dimensional arrangement of sound frequency coding.

1.10.3.3.6 Molecular specification of cortical areas through axon guidance There are several possible explanations of how the functional identity of each cortical area is established. Thalamic projections are well targeted during pathfinding (Crandall and Caviness, 1984; Miller *et al.*, 1993), forming sharp boundaries between projections to adjacent cortical areas, and thus could instruct cortical identity. How these precise projections of thalamocortical afferents are targeted remains to be determined, though previous studies have pointed to the ventral telencephalon and the cortical subplate as containing important guidance cues (Ghosh *et al.*, 1990; Catalano and Shatz, 1998; López-Bendito and Molnar, 2003), and to matching adhesion factors (cadherins) in thalamus and cortex (Gil *et al.*, 2002; Poskanzer *et al.*, 2003). Information intrinsic to cortex, such as transcription factors and markers of positional identity, may be sufficient to set up guidance of TCAs to their appropriate final cortical targets or to establish other unique features. Conversely, cortex may be a *tabula rasa* that is instructed by the TCAs to develop in a particular fashion. More likely the answer lies in between. Our recent effort has been to explore the expression and action of genes identified as candidates for a role in the specification of cortical identity.

Regionally patterned transcription factors and adhesion molecules are thought to be responsible for areal patterning, via specification of both positional information and TCA targeting (Grove and Fukuchi-Shimogori, 2003). Patterned gene expression is known to be involved in the specification of axonal projection patterns in the hindbrain (Keynes and Krumlauf, 1994) and spinal cord (Stoeckli and Landmesser, 1998). This protomap idea (Rakic, 1988) has been tested by cortical gene knockout studies (Bishop *et al.*, 2000; Mallamaci *et al.*, 2000) and by examination of mutants in which thalamocortical projections do not form or form incorrectly (Miyashita-Lin *et al.*, 1999; Nakagawa *et al.*, 1999; Tuttle *et al.*, 1999; Garel *et al.*, 2002). The finding that many patterning genes are expressed whether or not the TCAs are present

supports the idea that they instruct TCA pathfinding. None of the genes found so far are restricted to areal boundaries in their expression, however (Donoghue and Rakic, 1999). Thus, a combinatorial, threshold effect, similar to that for guidance of retinal axons by the repulsive molecules called ephrins (Hansen *et al.*, 2004; Tadesse *et al.*, 2004), has been suggested (Donoghue and Rakic, 1999).

Knockout of the transcription factor gene *Pax6*, which is expressed in a rostrolaterally increasing gradient, results in an apparent caudalization of the remaining cortical epithelium, as defined by rostral shifts in expression of the adhesion factor gene *Cad8* and other caudal marker genes (Bishop *et al.*, 2000; Mallamaci *et al.*, 2000). Conversely, knockout of *Emx2*, another transcription factor gene that is expressed in an opposite gradient to *Pax6*, anteriorizes positional identity in cortex, and is associated with caudal shifts in the normally anteriorly restricted expression of *Cad6* and other anterior marker genes. In addition, expansion or contraction of an *FGF8* expression domain induced by manipulating *Emx2* levels results in opposite shifts in the location of the somatosensory barrel field (Fukuchi-Shimogori and Grove, 2001, 2003). If there were corresponding defects in TCA pathfinding, then a role for the genes in areal specification via TCA guidance would be supported. In *Emx2* mutants, a caudal shift of TCAs to somatosensory cortex occurs, and the projection of TCAs to visual cortex is reduced concomitantly (Bishop *et al.*, 2000; Mallamaci *et al.*, 2000), prompting a suggestion that the loss of *Emx2* redirects TCAs through a change in positional identity of caudal cortex. However, this interpretation has been challenged by reports that TCAs become mistargeted in the ventral telencephalon, before they come into contact with the altered cortical regions (Caric *et al.*, 1997; Garel *et al.*, 2002; Molnar, 2000). Furthermore, another study has shown that similar Fgf8-induced shifts in patterned gene expression, and a resulting caudalization of the cortical epithelium, are not associated with any shift in TCA projection patterns in neonates (Garel *et al.*, 2003). It is possible that, rather than respecifying cortical identity, loss of Pax6 or Emx2 transcription factors interferes with the generation of neurons in cortex. Indeed, the cortex is smaller in the mutants (Bishop *et al.*, 2000), supporting this alternative interpretation. Another complication is that the knockout method eliminates gene function everywhere, throughout life, but these genes likely play multiple roles in development across space and time. In addition, the mutants die before the borders of cortical areas can be confirmed functionally, or have other profound defects that complicate interpretation of the data (Caric *et al.*, 1997; Molnar, 2000; Pratt *et al.*, 2000; Garel *et al.*, 2002). Thus the question of the role of these genes in cortical specification requires further study, with complementary approaches.

Our approach affords the unique advantage of combining physiological and molecular investigations in an animal, the ferret, with protracted postnatal development and well-characterized cortical physiology, providing a model that has major advantages over mice. We combined quantitative reverse transcriptase polymer chain reaction (RT-PCR) with neuroanatomical tracing of both TCA and corticocortical projections in ferrets, comparing A1 and V1 in normal animals in topography and levels of gene expression in relation to the timing of axon ingrowth. We found that, in normal animals, Pax6 and Emx2 expression gradients were declining by postnatal day 10 (P10) to P14, prior to TCA ingrowth. Differential expression of *Cad6* and *Cad8*, however, was maximal during the period of TCA targeting and synapse formation (P14 to P25) and overlapped with the later development of specific corticocortical connectivity (Xu *et al.*, 2003; Figure 6). The expression patterns of Pax6 and Emx2 across development and between cortical areas are consistent with a role in orchestrating gradients of neurogenesis, providing early regional patterning in cortex, and/or triggering a signal cascade that continues in their absence, but not with direct specification of axon targeting to cortex. These genes likely trigger expression of downstream genes such as the cadherins that could be more directly involved with correct pathfinding by TCA axons. The expression patterns between different cortical areas, correlated with the timing of afferent ingrowth, provided the impetus for our further investigations into the roles of cadherins in cortical plasticity.

Individual functional areas within the cerebral cortex have characteristic connectivity patterns which exhibit experience-dependent development and plasticity. Another of our efforts has been to look for a relationship between connectivity patterns and gene expression patterns. The classical cadherins may instruct, or be instructed by, thalamocortical ingrowth (Bishop *et al.*, 2000; Mallamaci *et al.*, 2000; Gil *et al.*, 2002). We tested the hypothesis that, if cadherins are instructive for TCA guidance, then in experimental models producing mistargeting of TCAs, cadherins should be misexpressed. Ferrets deafened prior to the onset of hearing exhibit mistargeting of both retinal and thalamocortical projections (see above and Pallas and Moore, 1997; Pallas *et al.*, 2002). We predicted that these deaf

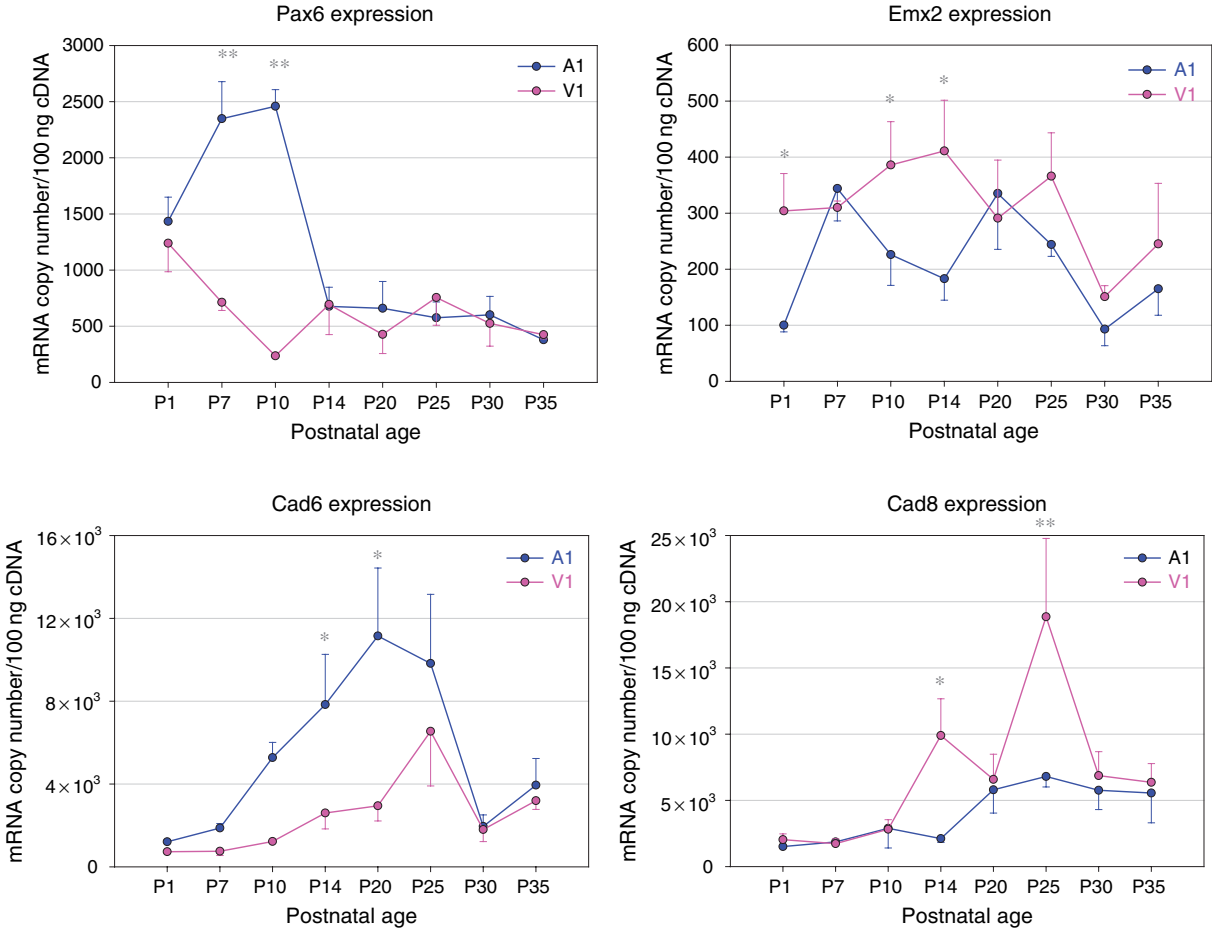

Figure 6 Expression levels of four putative cortical patterning genes were measured with quantitative RT-PCR. Top: Early in development, prior to TCA innervation, *Pax6* is high in auditory cortex and *Emx2* is higher in visual cortex, potentially providing positional information about regional cortical identity. As the TCAs are growing into the cortical plate and corticocortical connections are being formed, *Pax6* and *Emx2* levels drop, but the cadherin pair Cad6 and Cad8 form similar opposing gradients. The cadherins are thought to facilitate patterning of connections throughout the brain. Adapted from Xu, M., Baro, D. J., and Pallas, S. L. 2003. A quantitative study of gene expression topography in visual and auditory cortex during thalamocortical development in postnatal ferrets. *Soc. Neurosci. Abstr.* 29, 673–677.

ferrets with ectopic projections between visual thalamus (LGN) and auditory cortex would exhibit alterations in the spatial pattern of cadherin expression. However, this was not the case. Results from quantitative RT-PCR of cadherin mRNA and Western blots of cadherin protein were similar in normal and deafened ferrets. These data could be interpreted as evidence against a role for cadherins in TCA targeting. The LGN to A1 projections are vastly outnumbered by normal MGN to A1 projections in the deafened ferrets, however, and thus any alteration of cadherin expression may be undetectable with our method. Further study is warranted to uncover how TCA pathfinding strategies can respond to deafferentation of neighboring cortical areas, because it could provide an important basis for the recovery of function seen in humans with impaired sensory ability such as blindness or deafness (Neville, 1990; Sadato *et al.*, 1996; Cohen *et al.*, 1997; Finney *et al.*, 2001; Bavelier and Neville, 2002).

In contrast to this negative result, examination of cadherin mRNA and protein expression in cross-modal animals with altered corticocortical connectivity patterns revealed intriguing changes in cross-modal compared to normal animals. The patterning of corticocortical connectivity reflects the modular organization of the information-processing circuitry of neocortex and is essential to sensory perception. Connectivity within and between cortical areas starts out somewhat diffusely organized and refines during development, at least partly under the influence of activity (Innocenti, 1981; Feng and Brugge, 1983; Callaway and Katz, 1990; Callaway and Katz, 1991; Schlaggar and O'Leary, 1991). There is also evidence for intrinsic specification of corticocortical connectivity patterns by

restricted distribution of gene products, including cadherins (Korematsu and Redies, 1997; Suzuki *et al.*, 1997; Bekirov *et al.*, 2002; Huffman *et al.*, 2004). We reasoned that, if adhesion factors such as the cadherins are involved in targeting of cortico-cortical projections, at the direction of upstream transcription factors such as Pax6 and Emx2, then the cross-modal ferrets with experience-dependent alterations in horizontal and callosal connectivity patterns described above might have cadherin expression patterns reflective of that change. Specifically, the cadherin expression pattern in cross-modal A1 should resemble that in normal V1, given that the connectivity patterns in A1 are organized as they are in V1. Consistent with this idea, we found that expression levels of cadherin 6 and 8 mRNA and protein are similar across A1 and V1 of cross-modal ferrets, and no longer exhibit the graded differential expression seen in normal animals (Xu and Pallas, 2005). Thus cadherins could no longer provide information that could be used to differentiate auditory from visual cortex during the development and refinement of connectivity patterns.

1.10.3.4 Evolutionary Evidence of Cross-Modal Respecification of Cortical Identity

The ability to induce cross-modal projections in an experimental situation is useful only to the extent that it can model a natural event. In addition to the parallels between clinical reports of sensory substitution in the deaf and blind (Bavelier and Neville, 2002), and our findings in cross-modal ferrets, there are evolutionary experiments that also show parallels. Perhaps most well studied are the blind mole rats of the Middle Eastern desert, *Spalax ehrenbergi*. These fossorial creatures are born with a rudimentary eye that does not form images (Bronchti *et al.*, 1991). Wollberg and colleagues have shown that visual cortex in these rodents is activated by auditory stimuli (Bronchti *et al.*, 1989, 2002; Sadka and Wollberg, 2004). This evolutionary inverse of our cross-modal manipulation in ferrets provides the proof of principle that peripheral alterations that occur in evolution can be seamlessly incorporated by plasticity inherent in sensory cortex.

1.10.4 Summary and Conclusions

The three types of investigations discussed above have clearly documented the remarkable plasticity of the developing brain. In the hamster retinocollicular system, removal of more than half of the central target and/or blockade of NMDAR-based coincidence detection have no effect on visual stimulus coding. This conservation of function occurs through compensatory compression of the retinotopic map and rearrangement of inhibitory connections. Loss of sensory input, through neonatal cochlear ablation in ferrets, results in compensatory sprouting of visual thalamic axons into the silenced auditory cortex. Such sensory substitution may underlie the expanded visual capacity of humans with early hearing loss. Cross-modal rewiring of the ferret auditory cortex results in a compensatory alteration of the structure and function of auditory cortex in a way that provides it with visual processing capacity. Taken together, these studies provide insight into how developmental mechanisms such as compensatory innervation could provide an important substrate for evolutionary changes involving alterations in sensory structures or their targets. Our work and that of many others has shown that developmental studies provide a window into the past, providing insights into how the increasing complexity of mammalian sensory systems may have evolved.

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1.11 Neuronal Migration

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Glossary

cortex	Laminar neuronal structure that is formed at the surface of the central nervous system and that includes structures such as the cerebellum and cerebral cortex.
interneuron	Local circuit neuron, sometimes also referred as a Golgi type II neuron. In a general sense, any neuron that lies between an afferent neuron and an effector neuron.
leading process	Cell extension that is located in front of the nucleus and that directs the migration of neurons.
neocortex	The six-layered part of the dorsal pallium, more properly known as the isocortex.
nucleokinesis	The process of nuclear translocation in cells, including interkinetic movements during the cycle of epithelial cells or during cell migration.
pallium	Roof of the telencephalon; it contains both cortical (e.g., hippocampus and neocortex) and deep-lying nuclear structures (e.g., claustrum and parts of the amygdala). Pallium is not synonymous with cortex.
striatum	A part of the subpallium and one of the components of the striatopallidal complex. It comprises deep (caudate nucleus, putamen, and nucleus accumbens) and superficial (olfactory tubercle) parts.
subpallium	Base of the telencephalon; it consists primarily of the basal ganglia (e.g., striatum, globus pallidus, and parts of the septum and amygdala).

1.11.1 Introduction

Neurons of the central nervous system are natural migrants, as most of them originate far from the place where they will eventually perform their normal function. Indeed, the large majority of neurons are generated from precursor cells that line the walls of the ventricular system, from where they migrate until they settle at their final position. Thus, after the genesis of specific cell types through an exquisite and controlled process of patterning and regionalization, neurons of the brain are set to migrate. In some cases, new neurons migrate for relatively short distances to settle, for instance, in the ventral horn of the spinal cord, where they become somatic motor neurons. In other cases, neurons migrate for incredibly long distances, sometimes up to thousands of times their own size, to settle in remote regions of the brain, as in the case of the interneurons of the cerebral cortex or the olfactory bulb. Thus, independent of the neuronal type, location, or function, neuronal migration is always a fundamental step in brain development.

The complexity of the brain in vertebrates is proportional, to a large extent, to the elaboration of the mechanisms controlling neuronal migration. This is particularly evident in the mammalian forebrain and, more specifically, in the telencephalon, where the development of the isocortex has been accompanied by an enormous increase in the distance covered by migrating neurons from the ventricular zone to their final destination. This is in sharp

contrast with the situation found in amphibians, for example, in which neurons barely migrate away from the place they originate. Thus, as a mechanism that shapes the development of the brain, changes in neuronal migration have greatly contributed to its diversification during evolution.

In this article, we review concepts on neuronal migration through evolution, with a focus on the central nervous system (CNS). Whenever possible, we will refer to the development of the cerebral cortex as a model system for studying the cellular and molecular mechanisms controlling neuronal migration. Of note, although the general principles that control migration in the peripheral nervous system are essentially identical to those in the CNS, this subject is beyond the scope of this article. To learn more about this, the reader is referred to reviews focusing on the mechanisms controlling neural crest migration (Robinson, *et al.*, 1997; Locascio and Nieto, 2001; Kalcheim and Burstyn-Cohen, 2005).

1.11.2 Cellular Mechanisms in Neuronal Migration

Despite prominent differences in the distance covered by distinct neuronal types until their final settlement in the brain, or even fundamental discrepancies in the primary mode of migration used by different populations of neurons (discussed in detail in the next section), migrating neurons appear to use a basic set of cellular mechanisms that is roughly similar to those used by other cell types during vertebrate morphogenesis. In that sense, neuronal migration can be considered a cyclic process, in which polarization of the cell is followed by the extension of cell protrusions and differential rearrangements in the adhesion properties of the plasma membrane leading to the movement of the neuron, including its nucleus (nucleokinesis). Moreover, because cell migration is fundamental not only during vertebrate development, but also to plants and even single-celled organisms, the molecular mechanisms underlying this process are likely to be highly preserved throughout evolution.

1.11.2.1 Polarization of Migrating Neurons

The initial response of an immature neuron to a migration-promoting factor is similar to that of other cell types in different organs and organisms and includes the polarization and extension of protrusions in the direction of migration. In other words, the molecular processes occurring at the front and the back of a neuron become distinct during migration, although we still do not understand the fundamentals of these differences. In migrating neurons, the polarized protrusion in the direction of movement is known as the leading process, which appears to behave similarly to extending axons during axon growth and guidance. As growing axons, migrating neurons typically have a single leading process that constitutes the compass reading structure driving directed neuronal migration. In some cases, however, such as, for example, immature cortical interneurons, two or more leading processes seem to act coordinately to direct cell movement (Marín and Rubenstein, 2001). Moreover, although the leading process in migrating neurons is typically only a few cell diameters in length, an extremely long leading process (up to more than 1 mm long) characterizes some populations of migrating neurons. This is the case for example of basilar pontine neurons, which are born in the dorsal hindbrain and migrate to the ventral midline, where they finally reside (Yee *et al.*, 1999).

Despite the close resemblance of the leading process – in particular, of long leading processes – to growing axons, marker analysis suggests that these two structures are molecularly different to a large extent, bearing strong similarities only at their more distant tip, the growth cone (Ramóny Cajal, 1911). For example, the processes of basilar pontine neurons stain with antibodies against transiently expressed axonal surface glycoprotein-1 (TAG-1), but do not express any of the common neuronal markers associated with axons, including growth-associated protein 43 (a molecule expressed by immature axons), microtubule-associated protein-2, or neurofilament 200. Thus, leading processes and growing axons seem to represent distinct cellular specializations used by neurons at different stages of development (Figure 1).

The polarization of migrating neurons (i.e., the extension of a leading process) depends on chemotactic responses to external cues, which seem to control also the orientation of the leading process and therefore the subsequent direction of movement. The molecules that influence the behavior of migrating neurons also typically control axon pathfinding, suggesting that the mechanisms underlying the polarization of both migrating neurons and axons are very similar. For example, Netrin-1, a prototypical axon guidance molecule (Serafini *et al.*, 1994), also promotes the extension of leading processes during neuronal migration and influences the direction of migration in multiple neuronal populations through the CNS. Similarly, Slit proteins prevent axon growth into undesirable regions

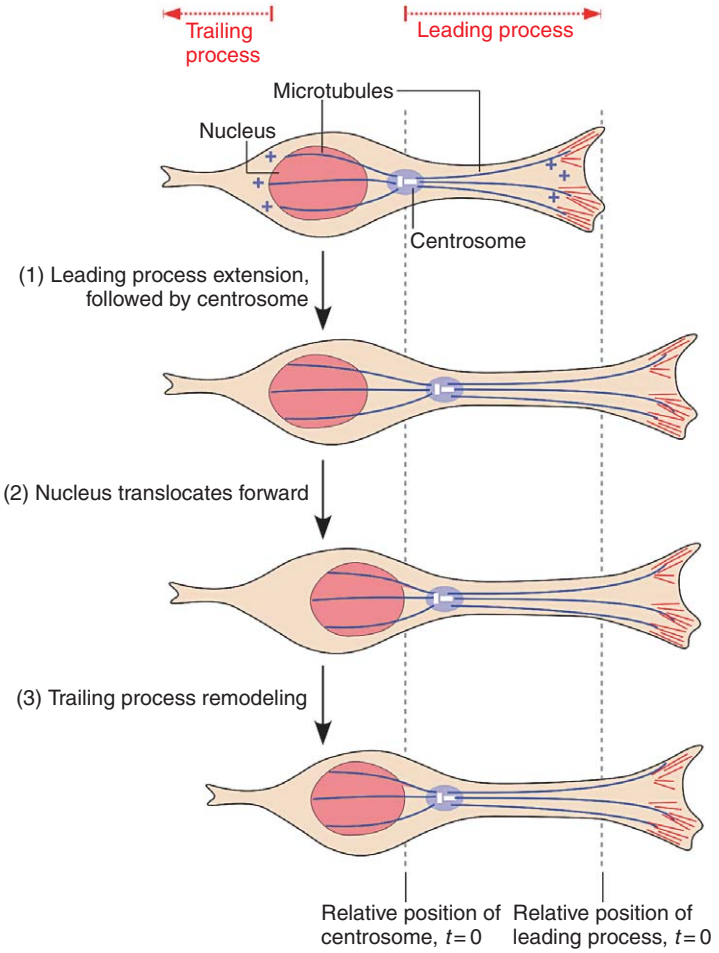

Figure 1 Steps in neuronal migration and the molecules involved. A prototypical migrating neuron contains distinct subcellular domains: the leading process, the perinuclear domain, and the trailing process. Neuronal migration involves repeated cycles of (1) polarized extension of the leading process, followed by movement of the centrosome forward, (2) a highly coordinated movement of the nucleus closer to the centrosome (nucleokinesis), and finally (3) a trailing process remodeling.

and direct neuronal migration through the CNS, acting as chemorepellent factors for both migrating axons and neurons (Brose and Tessier-Lavigne, 2000) (see also Section 1.11.5).

During chemotaxis, migrating cells – including neurons – appear to detect very small differences in chemical gradients and therefore it is likely that the process of polarization requires their amplification through steeper intracellular gradients that allow appropriate cellular responses (Figure 1). In *Dictyostelium* cells, this process involves the polarization of phosphoinositides (such as phosphatidylinositol-triphosphate (PIP3) and phosphatidylinositol (3,4)-biphosphate ($PI(3,4)P_2$)P_2) across the cell and is mediated by localized accumulation at the front of the cell of phosphoinositide 3-kinase (PI3K), which generates phosphoinositides, and restricted localization and activation at the rear of the phosphatase and Tensin homologue deleted on chromosome 10 (PTEN), which removes them (Funamoto *et al.*, 2002). In neurons, however, very little is known about how these molecules control directed polarization. Nevertheless, PI3K is required for the chemotaxis of neurons in response to neurotrophins (Polleux *et al.*, 2002) and perturbation of PTEN function causes abnormal neuronal migration (Li *et al.*, 2003).

Directed migration also requires the polarization of several organelles in slow-moving cells such as neurons. Specifically, the microtubule-organizing center (MTOC) and the Golgi apparatus are normally localized ahead of the nucleus and plays a role in defining the direction of movement. (This is not the case for fast-moving cells such as neurotrophils, in which the MTOC is behind the nucleus.) In other cell types, the small Rho GTPase Cdc42 is active toward the front of migrating cells during chemotactic responses and plays a role in localizing the MTOC ahead of the nucleus, although its contribution to the polarization of migrating neurons is still

unclear. Nevertheless, inactivation of Cdc42 appears to be required for Slit repulsion of migratory cells from the subventricular zone (SVZ) of the telencephalon (Wong *et al.*, 2001), suggesting that Cdc42 may normally help to polarize migrating neurons toward a chemoattractant source but is inactivated during chemorepulsion. Another Rho GTPase, Rac, is also polarized to the front of migrating cells and is involved in promoting directional extension of protrusions through a signaling loop that involves also Cdc42 and PI3K products. In neurons, the cyclin-dependent kinase 5 (Cdk5) and its neuron-specific regulator p35 localize with Rac during the extension of neurites and are part of the signaling machinery that may help neurons to engage in directional migration (Nikolic *et al.*, 1998). The direct interaction of Rac and possibly other small Rho GTPases with the cytoskeleton at the front of the cell appears to constitute the final effector mechanism that mediates the extension of migrating cells in a specific direction.

1.11.2.2 Nucleokinesis

One of the main differences that distinguish axon guidance from cell translocation is, obviously, the coordinated movement of the nucleus during cell migration. Thus, nucleokinesis is a fundamental step in the cycle that leads to directed cell migration and neurons are no exception to this rule. Indeed, disruption of nuclear translocation systematically leads to prominent defects in neuronal migration (Xie *et al.*, 2003; Shu *et al.*, 2004; Solecki *et al.*, 2004; Tanaka *et al.*, 2004).

Nucleokinesis in migrating neurons critically depends on the microtubule network, which plays a part in positioning the nucleus during translocation (Rivas and Hatten, 1995). As briefly mentioned in the previous section, polarization of neurons during migration includes the location of the MTOC ahead of the nucleus, an event that appears to be necessary for normal movement of the nucleus (Figure 1). This process relies on the interaction between the MTOC and the nucleus through a specialized network of perinuclear microtubules and microtubule-associated proteins, such as doublecortin (DCX) and lissencephaly-1 (LIS1). Both of these proteins bind to microtubules and appear to regulate their polymerization, bundling, and/or stabilization in migrating neurons. In humans, mutations in DCX cause an X-linked type of lissencephaly known as double cortex syndrome (also called subcortical band heterotopia), whereas mutations in the *Lis1* gene cause classic lissencephaly, the Miller–Dieker syndrome (Ross and Walsh, 2001).

As expected from their crucial function in nuclear movement, proteins involved in this process are highly conserved throughout evolution. For example, the *Lis1* homologue in the filamentous fungus *Aspergillus nidulans* is a nuclear migration gene. During development of the fungus, cells become multinucleated through several rounds of divisions and it becomes crucial that nuclei disperse uniformly within the cell for normal growth to occur. This process of nuclear migration in fungi also depends on the network of microtubules and is regulated by proteins that associate with the microtubules, such as that encoded by the *nud*F gene. (Proteins related to nuclear movement in *A. nidulans* were isolated through a screen for nuclear distribution mutants, for which they are named.) *nud*F shares 42% sequence identity with *Lis1* and both genes are considered orthologues. Analysis of other nuclear distribution mutants similar to *nud*F has helped to define the molecular mechanisms mediating the function of this protein in fungi and, by extension, in migrating neurons. For example, *nud*F closely interacts with *nud*A, a gene that encodes the heavy chain of cytoplasmic dynein and is directly involved in nuclear translocation. Another protein that appears to act as a downstream effector of *nud*F is NUDE, two homologues of which have been isolated in mammals, mNudE and NUDEL. Both of these proteins localize to the MTOC and appear to be important in controlling the movement of the nucleus through their association with other proteins, such as γ-tubulin or dynein. Indeed, Lis1, dynein, or NUDEL loss of function results in defects of centrosome–nucleus coupling during neuronal migration (Shu *et al.*, 2004; Tanaka *et al.*, 2004). In summary, these findings illustrate how the identification of homologous proteins in model systems such as *A. nidulans* is greatly contributing to the identification of the function of vertebrate proteins associated with neuronal migration and, more specifically, nucleokinesis (Feng and Walsh, 2001).

In addition to proteins that directly associate with the microtubule network encaging the nucleus during nuclear translocation, other signaling proteins appear to be crucial for normal nucleokinesis. One of these proteins is Cdk5, a serine/threonine kinase that phosphorylates proteins that maintain cytoskeletal structures and promote cell motility. Mice deficient in *Cdk5* or its activating subunits, *p35* and *p39*, exhibit prominent laminar defects in the cerebral cortex, suggesting that this signaling pathway is crucial for neuronal migration (reviewed in Dhavan and Tsai, 2001). For instance, NUDEL is a physiological substrate of Cdk5 (Niethammer *et al.*,

2000; Sasaki *et al.*, 2000). Another case is the focal adhesion kinase (FAK), which is localized in a Cdk5 phosphorylation-dependent manner to the perinuclear network of microtubules where it contributes to normal nuclear movement (Xie *et al.*, 2003). Another example is mPar6α, a protein that associates with different forms of protein kinase C and localizes to the MTOC, where it contributes to promote the polarization of the centrosome in the direction of the movement. Because movement of the centrosome precedes that of the nucleus itself, the function of proteins such as mPar6α is essential for determining the direction of nucleokinesis.

In summary, multiple components of the cellular machinery involved in nucleokinesis have been already identified and a model for understanding nucleokinesis in migrating neurons is starting to emerge (Figure 1). As in the past few years, it is expected that the discovery of other proteins involved in this process may arise through additional homology analyses, since it is clear now that the cellular mechanisms underlying nuclear migration are similar throughout evolution, from unicellular organisms to humans.

1.11.3 Modes of Migration in the Developing Brain

1.11.3.1 Two Primary Modes of Migration in the Developing CNS

As discussed in the previous section, the cellular mechanisms underlying the migration of neurons are likely to be similar to those in other cell types. Despite these molecular similarities, two different modes of migration are classically distinguished within the developing brain, radial and tangential migration. In a general sense, radial migration refers to neurons that migrate perpendicularly to the surface of the brain. In contrast, tangential migration is defined by neurons that migrate in a direction that is parallel to the surface of the brain (in either the rostrocaudal axis or the dorsoventral axis) and that is therefore perpendicular to radially migrating neurons. Although this subdivision is primarily based on the orientation of migrating neurons in relation to the neural tube coordinates, it also implicitly reflects the dependence of different classes of neurons on substantially distinct substrates for migration, as we discuss in the next section. In any case, the existence of these different modes of migration in the developing CNS does not indicate that the molecular and cellular mechanisms underlying radial and tangential migration are essentially different. In other words, independent of the mode of migration, alternating cycles of polarization and nucleokinesis are common events to any migrating neuron.

Radial migration is the principal mode of migration within the CNS. In a general sense, radial migration allows the transfer of topographic information from the ventricular zone to the underlying mantle, since neurons that are born nearby tend to occupy adjacent positions in the mantle when using radial migration to reach their final destination. This has important consequences for the organization of the brain. First, radial migration is essential to generate and maintain distinct neurogenetic compartments in the developing neural tube, which is ultimately necessary for the establishment of different cytoarchitectonic subdivisions within the brain (Figure 2). That is, different progenitor regions generate distinct structures in the CNS largely because progenitor cell dispersion is restricted in the ventricular zone (Fishell *et al.*, 1993; Lumsden and Krumlauf, 1996) and migrating neurons from different compartments do not intermingle during their migration. Second, the transfer of positional information from the ventricular zone to the mantle of the brains allows the formation of topographically organized projections, which are crucial for the proper function of the brain. For this reason, radial migration is the basic mechanism preserved throughout evolution to segregate neurons in all regions of the CNS.

Radial migration contributes to the formation of both cortical (i.e., laminar, such as the cerebral cortex, hippocampus, or cerebellum) and nuclear structures (e.g., striatum, red nucleus), although the development of laminar structures is perhaps the most remarkable example on how radial migration may contribute to the formation of complex circuits in the brain. Laminar structures are found in the brain of all vertebrates and, although the most sophisticated example is the mammalian isocortex, the optic tectum of amphibians, reptiles, or birds is a prominent antecedent of this structure. In contrast to brain nuclei, laminar structures are organized to segregate complex patterns of afferent and efferent connections. Because of this organizing principle, the formation of cortical–laminar structures requires the perfect synchronization of proliferation, cell fate, and radial migration mechanisms to determine the number of layers, as well as their cell density and arrangement.

In contrast to radial migration, which appears to play a general role in the formation of major subdivision in the brain, tangential migration is thought to increase the complexity of neuronal circuits because it allows neurons born from distinct

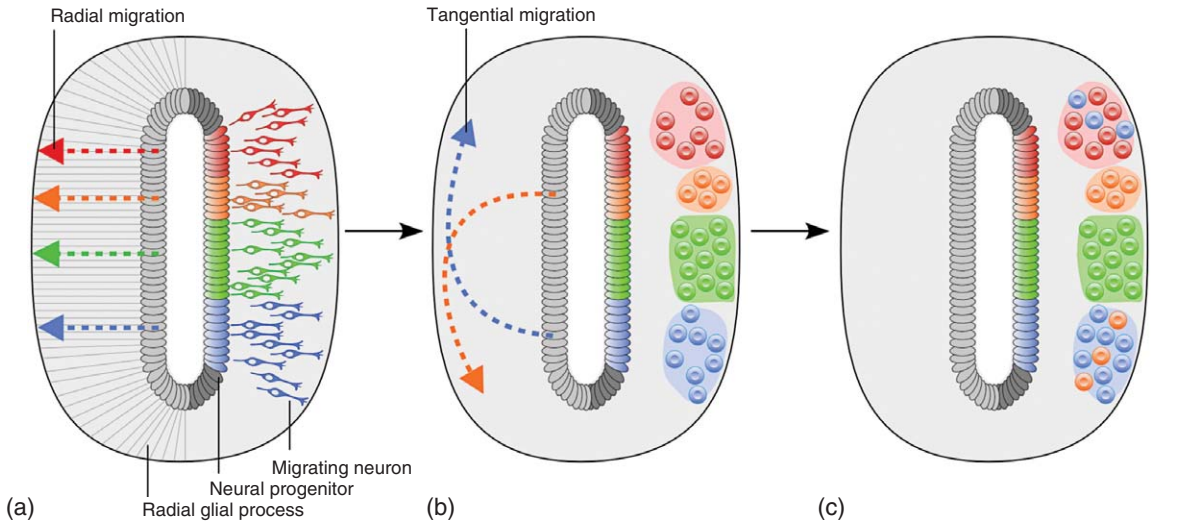

Figure 2 Radial and tangential migration in the central nervous system. a and b, Radial glial cells provide structural support for radial migration, a process that results in the generation of different nuclei that are topographically organized in relation to their place of origin. b and c, Tangential migration is independent of radial glial processes and therefore does not respect topographical references. As a result, tangential migration produces an increase in the complexity of different nuclei by providing cell types distinct from those that are locally generated.

ventricular zones to intermingle and occupy a final common destination (Marín and Rubenstein, 2001) (Figure 2). Tangential migration is likely to be a relatively modern mechanism compared to radial migration, but may have been successfully maintained during evolution because it inherently adds complexity to brain circuits through the incorporation of new cell types with those already present in each region (Figure 2).

Compared to radial migration, the existence of tangential dispersion of neurons in the developing brain has only begun to receive much attention, so it may give the impression of being a relatively contemporary discovery. During the last 30 years of the past century, the predominant view on brain development was based on the idea that radial migration was the sole mechanism allowing the movement of neurons from the progenitor regions to their final destination (Rakic, 1990). This idea was consistent with the basic notion of developmental segmentation in the brain because, as discussed earlier, radial migration contributes to the establishment of segregated cytoarchitectonic regions (Lumsden and Keynes, 1989; Puelles and Rubenstein, 1993). Nevertheless, it was clear from early studies using Golgi-stained sections or electron microscopy that some neurons within the developing brain are oriented tangentially in directions inconsistent with radial migration (Stensaas, 1967; Morest, 1970; Shoukimas and Hinds, 1978). Since then, tangential dispersion has been observed in virtually every subdivision of the developing CNS, from the spinal cord and hindbrain (Bourrat and Sotelo, 1988; Ono and Kawamura, 1989; Leber *et al.*, 1990; Marín and Puelles, 1995; Phelps *et al.*, 1996) to the telencephalon (Austin and Cepko, 1990; Halliday and Cepko, 1992; Walsh and Cepko, 1992; O'Rourke *et al.*, 1992, 1995; Tan and Breen, 1993; De Carlos *et al.*, 1996). In the case of the cerebral cortex, the most compelling experimental evidence supporting the existence of two general modes of cell dispersion, radial and tangential, came from analysis of clonally related cells using retroviral-mediated transfer or highly unbalanced chimeras (Walsh and Cepko, 1992; Tan and Breen, 1993), unequivocally demonstrated by pioneer time-lapse studies (O'Rourke *et al.*, 1992). The main conclusion from all these studies confirms a general principle in our view of brain development: the organization of distinct cytoarchitectonic regions in the CNS most frequently depends on two mechanisms of cell allocation: radial mosaicism and tangential migration.

The existence of two basic modes of migration within the CNS may lead to the erroneous conclusion that there are two major populations of neurons in the developing brain: those that migrate radially and those that use tangential migration to reach their final destination. Indeed, radial and tangential migrations are just two different mechanisms of cell dispersion that the same population of neurons may use indistinctly to reach their final position within the brain. The stereotyped behavior of the facial branchiomotor (fbm) neurons in the hindbrain perfectly illustrates this point (Figure 3). In the mouse, fbm

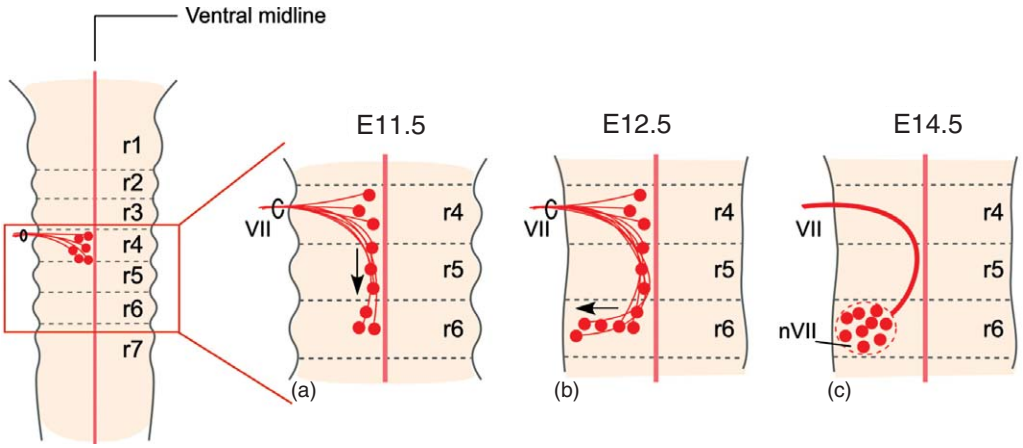

Figure 3 Facial branchiomotor (fbm) neurons adopt tangential and radial modes of migration. Schematic representation of the hindbrain showing the migration of fbm neurons (red circles) during mouse development. At embryonic (E) day 11.5 (E11.5), fbm neurons migrate tangentially (in the caudal direction) from r4, where they originated, to r6. Later, they migrate tangentially within r6, from ventral to dorsal. Finally, they adopt a radial mode of migration to finally form the facial motor nucleus laterally (nVII).

neurons are born in the basal plate of rhombomere 4 (r4), but they finally come to reside in r6. To reach their destination, fbm neurons first migrate tangentially in the caudal direction until they reach r6. Then, they turn 90° and migrate tangentially in the dorsal direction toward the alar–basal boundary. Finally, they turn 90° again and migrate radially toward the pial surface, where they settle to form the facial motor nucleus (for references, see Garel *et al.*, 2000). Similar examples of switching migratory behaviors are present throughout the CNS (cerebellar granule cells, olfactory bulb, and cerebral cortex interneurons, etc.), suggesting that this is a general trend during development. In summary, the same population of neurons may use radial and tangential migration strategies to reach their final destination, likely depending on the extracellular environment available for their dispersion.

1.11.3.2 Evolutionary Advantages of Different Modes of Migration

The development of the cerebral cortex nicely illustrates how the different modes of neuronal migration contribute to the formation of complex circuits in the CNS. The cortex contains two main classes of neurons, the glutamatergic pyramidal neurons and the γ-aminobutyric acid (GABA)-containing neurons. Both classes of neurons use largely different modes of migration to reach their final position in the cortex during development (reviewed in Corbin *et al.*, 2001; Marín and Rubenstein, 2001). Thus, pyramidal neurons migrate radially from the progenitor zones of the pallium to their final position in the cortex. In contrast, interneurons are largely born in progenitor regions of the subpallium and therefore have to migrate tangentially to reach the pallium. Once in the pallium, interneurons change their mode of migration from tangential to radial to reach their final destination in the cortex. Thus, projection neurons and interneurons use different modes of migration to arrive at the cerebral cortex largely because they derive from segregated progenitors within the telencephalon.

What advantage might there be in producing different classes of neurons at distant places in the CNS instead of producing all of them locally for each brain structure? This question might be answered if we consider that cell patterning and migration are intimately linked during the development of the CNS throughout evolution. In the telencephalon, for example, early dorsoventral patterning specifies distinct domains that produce neurons synthesizing different classes of neurotransmitters (reviewed in Wilson and Rubenstein, 2000; Campbell, 2003). Thus, the dorsal region of the telencephalon – the pallium – becomes patterned to produce glutamatergic neurons, whereas the subpallium is specified to generate GABAergic and cholinergic neurons. This organization is a primitive trend of the telencephalon in vertebrates, since it seems to be present in the different classes of living vertebrates (Puelles *et al.*, 2000; Frowein *et al.*, 2002; Gonzalez *et al.*, 2002; Brox *et al.*, 2003) and appears to represent an efficient way to pattern neural progenitors to produce different classes of neurons using a limited number of morphogenetic centers. Thus, patterning mechanisms that have been preserved throughout evolution appear to limit to some extent the generation of multiple classes of neurons in the exact same region of the brain, at least from the perspective of the neurotransmitter phenotype, and tangential

migration may have evolved, among other things, to overcome this limitation.

In mammals, the balance between excitatory (glutamatergic) and inhibitory (GABAergic) synaptic activity is critical for the normal functioning of the cerebral cortex. As a result, inherited disruption of this balance leads to important behavioral dysfunction in animal models (Liu *et al.*, 2000; Steinlein and Noebels, 2000; Powell *et al.*, 2003) and in severe neurological disorders in humans (Keverne, 1999; Lewis, 2000; Sanacora *et al.*, 2000; Holmes and Ben-Ari, 2001). In that context, the introduction of GABAergic interneurons from an external source to the population of cortical neurons may have played a pivotal role in shaping up neural circuits during the expansion of the cerebral cortex through evolution. A recent study by López-Bendito *et al.* (2006) has strongly suggested the convergence of these phenomena in the development of the thalamocortical system. This study has demonstrated the existence of a new tangential migration of GABAergic cells within the ventral telencephalon that mediates the navigation of thalamic axons toward their final destination in the neocortex. Specifically, tangential migration from an evolutionarily primitive intermediate target, the striatum, contributes to form a permissive bridge for the extension of thalamocortical axons through nonpermissive regions of the ventral telencephalon. In a more general sense, whereas radial migration has been preserved as the mechanism conferring regional identity to distinct structures in the CNS, tangential migration may represent a paradigm to increase the complexity of neuronal circuits during evolution. For instance, the casual incorporation of a migratory route that brings a new population of neurons into an established structure (e.g., through a mutation that induces the expression of a receptor for a guidance molecule in that specific population of neurons) may lead to a complete dysfunctional brain or, occasionally, to a modification of the normal function of the structure representing a competitive evolutionary advantage for the species. Such a mechanism may explain, for example, the differences observed in the number of GABAergic interneurons in the dorsal thalamus of primates – in particular humans – compared to other vertebrates (Letinic and Rakic, 2001). Moreover, the identification of a neocortical origin for a population of GABAergic neurons in the developing human cortex reinforces the existence of such evolutionary trend (Letinic *et al.*, 2002).

1.11.4 Mechanisms of Radial Migration

Radial migration has classically been known as glial-guided cell migration because during this process neurons move along the processes of specialized glial cells known as radial glia (Rakic, 1971a, 1971b, 1972; Rakic *et al.*, 1974; Edmondson and Hatten, 1987). Despite their name, however, radial glial cells do not simply function as static supportive elements. Instead, radial glial cells represent an intermediate stage in the stem cell lineage of the CNS (reviewed in Alvarez-Buylla *et al.*, 2001) and undergo mitosis to produce new neurons (Noctor *et al.*, 2001). In addition, radial glial cells have a process that spans the wall of the neural tube and reaches the pial surface (Bergman glial cells being one exception to this rule), where it is anchored to the basal membrane. This process establishes a point-to-point relation between the ventricular zone and the surface of the brain, supporting neuronal movement during radial migration. Genetic defects affecting the development of radial glia cells lead to abnormal neuronal migration in the CNS (reviewed in Ross and Walsh, 2001; Marín and Rubenstein, 2003), suggesting that radial glia integrity is fundamental for radial migration.

Although radial glia integrity is largely essential for radial migration, there seem to be exceptions to the rule described above. During early stages of corticogenesis, for example, new neurons undergo radial migration through a process known as somal translocation (described as perikaryal translocation by Morest, 1970), which appears to be largely independent of radial glial cells (reviewed in Nadarajah and Parnavelas, 2002). During somal translocation, the leading process of migrating cells terminates at the pial surface and it becomes progressively shorter as the cells approach their final position. This is also observed in cells moving through glial-guided radial migration as they approach the pial surface. Thus, for some cell types or specific developmental periods, radial migration may not directly depend on radial glial cells.

Radial migration has been preferentially studied during the development of the cerebral cortex and the cerebellum and thus most of our knowledge on the mechanisms that control radial migration derives from the analysis of these structures. *In vitro* and *in vivo* studies of radial cell migration have identified a number of molecules that mediate this mode of migration. These molecules belong to multiple categories, including motogenic factors (i.e., factors that promote migration), cell adhesion molecules, receptors, and secreted factors, some of which are described below. We have excluded from this list molecules controlling those aspects of migration that are likely to be common to any type of neuronal migration (e.g., LIS1, DCX; see Section 1.11.2), even though they have been

classically associated with radial migration defects. In that context, it is worth noting that neuronal migration abnormalities are likely to be more easily identified in laminar than in nuclear structures; this does not exclude, however, a role for these molecules in other types of migration (see, for example, McManus *et al.*, 2004b; Pancoast *et al.*, 2005).

Several classes of molecules have been described to stimulate radial migration. In the cerebral cortex, for example, members of the neurotrophin family such as brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) promote the motility of cortical cells through their high-affinity receptor tyrosine kinase B (TrkB) (Behar *et al.*, 1997; Brunstrom *et al.*, 1997). Other factors, such as (GABA) and glutamate, also appear to promote the migration of cortical neurons *in vitro*. These neurotransmitters are released independently of the conventional soluble N-ethylmaleimide sensitive fusion protein attachment protein receptor (SNARE)-dependent mode of secretion – probably through a paracrine mechanism – and mediate their effects primarily through the activation of $GABA_A$ and N-methyl-D-aspartate (NMDA) receptors (Komuro and Rakic, 1993; Behar *et al.*, 1996, 1999, 2001; Manent *et al.*, 2005).

To a large extent, most factors directly involved in controlling radial migration are molecules that regulate the interaction between migrating neurons and radial glial. This is the case of Astrotactin-1 (Astn1), which was first identified as an activity mediating the interaction of neurons and radial glial processes in cerebellar cultures (Edmondson *et al.*, 1988). Astn1 is a glycoprotein expressed by migrating neurons both in the cerebellum and in the cerebral cortex and it is required for normal migration of neuroblasts along glial processes (reviewed in Hatten, 2002). Integrins constitute another family of factors implicated in the association between migrating neurons and radial glia. Thus, function-blocking antibodies against α3, αv, and β1 integrins perturb the interaction between neurons and radial glial cells *in vitro* (Anton *et al.*, 1999; Dulabon *et al.*, 2000). Moreover, radial migration is altered in the cerebral cortex of α3, α6, or β1 integrin mutant mice (Georges-Labouesse *et al.*, 1998; Anton *et al.*, 1999; Graus-Porta *et al.*, 2001), although the precise function of integrins during *in vivo* radial migration remains unsettled. In the case of α3 integrin, however, it has been suggested that signaling through this receptor may directly control actin dynamics and consequently influence the ability of migrating neurons to search and respond to guidance cues in the developing cortex (Schmid *et al.*, 2004).

The interaction between migrating neurons and radial glial fibers may also be controlled through intracellular signaling cascades. For example, correct apposition of neurons to radial fibers may largely depend on the morphology of migrating neurons. In the cerebral cortex, migrating neurons are largely bipolar, which possibly facilitates their interaction with radial glial processes. In the absence of p35, a regulatory activator of Cdk5 that controls the function of many proteins associated with the cytoskeleton, the leading process of radially migrating neurons is branched, and this associates with an impaired neuronal–glia interaction and perturbed migration (Gupta *et al.*, 2003). Thus, the morphological organization of migrating neurons might be an important factor in determining their mode of migration.

The interaction between migrating neurons and radial glial processes is important not only for the initiation and maintenance of radial migration, but also for the control of its finalization. The precise termination of radial migration is crucial for the normal organization of brain structures. This is more evident in cortical structures, in which the pattern of radial migration termination determines the establishment of the laminar organization. In the case of the isocortex, birth-dating studies have shown that layers in the cortical plate (future cortical layers 2–6) are established according to an inside–outside pattern, where the deeper layers contain cells that become postmitotic earlier than the cells in more superficial layers (Angevine and Sidman, 1961; Rakic, 1974). During development, new neurons migrate radially toward the surface of the cortex, passing through cohorts of previously born neurons, and detach from radial glia as they approach the marginal zone. Analysis of mutations in mice and humans has revealed that the interaction between migrating neurons and Cajal–Retzius cells, a specialized cell type present in the embryonic marginal zone, is essential for controlling the detachment of migrating neurons from radial glia and, subsequently, the normal laminar organization of the cortex (reviewed in Gupta *et al.*, 2002; Marín and Rubenstein, 2003).

The interaction between Cajal–Retzius cells and radially migrating neurons is mediated, at least in part, by Reelin, a large glycoprotein secreted by Cajal–Retzius cells during early stages of the development of the cortex. Reelin is expressed in many regions of the developing brain and in many species of vertebrates, but its function has been most extensively studied in the developing cortex. (see Reelin, Cajal-Retzius Cells, and Cortical Evolution for more on Reelin and its function in neuronal migration.) Reelin is a high-affinity ligand for two members of

the LDL family of lipoprotein receptors, the very low-density lipoprotein receptor (VLDLR) and the low-density lipoprotein receptor-related protein 8 (LRP8, also known as ApoER2), which are expressed by radially migrating cortical neurons (D'Arcangelo *et al.*, 1999; Hiesberger *et al.*, 1999). Signaling through VLDLR/LRP8 mediates tyrosine phosphorylation of the mouse homologue of the *Drosophila* protein Disabled (DAB1). DAB1 is a cytoplasmic adapter protein that interacts with the cytoplasmic tails of VLDLR and LRP8 and is linked to events related to the reorganization of the cytoskeleton.

Although many aspects of the function of the Reelin–VLDLR/ApoER2–Dab1 pathway in radial migration remain unsettled, it is clear that Reelin signaling is involved in the final events that lead to the detachment of migrating neurons from radial glia. Loss of Dab1 function, for example, results in an impairment of the adhesive properties of radially migrating neurons, which fail to detach normally from the glial fiber in the later stage of migration (Sanada *et al.*, 2004). Importantly, the influence of Reelin on the adhesive properties of radially migrating neurons may be the result of its interaction with other proteins, such as $\alpha 3\beta 1$ integrin receptors, which are expressed in radially migrating neurons (Dulabon *et al.*, 2000). It should be noted, however, that Reelin function is likely not restricted to controlling the interaction between migrating neurons and glial fibers.

It is likely that the Reelin–VLDLR/ApoER2–Dab1 pathway is just one of many signaling routes controlling neuronal detachment from radial glia and movement termination in the cerebral cortex. Thus, other proteins that are specifically expressed in radial glial processes at the level of the cortical plate are also candidates for the regulation of this process. One of these proteins is secreted protein acidic and rich in cysteine-like 1 (SPARC-like 1), which appears to function in ending neuronal migration by reducing the adhesiveness of neurons to glial fibers in the cortical plate (Gongidi *et al.*, 2004).

1.11.5 Mechanisms of Tangential Migration

Tangential migration, defined as a nonradial mode of migration, includes distinct types of cell movement that differ in the type of substrate used by migrating cells. Regardless of the substrate employed, tangentially migrating cells share an important common feature: they do not respect regional forebrain boundaries. Thus, cell populations engaged in tangential migration normally move over long distances and follow complex trajectories before reaching their final destination. These migrations usually involve multiple changes in the direction of the movement, which depend on changes in the environment and/or the responses of migrating neurons. In the past few years, many studies have demonstrated the existence of environmental cues that can act as contact or diffusible attractants or repellents that provide directional information to tangentially migrating neurons through interactions with cell-surface receptors (see Table 1). Here, the cellular and molecular mechanisms controlling tangential migration are reviewed using as examples two well-characterized tangential migratory populations, cortical interneurons and facial branchiomotor neurons.

1.11.5.1 Migration of Cortical Interneurons

The tangential migration of cortical interneurons to the cortex is, most likely, one of the most intensively studied cell populations of the developing brain since the seminal discovery of their subpallial origin in mammals (Anderson *et al.*, 1997). Since then, several other studies have shown that a subpallial origin of cortical interneurons is a common feature to, at least, tetrapod vertebrates (Cobos *et al.*, 2001; Gonzalez *et al.*, 2002; Brox *et al.*, 2003), suggesting that this is a highly conserved trait in cortical evolution.

Cells migrating tangentially to the cortex have multiple origins within the subpallium (reviewed in Corbin *et al.*, 2001; Marín and Rubenstein, 2001), although most GABAergic interneurons seem to derive from the medial ganglionic eminence (MGE). Interestingly, the MGE is also the source of interneurons for other forebrain structures, such as the striatum (Marín *et al.*, 2000; Wichterle *et al.*, 2001). Consequently, most of our knowledge on the mechanisms controlling the migration of cortical interneurons refers to MGE-derived cells and it is likely that different molecules may control the migration of interneurons generated in other subpallial structures, such as the caudal ganglionic eminence (Nery *et al.*, 2002).

There are several key decision points affecting the migration MGE-derived cortical interneurons. First, interneurons initiate their migration in response to factors that stimulate their movement. Second, interneurons refrain from migrating in ventral and ventromedial regions – thus avoiding the preoptic area and the septum – directing instead their movement in a dorsal direction. Third, cortical interneurons actively avoid entering the developing striatum, a target for other classes of MGE-derived interneurons. Early during development, interneurons

Table 1 Guidance factors and neuronal migration in the CNS

Gene	*Function*	*Neuronal population*	*Refs.*
BDNF, NT4	Growth factor; motogenic; promotes neuronal migration	Cortical interneurons, cortical projection neurons	*a*
SDF1	Chemokine; chemoattractant	Cerebellar granule cells, dentate granule cells, cortical interneurons	*b*
EphrinB2, EphrinB3	Guidance molecules; chemorepellents	Rostral migratory stream	*c*
GABA	Neurotransmitter; chemoattractant	Cortical interneurons, cortical projection neurons	*d*
Glutamate	Neurotransmitter; promotes neuronal migration	Cortical projection neurons	*e*
Gdnf	Growth factor; motogenic; promotes neuronal migration	Cortical interneurons	*f*
Hgf	Growth factor; motogenic; promotes scattering of neurons	Cortical interneurons	*g*
Netrin-1	Guidance molecule; chemoattractant and chemorepellent	Basilar pontine neurons, precerebellar nuclei, cerebellar granule cells, striatal projection neurons	*h*
Nrg1	Guidance molecule; chemoattractant and permissive factor	Cortical interneurons, rostral migratory stream	*i*
Sema3A, Sema3F	Guidance molecules; chemorepellents	Cortical interneurons	*j*
Somatostatin	Motogenic; movement promotion and termination	Cerebellar granule cells	*k*
Slit1, Slit2	Guidance molecules; chemorepellents	Rostral migratory stream, different classes of neurons derived from the subpallium	*l*

[a]Brunstrom *et al.* (1997); Polleux *et al.* (2002).
[b]Zou *et al.* (1998); Bagri *et al.* (2002); Stumm *et al.* (2003).
[c]Conover *et al.* (2000).
[d]Behar *et al.* (1996, 2000); López-Bendito *et al.* (2003); Luján *et al.* (2005).
[e]Hirai *et al.* (1999).
[f]Pozas and Ibañez (2005).
[g]Powell *et al.* (2001).
[h]Bloch-Gallego *et al.* (1999); Yee *et al.* (1999); Alcántara *et al.* (2000); Hamasaki *et al.* (2001).
[i]Anton *et al.* (2004); Flames *et al.* (2004).
[j]Marín *et al.* (2001); Tamamaki *et al.* (2003).
[k]Yacubova and Komuro (2002).
[l]Hu (1999); Wu *et al.* (1999); Zhu *et al.* (1999); Gilthorpe *et al.* (2002); Marín *et al.* (2003).

tend to migrate superficial to the striatal mantle. However, as development proceeds and the dorsal striatum becomes a large structure in the basal ganglia, cortical interneurons migrate preferentially deep to the striatal mantle (i.e., through the interface between the SVZ of the lateral ganglionic eminence and the striatal mantle). Fourth, interneurons cross the subpallial–pallial boundary, invading the pallium through highly stereotyped routes of migrating, which include the marginal zone, the subplate, and the cortical SVZ. And fifth, interneurons invade the cortical plate and integrate in their appropriate layer according to their birth date. Thus, the migration of cortical interneurons is a complex and well-orchestrated event in the developing forebrain. So, what are the molecular cues that regulate each of these decisions?

Cortical interneurons initiate their movement and engage in long-distance migration probably because they respond to motogenic/scatter factors along their pathway. Several such factors have been identified in the past few years, all of which have in common their ability to also influence the maturation and final differentiation of cortical interneurons. Thus, the neurotrophins BDNF and neurotrophin-4, the scattered factor/hepatocyte growth factor, and the glial-derived neurotrophic factor (GDNF) all have the ability to promote interneuron migration to the cortex (Powell *et al.*, 2001; Polleux *et al.*, 2002; Pozas and Ibanez, 2005).

Cortical interneuron migration to the cortex is strongly influenced by molecular activities that prevent interneuron invasion of unsolicited regions. This is the case in the preoptic area and the septum, where the molecular nature of the repulsive activity preventing the migration of interneurons is still unknown (Marín *et al.*, 2003). The striatum constitutes a nonpermissive territory for the migration of cortical interneurons because it expresses class 3 semaphorins (Sema3A and Sema3F) and cortical interneurons

express neuropilin receptors for these repellent cues (Marín *et al.*, 2001; Tamamaki *et al.*, 2003).

Cortical interneuron migration is also controlled by permissive and attractive factors that direct interneurons in a dorsal direction from the MGE to the cortex (Marín *et al.*, 2003; Wichterle *et al.*, 2003). However, for the first time, a chemoattractive effect on cortical interneurons by a molecule expressed at the cortex has been described. This cue is Neuregulin-1 (NRG1), a member of the neuregulin family of proteins. Of note, different isoforms of NRG1 are differentially expressed in the developing telencephalon, thus controlling distinct aspects of the migration of cortical interneurons (Flames *et al.*, 2004). Thus, membrane-bound forms of NRG1, Cystein-rich domain (CRD)-NRG1, are expressed in the route of interneuron migration from the MGE to the pallial–subpallial boundary, and it seems to create a permissive corridor for interneuron migration toward the cortex. In addition, diffusible forms of NRG1, Ig-NRG1, are specifically expressed in the cortex, from where they appear to attract interneuron migration. Other factors are likely to attract interneuron migration to the cortex. For example, GDNF also acts as an attractive cue for interneuron migration *in vitro* (Pozas and Ibañez, 2005), although its wide distribution in the telencephalon suggests that it may rather act as motogenic factor *in vivo*.

It has been suggested that cortical interneurons may use corticofugal axons as a substrate for their migration to the cortex. Axons have been proposed as substrates for other tangentially migrating cell populations, such as gonadotropin-releasing hormone neurons (Wray, 2002), and *in vitro* evidence suggests that axons may serve as substrates for the migration of cortical interneurons (McManus *et al.*, 2004a). Moreover, molecules specifically expressed in corticofugal axons appear to influence interneuron migration *in vitro* (Denaxa *et al.*, 2001). At the peak of interneuron migration, however, most cells migrate through axon-poor regions such as the cortical SVZ, suggesting that axons may influence primarily early stages of interneuron migration to the cortex.

The guidance of cortical interneurons may also be influenced by neuronal activity. In agreement with this hypothesis, several studies have described the early expression of GABA and glutamate receptors at the cerebral cortex before the formation of synapses (Métin *et al.*, 2000; López-Bendito *et al.*, 2002a, 2002b; Luján *et al.*, 2005). The function of some of these receptors has been tested *in vitro*. For example, stimulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in slice cultures induces GABA release in tangentially migrating cells (Poluch and Konig, 2002). In contrast, *in vitro* blockade of $GABA_B$ receptors leads to a derailment of GABAergic interneurons within the neocortex (López-Bendito *et al.*, 2003). Others neurotransmitter receptors, such as NMDA, AMPA/Kainate, and $GABA_A$, are also functional on tangentially migratory interneurons (Métin *et al.*, 2000; Soria and Valdeolmillos, 2002), suggesting that they also influence the migration cortical interneurons through a yet unknown mechanism.

Once interneurons reach the cortex, they invade the cortical plate and distribute through the different cortical layers. Interestingly, invasion of the cortical plate does not occur automatically as interneurons reach the cortex, but rather seems to be a highly stereotyped process designed to allow the homogeneous dispersion of cortical interneurons throughout the whole rostrocaudal and mediolateral extent of the cerebral cortex (G. López-Bendito and O. Marín, unpublished observations). In addition, invasion of the cortical plate by interneurons may depend on radial glia (Ang *et al.*, 2003; López-Bendito *et al.*, 2004; Tanaka *et al.*, 2003) and thus on the mechanisms described for cortical pyramidal cells (see Section 1.11.4). Nevertheless, some of the molecules that influence migration of projection neurons, such as Cdk5, do not seem to influence the tangential migration of cortical interneurons or their subsequent movement into the cortical plate (Gilmore and Herrup, 2001).

1.11.5.2 Migration of Facial Branchiomotor Neurons

Tangential cell movements during CNS development are not restricted to forebrain. Indeed, tangential migration is present at all rostrocaudal levels of the neural axis. In the hindbrain, for example, the facial (nVII) branchiomotor neurons of several vertebrates, including fish and mammals, follow a large stereotyped migration that includes tangential migration from their origin in r4 to caudal r6 or r7 (reviewed in Chandrasekhar, 2004). Tangentially migrating fbm neurons use a mode of migration very similar to the somal translocation described in the cortex (Book and Morest, 1990), in which the nucleus moves along a large leading extension as the migrating cell leaves behind an axonal process that reflects the migratory path.

It has been shown that environmental cues present in r5 and r6 mediate fbm neuronal tangential migration. Interestingly, chick fbm neurons undergo limited caudal migration naturally; however,

transplantation studies have demonstrated that these cells have the ability to migrate caudally when transplanted into mouse r4, demonstrating that the cues necessary for the initiation, and perhaps maintenance, of caudal migration are absent in the chick hindbrain (Studer, 2001). Additional evidence for environmental cues regulating fbm migration comes from genetic and molecular studies in zebra fish. In the zebra fish mutant *trilobite* (*tri*), fbm neurons fail to migrate tangentially into r5–7 (Bingham *et al.*, 2002). This phenotype, however, can be rescued when *tri* mutant fbm neurons are transplanted into a wild-type environment, whereas wild-type fbm neurons fail to migrate caudally in a mutant context. What molecules are responsible for this behavior? Tangentially migrating fbm neurons regulate the expression of genes encoding the cell membrane proteins, such as TAG-1, Ret, and Cadherin-8, and this regulation is dependent on their location at r4, r5, or r6 (Garel *et al.*, 2000). Interestingly, in embryos deficient for *Ebf1* or *Nkx6-1*, fbm neurons either fail to migrate or undergo an incomplete caudal migration, prematurely expressing an abnormal combination of markers (Garel *et al.*, 2000). These data suggest that fbm neurons adapt to their changing environment by switching on and off specific genes.

Finally, studies have shown that tangential migration of fbm neurons is controlled by neuropilin receptors, as is the case for cortical interneurons. Thus, loss of Neuropilin-1 (Nrp1) in the mouse compromises the tangential migration of fbm neurons, causing the formation of misshapen and malpositioned facial motor nuclei. In contrast to cortical interneurons, however, which rely on class 3 semaphorins for their guidance, soma migration of fbm neurons relies on the presence of a structurally unrelated Nrp1 ligand, an isoform of vascular endothelial growth factor (VEGF) termed VEGF164 (Schwarz *et al.*, 2004).

1.11.6 Migration in the Postnatal Brain

Neuronal precursor cells persist in the adult vertebrate forebrain and thus new neurons are continuously added to restricted regions, such as the olfactory bulb and hippocampus in mammals. Consequently, neuronal migration is not restricted to the embryonic milieu but also exists in an adult brain environment. Because the latter is thought to be largely a nonpermissive territory for cell movement – with the obvious exception of cancer cells – migration is restricted to very specific permissive pathways within the brain.

Perhaps the best-known example of adult neurogenesis and neuronal migration is among the first discovered, that of the adult songbird forebrain (Goldman and Nottebohm, 1983). Songbirds display widespread neurogenesis and migration during adulthood, most remarkably in an area of the telencephalon involved in song learning, the higher vocal center (HVC). Studies carried out by Alvarez-Buylla and colleagues (Alvarez-Buylla and Nottebohm, 1988) showed that neurons originating in the SVZ migrate to the cortex when new neurons are added to the songbird hippocampus and HVC, in a process involving the guidance of radial fibers. Moreover, both diffusible and substrate-bound molecules control this migration through a set of hormonally regulated short-distance cell–cell interactions.

In contrast to the relatively widespread neurogenesis found in songbirds, the adult mammalian forebrain utilizes progenitors to generate new neurons destined for very few regions. Specifically, the SVZ of the lateral ventricle and the dentate gyrus subgranular zone (SGZ) of the hippocampus are the regions where adult neurogenesis has been demonstrated (reviewed in Gage, 2000; Alvarez-Buylla and Lim, 2004). The adult SVZ produces new GABAergic interneurons for the olfactory bulb, whereas the SGZ gives rise to granule cells of the hippocampus. Despite the hostile territory that the adult brain represents for migration, new neurons in the hippocampus have a relatively easy path to their final destination, because they are very close to their final location. A different case is the migration of olfactory interneurons, which need to navigate through an extremely long distance from the SVZ to the olfactory bulb.

In contrast to the findings regarding neurogenesis and neuronal migration in songbirds, radial glia cells do not guide the postnatal migration of newly born cells. Instead, olfactory interneurons migrate using a cellular process called chain migration, which involves homotypic interactions between the migrating cells and tubular structures formed by specialized astrocytes (Lois *et al.*, 1996). This migration occurs through a highly restricted route termed the rostral migratory stream (RMS). Like other cell populations in the embryonic brain, tangentially migrating olfactory interneuron precursors change the direction of movement on arriving in the olfactory bulb, migrating radially into specific layers.

Defining the diffusible or membrane-bound factors that guide the tangential migration of new interneurons from the adult SVZ to the olfactory bulb is a very active field in developmental neurobiology. A polysialated glycoprotein neuronal cell

adhesion molecule (PSA–N-CAM) is highly expressed on the surface of olfactory migrating neurons and it has been shown that deletion of the gene for N-CAM or enzymatic removal of PSA results in deficits in the migration of olfactory interneurons and a reduction in the size of the olfactory bulb (Cremer *et al.*, 1994; Ono *et al.*, 1994). Evidence suggests that PSA and/or N-CAM may not be essential for chain formation but, without them, there are several alterations in the nature of the chains that may inhibit the migration of neuronal precursors (Hu *et al.*, 1996). Several additional adhesion molecules have been identified in the migratory route of olfactory interneuron precursors. For example, Tenascin-C, a ligand for αvβ3 and αvβ6 integrins, is strongly expressed in the astrocytes that form the tubes through which olfactory precursors migrate in the RMS (Jankovski and Sotelo, 1996), and αv-, β3-, and β6-integrin subunits are also present in the postnatal RMS. Nevertheless, the lack of abnormalities in the olfactory bulb of mice with individual mutations for some of these molecules prevents a more definitive evaluation of the function of these proteins *in vivo*.

The molecular mechanisms guiding the highly directed migration of olfactory interneurons in the RMS are still unclear, although both attractive and repulsive guidance cues have been proposed to mediate this process. Among the repellents, Slit proteins have been shown to repel SVZ-derived cells *in vitro* (Hu, 1999; Wu *et al.*, 1999). In addition, evidence demonstrates that the activation of the receptor tyrosine kinase ErbB4 is essential for regulating the organization of neural chains in the RMS and therefore their migration (Anton *et al.*, 2004). It seems evident that other molecules are likely to be involved in this process – future experiments will determine their molecular nature.

1.11.7 Conclusions

Studies on the cell biology of neuronal migration suggest that migrating neurons share many common mechanisms with other migrating cell types in the vertebrate body, although additional experiments are required to comprehensively decipher the cellular and molecular components of the migratory machinery in neurons. Regardless of the cell biological mechanisms, distinct modes of migration exist in the embryonic and adult brains, which seem to be adapted to fulfill different functions during evolution. Thus, whereas radial migration may have evolved as a mechanism to preserve the identity of different regions in the developing brain, tangential migration may have provided a means to increase the complexity of neural circuits during evolution.

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1.12 Axon Pathfinding

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Glossary

BMP	Bone morphogenetic protein.
CAM	Cell adhesion molecule.
C. elegans	*Caenorhabditis elegans*.
CNS	Central nervous system.
Comm	Commissureless.
CSPG	Chondroitin sulfate proteoglycan.
DCC	Deleted in colorectal cancer.
DRG	Dorsal root ganglion.
FGF	Fibroblast growth factor.
frz	Frizzled.
GDNF	Glial cell line-derived neurotrophic factor.
GFP	Green fluorescent protein.
GPI	Glycosyl-phosphatidyl.
Hh	Hedgehog.
HSPG	Heparan sulfate proteoglycan.
LPA	Lysophosphatidic acid.
MAP	Microtubule-associated protein.
NGF	Nerve growth factor.
ONH	Optic nerve head.
RGC	Retinal ganglion cell.
Robo	Roundabout.
SFRP	Secreted Frizzled-related protein.
Ti	Tibial.
Wnt	Wingless.

1.12.1 Introduction

The formidable task of accurately establishing the connections of the central nervous system (CNS) is shared by both vertebrates and invertebrates and appears to be conserved throughout phylogeny. For example, in adult humans, the nervous system consists of more than a billion neurons, each connecting to over 1000 target cells in intricate circuits (see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates, Primate Brain Evolution in Phylogenetic Context, The Evolution of Parallel Visual Pathways in the Brains of Primates, Brain Size in Primates as a Function of Behavioral Innovation, Constraints on Brain Size: The Radiator Hypothesis). The very simple nervous system of the nematode *Caenorhabditis elegans* contains only 302 neurons, yet faces similar challenges with respect to forming appropriate neuronal connections. Indeed, despite millions of years of evolutionary separation, most of the key molecules that mediate the formation of the nervous system in organisms as different as humans and *C. elegans* are highly conserved. These neuronal connections are

generated during embryogenesis in a highly specific and precise manner, which is fundamental for the proper functioning of the adult nervous system.

As development progresses, neurons connect to each other in their local environment with dendrites and to distant targets through the extension of an axon. The process of dendritogenesis is a very complex field (reviewed in Sanes *et al.*, 2000) and, in this article, we will focus solely on axon development. Observations of developing axonal projections *in vivo* have revealed that axons extend toward their targets in a highly stereotyped and direct manner (reviewed in Sanes *et al.*, 2000). Axon pathfinding is controlled by proteins present in the environment explored by each growing axon, which is tipped at its leading edge by a specialized structure called the growth cone (Ramon y Cajal, 1890). The growth cone receives and integrates local attractive and repulsive signals presented by cells in the environment, resulting in directed guidance toward its appropriate target.

How does the growth cone accomplish this? Understanding the molecular interactions between a growth cone and the environment presents a great challenge for neurobiologists. Our current knowledge comes from studies of both vertebrates and invertebrates, with each group of organisms offering specific advantages toward the elucidation of this process. For example, invertebrates are commonly used for genetic mutation screens to identify genes of interest in axon pathfinding, whereas vertebrate models have provided useful systems in unraveling the functional mechanisms of these genes.

In this article we will describe the general aspects of axon pathfinding. We will first discuss the molecular characteristics of the growth cone and some general concepts of axon guidance; second, we will describe the main families of guidance cues; third, we will focus on two model systems commonly used to study axon pathfinding, the vertebrate visual system and the CNS midline choice point; and finally, we will discuss some mechanisms known to modulate axon pathfinding.

1.12.2 The Growth Cone: A Central Player in Axon Pathfinding

The growth cone structure was first characterized more than a century ago by the Spanish neuroanatomist, Ramon y Cajal. He imagined the growth cone as a "soft battering ram" that extending axons used to force their way through the embryonic brain (Ramon y Cajal, 1890). Following this discovery, it was shown that axons of embryonic neural tube tissue are tipped with growth cones and are able to grow along a glass coverslip (Harrison, 1910). These very active structures have now been characterized using imaging techniques in both invertebrates and vertebrates and their structure shows a remarkable degree of conservation among species (reviewed in Sanes *et al.*, 2000).

1.12.2.1 The Growth Cone is a Highly Dynamic Structure and Changes Its Shape in Response to Its Environment

1.12.2.1.1 Structure and organization of the growth cone The growth cone is a highly dynamic, actin-rich structure capable of recognizing and responding to a variety of guidance cues (Figure 1). It consists of two major domains, the peripheral and central domains, which are characterized by specific cytoskeletal components. The peripheral domain is rich in actin filaments, while the central domain mainly contains microtubules, mitochondria, and various other organelles. In the peripheral domain, the actin filaments are organized into different types of membrane structure: they can extend into finger-like protrusions (filopodia), remain closely associated with the substratum (lamellipodia), or appear at the apical surface of the growth cone (membrane ruffles).

Microtubules present in the central domain are oriented with their plus ends toward or within the peripheral domain (Gordon-Weeks, 1993) and serve as the major cytoskeletal structure on which rapid vesicular transport occurs (Lockerbie *et al.*, 1991). Such vesicles deliver the molecules needed for neurite elongation, including structural proteins and lipids.

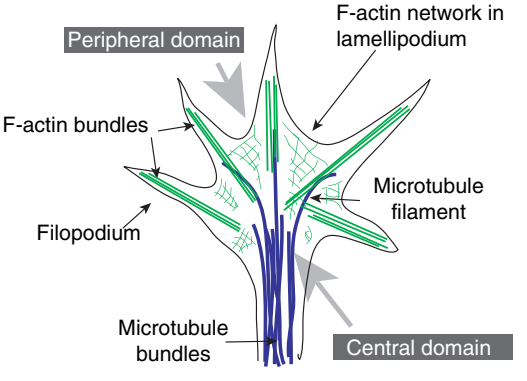


Figure 1 Structure of the growth cone. The growth cone is the highly dynamic tip of the axon that is involved in pathfinding. The growth cone consists of a central and a peripheral domain. The central domain contains stable microtubule bundles as well as membranous organelles. The peripheral domain is rich in actin filaments, which comprise either the F-actin network found in lamellipodia, or the F-actin bundles of filopodia. The growth cone cytoskeleton can be rapidly remodeled in response to environmental cues. Adapted from Dickson, B. J. 2002. Molecular mechanisms of axon guidance. *Science* 298, 1959–1964.

1.12.2.1.2 The dynamic cytoskeleton The growth cone cytoskeleton plays an important role in the process of cell movement (Reinsch *et al.*, 1991; Bentley and O'Connor, 1994; Heidemann *et al.*, 1995; Heidemann, 1996; Letourneau, 1996). The most important cytoskeleton components for axon pathfinding are actin, tubulin, and several actin- and microtubule-associated proteins (MAPs). Tubulin and actin polymerize at the distal tip to generate microtubules and microfilaments respectively. Associated proteins are involved in the assembly, disassembly, and stabilization of actin and tubulin as well as in the anchoring of actin and microtubules to the cell membrane or to other cytoskeletal components. For example, myosin, an actin-associated protein, is able to generate a vectorial force in the growth cone by pulling on the actin filaments, a mechanism similar to muscle contraction.

The filopodia dynamics largely account for the sensory capacity of the growth cone. The length of the filopodia (tens of microns, in some cases) allows them to search within the environment and to navigate across cells and obstacles. Movement at the tips of growing filopodia is generated by the rapid assembly of actin filaments, whereas microtubule assembly is involved in the advance of the body of the growth cone. Importantly, experiments performed *in vitro* have demonstrated that a single filopodium making strong contact with an adhesive substrate is able to steer the growth cone by pulling it toward the adhesive substrate (Letourneau, 1996). Furthermore, when one filopodium is detached, the growth cone changes direction due to the release of tension from that side (Wessells, 1978).

Many of our insights into the role of cytoskeleton in axon pathfinding come from experiments using depolymerizing drugs that interfere with the normal function of microtubules and filopodia. For example, cytochalasin B, an actin-depolymerizing drug, prevents filopodia formation, resulting in axons either stopping growing or slowing dramatically (Bentley and Toroian-Raymond, 1986). Moreover, retinal axons of the amphibian brain treated with cytochalasin grow past a critical turning point and fail to find their targets (Chien *et al.*, 1993). Indeed, experiments using the tibial (Ti1) pioneer neuron in the grasshopper limb have shown that cytochalasin treatment of growth cones induces a loss of filopodia, but the lamellipodia are still functional, allowing the growth cone to advance slowly (Bentley and Toroian-Raymond, 1986). The role of filopodia in directing growth cones has been demonstrated in the pioneer axons of the grasshopper limb. When a single filopodium of a Ti1 neuron makes contact with a guidepost cell indicating the direction to choose, then this filopodium stays in contact while other filopodia retract. The filopodium in contact with the guidepost cell is stabilized and eventually becomes the shaft of the growing axon (Sabry *et al.*, 1991). These results indicate that filopodia play a critical role in axon growth and navigation during pathfinding.

Interfering with microtubule dynamics with pharmacological inhibitors has also provided evidence highlighting their role in axon growth. It has been shown that axons lacking filopodia by treatment with cytochalasin are still able to grow due to the addition of tubulin at the plus end of the microtubule at the distal process. However, axon elongation can be completely inhibited by the depolymerization of microtubules at the distal tip, the most sensitive region of the growing axon to drugs (Marsh and Letourneau, 1984; Bentley and Toroian-Raymond, 1986).

In addition to microtubules, axonal growth also involves several MAPs. For example, treatment of cultured neurons with the nerve growth factor (NGF), a growth factor that promotes neurite outgrowth, results in the upregulation of the MAPs Tau and MAP1B, and inhibition of Tau function can block neurite outgrowth, indicating the critical role of this protein (Letourneau, 1996). As the coupling between the sensory and motor capabilities of the growth cone is critical for axon pathfinding, it has been hypothesized that actin and microtubules may be associated to generate cell movement (Letourneau, 1996; Williamson *et al.*, 1996). Indeed, some MAPs may also bind actin, providing a mechanical link between these two structural components. However, the mechanism of growth cone steering and how these cytoskeletal components are regulated by the environmental milieu are still incompletely understood.

1.12.2.1.3 Growth cones change their morphologies in response to the environment En route to their target, growth cones advance within their environment and display a range of morphologies, from simple cigar shapes to highly complex structures, depending on their position (Tosney and Landmesser, 1985; Bovolenta and Mason, 1987). When axons are growing in fasciculated nerve bundles along straight tracts, they often display a streamlined form with one or two filopodia pointing in the direction of growth. Studies in mice and in *Xenopus* have shown that growth speed is about $55\,\mu m\,h^{-1}$ (Harris *et al.*, 1987; reviewed in Mason and Erskine, 2000). Growth is, however, often saltatory, meaning that rapid advance is frequently interrupted by pauses. These breaks are mainly observed at critical choice points, where decisions regarding subsequent directional growth are made.

At choice points, where growth cones display highly complex morphologies, they bear a widespread lamellipodium and multiple filopodia. Indeed, it has been shown that at key choice points in the retinal pathway (see Section 1.12.3), growth cones pause for periods of approximately 30 min to determine which direction to follow (Llirbat and Godement, 1999). These morphological changes indicate that the growth cone needs to integrate signals from the environment to advance along its pathway.

1.12.2.2 Axon-Pathfinding Concepts

We have seen that the growth cone serves as both an antenna that receives directional cues from the environment and as a motor structure that drives axon growth. How do axons find their target in the highly complex environment of the developing CNS? To visualize this complex process, axon pathfinding can be compared to a maze (Figure 2). During its long journey, the axon will have to choose which direction to take by interpreting attractive or repulsive signals from the environment at each decision point. To navigate through the maze of the developing embryo to reach their final destination, growing axons are guided by various diffusible or substrate-bound molecules, which they use as navigational cues. Interestingly, the decisions at choice points are not made by trial and error; instead, the growth cone pauses, samples the environment with filopodia, and adapts its direction accordingly, resulting in an almost error-free navigation. Importantly, when growth cones are cut from their cell bodies, they still continue to navigate appropriately in the brain and detect cues, demonstrating that growth cones can function independently of their cell bodies (Harris *et al.*, 1987).

Our understanding of axon pathfinding comes from a variety of experimental data generated in both invertebrate and vertebrate model systems. The nervous system architecture of invertebrates is relatively simple, which allows researchers to trace individual neurons, so providing a useful tool to perform *in vivo* experiments. Remarkably, axon-pathfinding concepts are very well conserved and have also been found in higher organisms.

An illustrative example of the concept of axon pathfinding is the experiment performed by Sperry in 1943. In this experiment, he severed the optic nerve in a frog and rotated the eye by 180° (Sperry, 1944). After regeneration and restoration of the retinotectal projection, the animal behaved as if it saw the world upside down and back to front. Therefore, Sperry proposed his chemospecificity hypothesis, which stated that each individual neuron had its specific chemical tag to find its target. Twenty years later he refined his theory and proposed that guidance cues are expressed in gradients, which would be more economical, requiring fewer molecules than individual labels would. Gradients would also have the advantage of being able to tell the axon the direction to go, removing the necessity of searching for the target randomly. Supporting this theory, rotation experiments performed in salamander hindbrains revealed that axons still find their way to their targets, suggesting that external cues are involved in this pathfinding rather than molecules secreted by the cell bodies (Hibbard, 1965).

Complementary evidence that growth cones are guided by external cues comes from experiments performed *in vivo* using the grasshopper leg (Keshishian and Bentley, 1983; Raper *et al.*, 1983). During development of the grasshopper, a pair of afferent neurons arises in the distal tip of each limb bud. These Ti1 pioneer neurons navigate to their

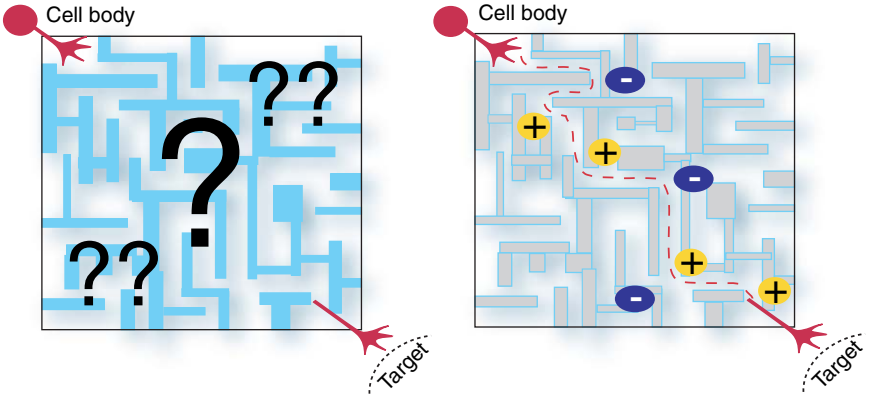


Figure 2 Axon pathfinding. The environment through which a growing axon must traverse to reach its target is analogous to a maze. The axon negotiates the maze of the developing nervous system by responding to specific positive or negative directional cues at intermediate choice points along its pathway.

final destination following a stereotypical pathway. Experiments using two sibling neurons have shown that growth cones of later-differentiating neurons elongate upon the axons of specifically labeled earlier-differentiating neurons (Raper *et al.*, 1983). When pioneer axons were ablated with a laser, the later axons could not find their way into the CNS (Keshishian and Bentley, 1983). To find their correct target, the pioneer Ti1 axons are guided by local cues or guidepost cells that are spaced at short distances (Bentley and Caudy, 1983). When these cells were obliterated using a laser, the Ti1 axons were unable to travel from one segment to another (Bentley and Caudy, 1983). In addition to these critical guidepost cells, adhesive molecules on the epithelium are also involved in directing the growth cone from one guidepost cell to the next and growth-inhibitory molecules prevent the growing axons from taking the wrong way (Singer *et al.*, 1995).

1.12.3 Growth Cone Guidance

As we previously mentioned, the process of axon guidance in both vertebrates and invertebrates has been conserved and occurs in a highly ordered and stereotyped manner (reviewed in Araujo and Tear, 2003). Studies in the past two decades have provided compelling evidence for cellular interactions between growth cones and their environment in directing axon pathfinding and have led to the identification of different families of guidance cues. Four types of basic mechanisms are thought to steer axon growth: contact attraction, chemoattraction, contact repulsion, and chemorepulsion (Figure 3; reviewed in Tessier-Lavigne and Goodman, 1996). However, these designations are dependent on the environmental context in which axon pathfinding is taking place, as some families of guidance cues have both diffusible and nondiffusible members and some individual cues themselves can act as attractants for some axons and repellents for others. Thus, axon pathfinding is directed by the coordinate action of multiple attractant and repellent cues integrated by the growth cone along the pathway. In this section, we will describe the characteristics of a guidance cue and the techniques widely used to test for guidance activity. We will then describe the main families of guidance cues identified to date.

1.12.3.1 Identification of Guidance Molecules

How are we able to identify molecules that regulate the process of axon pathfinding? A candidate protein has to fulfill several criteria to be classed as a guidance cue: (1) it has to be expressed at the right

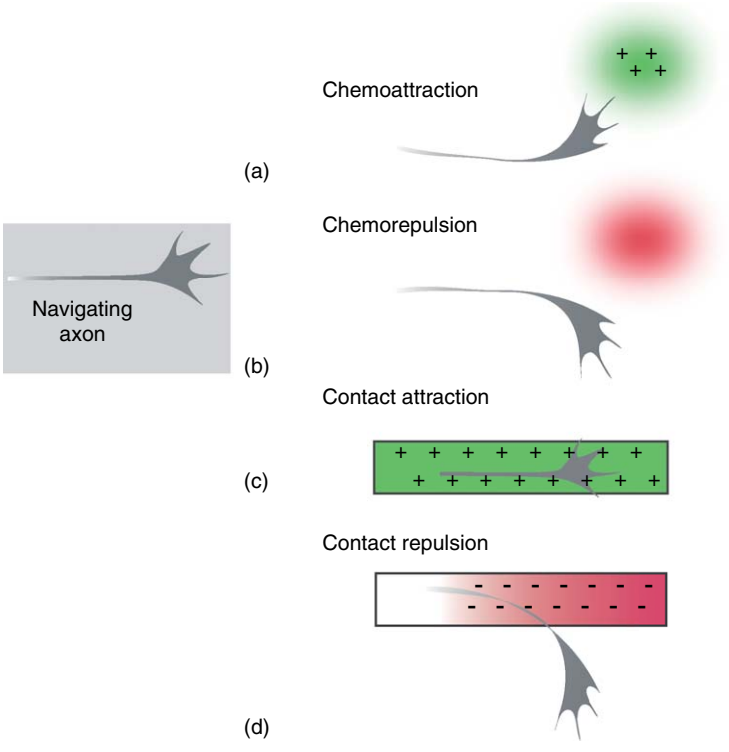


Figure 3 Mechanisms of growth cone guidance. Growth cone guidance is modulated by four main environmental stimuli: a, chemoattraction; b, chemorepulsion, mediated by soluble molecules; c, contact attraction; and d, contact repulsion, mediated by substrate-bound molecules.

time when axons meet with its region of expression; (2) it must be able to steer axons *in vitro* and *in vivo*; and (3) interfering with its function should result in axon guidance and targeting errors. The recent advent of powerful molecular and genetic techniques enabled these criteria to be met:

1. Analysis of the expression patterns of the protein of interest can be performed using antibody staining, application of tagged protein probes, or of RNA by *in situ* hybridization, screening through different developmental stages and target tissues.
2. To test for guidance activity *in vitro*, several experimental paradigms have been developed. One of them is called the stripe assay (Figure 4a). In this assay, axons are given a choice of growing on alternating lanes of guidance molecule versus control protein. If the guidance molecule tested is an attractive one, the axons will grow on the lanes containing the guidance molecule. If the guidance molecule tested acts as a repellent, then axons will avoid the lanes containing the guidance molecule and will grow in the control protein lanes.

Other *in vitro* guidance tests are also common. The growth cone collapse assay is a chemotropic assay for repulsive guidance cues, in which the protein of interest is applied globally to the growing axons for a certain amount of time (Raper and Kapfhammer, 1990). After application of the protein of interest, the percentage of collapsed growth cones is counted. Collapse is defined as a loss of the motile structures of the growth cone (the lamellipodia and the filopodia) and is often accompanied by cessation of growth and withdrawal of the growth cone (Figure 4b). Collapse *in vitro* is thought to be correlated with repulsion *in vivo*. An assay to test the guidance activity of substrate-adhered proteins, the bead assay, is also widely used. In this assay, a protein-coated latex bead is manipulated with the help of a laser tweezer (Kuhn *et al.*, 1995; Gallo *et al.*, 1997). The advantages of this system are that the concentration of the protein on the bead surface can be regulated, the beads can be immobilized accurately at the desired position, and the presentation on the bead surface may mimic the

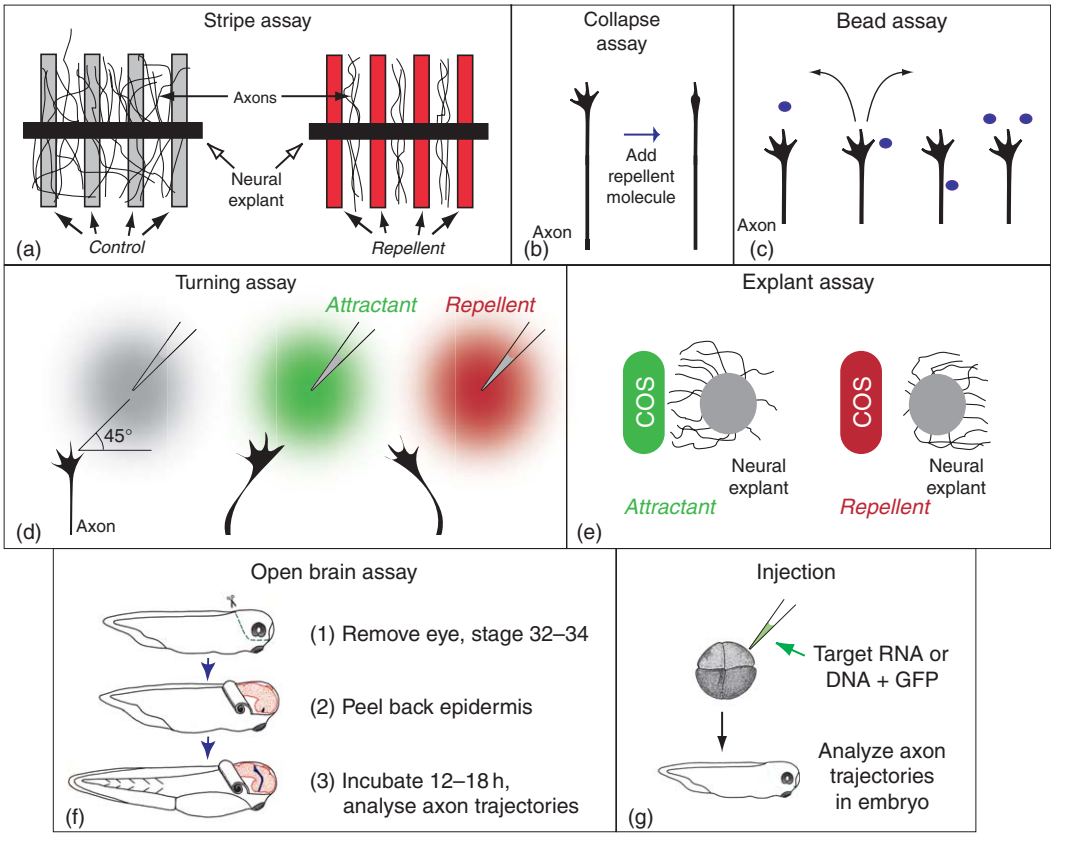


Figure 4 Common assays for axon guidance activity. A variety of *in vitro* and *in vivo* assays have been developed to assess the capacity of a molecule to influence axon guidance. Common *in vitro* assays include: a, the stripe assay; b, the collapse assay; c, the bead assay; d, the turning assay; and e, the explant assay. Two *in vivo* assays are: f, the open-brain assay; and g, the injection of RNA or DNA into blastomere-stage embryos (see text for details).

in vivo anchorage of certain proteins like the ephrins (Figure 4c).

Two techniques are also commonly used to analyze the activity of soluble guidance cues. In the turning assay soluble proteins are ejected out of a micropipette, resulting in stable diffusion gradient (Lohof *et al.*, 1992). An attractive cue will guide the growth cones toward the pipette, whereas a repellent molecule will result in turning away from the pipette (Figure 4d). A second test to the guidance activity of soluble proteins is the co-culture explant assay (Tessier-Lavigne *et al.*, 1988). Cultured cells expressing and secreting the protein of interest (for example, COS (cell line from African green monkey kidney) cells or the floorplate of the developing spinal cord) are grown near neural explants. Axons extending from the neural explant will grow toward the diffusible source if the protein secreted is an attractive one, and will grow away if the protein is exhibiting a repellent activity. Thus, there will be more axons on the side facing the cell explant in the case of an attractant and fewer axons in the case of a repellent molecule (Figure 4e).

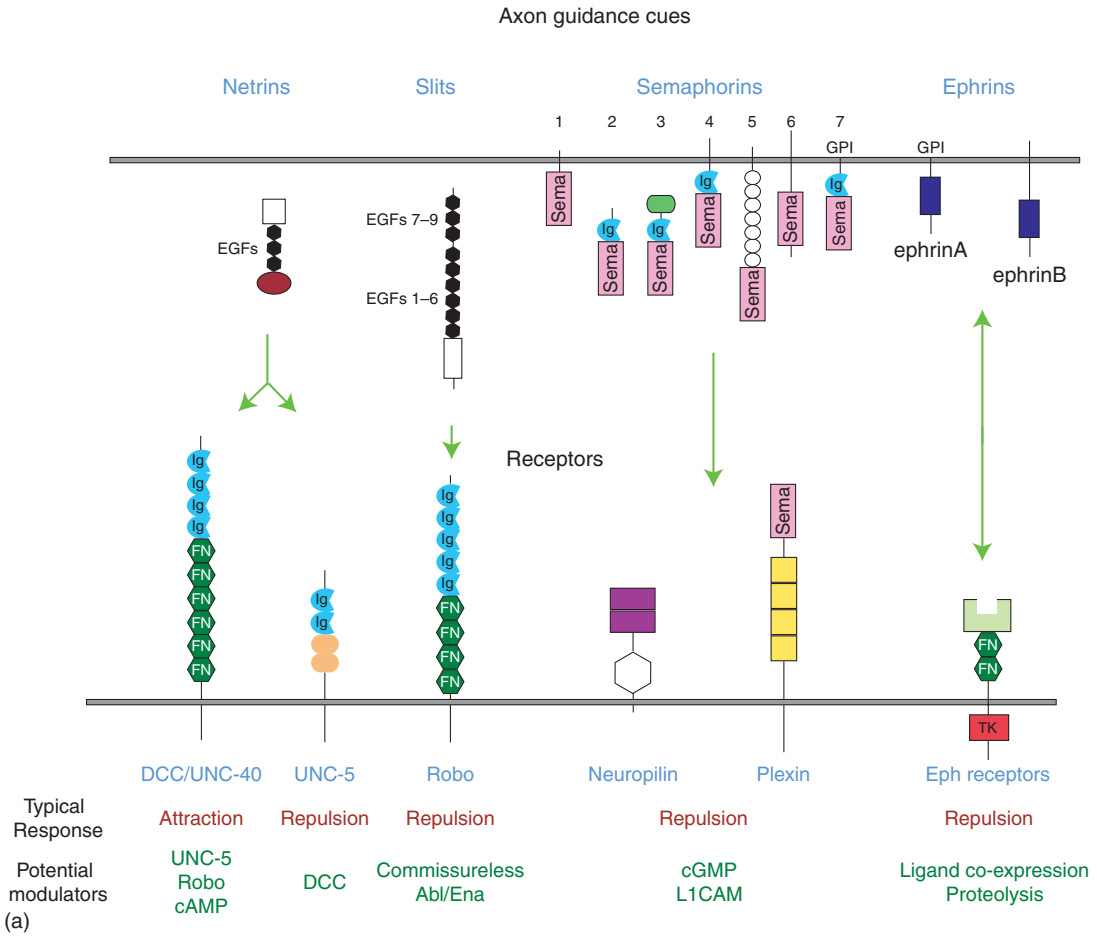
3. If the protein of interest is essential for axon pathfinding, then interfering with its function *in vivo* should result in pathfinding errors. Techniques to accomplish this include the overexpression or downregulation of proteins. For example, the recent development of knockin or knockout mice provides an elegant *in vivo* model for studying axon pathfinding and target-mapping defects. Other methods for studying axon pathfinding and target recognition in *Xenopus* are the open retina and open brain preparations (Figure 4f; McFarlane *et al.*, 1996). Either the retina or the tectum of living animals is exposed to the guidance cue of interest and the subsequent pathfinding of the axons is observed with *in vivo* time-lapse microscopy. Other methods for interfering with protein function are the use of siRNA or morpholino antisense oligonucleotides. These techniques are well established in *C. elegans*, *Xenopus,* and zebra fish model systems. RNA or DNA constructs interfering with the protein of interest are introduced by electroporation, injection, or lipofection into early blastomere or later-stage embryos along with the green fluorescent protein (GFP) to visualize the injected axons and to look for axon-pathfinding errors (Figure 4g).

1.12.3.2 Guidance Cue Families

Extracellular cues have long been postulated to provide positional information to axonal growth cones. Until recently the identity of the molecules responsible for this remained a mystery. However, in the last 10–15 years, the application of a variety of powerful genetic techniques in *Drosophila melanogaster* and *C. elegans* and the development of molecular and biochemical tools and assays to study axon growth in vertebrates have led to the identification of several families of axon guidance cues; the most prominent of them are the netrin-1s, semaphorins, Slits, and ephrins, which are discussed below (Figure 5). However, it should be noted that many other molecules have been implicated in axon guidance, such as the morphogens Wnt4 and Sonic Hedgehog, and growth factors like the fibroblast growth factor (FGF) and the glial cell line-derived neurotrophic factor (GDNF).

Perhaps the most remarkable aspect of the identification of these four families of molecules has been the extent to which their role in axon guidance has been conserved during evolution. For example, despite an evolutionary separation exceeding 600 million years, the netrin-1s have retained their role for attracting axons ventrally toward the midline in organisms as diverse as *C. elegans, Xenopus,* and human. A second interesting feature is the relatively small number of molecules that are used to generate the startling array of complexity found within the CNS. Here, we will describe the four major families of axon guidance molecules before discussing their multiple roles in axon pathfinding in the next section.

1.12.3.2.1 Netrin-1s and their receptors Chemoattractants are specifically expressed in the developing embryo to guide axons toward intermediate targets and to facilitate correct pathfinding to their ultimate destination. The netrin-1s ("one who guides," a derivation from Sanskrit) are the best-known chemoattractants, and have been implicated in the guidance of many developing axonal populations across a variety of species. Two studies, the first analyzing genes that regulate circumferential axon guidance in *C. elegans* and the second searching for secreted midline attractants involved in guiding spinal commissural axons in rodents, concurrently identified the first members of this family. Gene knockout studies in both these species have since verified the importance of this family of attractants in axon guidance. For example, one of the prominent phenotypes in mice lacking netrin-1 is a failure of the spinal commissural axons to reach the normally attractive floorplate (Serafini *et al.*, 1996). *C. elegans* and *Drosophila* contain one or two known members respectively (uncoordinated (UNC)-6 and netrin-1-A and netrin-1-B, reviewed in Tessier-Lavigne and



(a)

Nomenclature of guidance cues

	Netrins	Receptors	Slits	Receptors	Semaphorins	Receptors	Ephrins	Receptors
C.elegans	UNC-6	UNC-40	SLT-1	SAX-3	SMP-1, MAB-20	PLX-1,2	EFN1–4	VAB-1
Drosophila	Netrin-A,B	Frazzled	Slit	Robo, Robo2,3	Sema1,2	Plexin-A,B	2 ephrins	Dek
Vertebrates	Netrin1–3	DCC, neogenin	Slit1–3	Robo1,2, Rig-1	Sema3–7	Plexin-A1-4, B1-3, C1, D1, neuropilin 1,2	ephrinA 1–5, ephrinB 1–3	EphA 1–8, EphB 1–6

(b)

Figure 5 a, Axon guidance cues. The four major families of axon guidance cues identified to date are: the netrins, Slits, semaphorins, and ephrins. These molecules interact with specific receptors on the surface of the growth cone to control axon pathfinding. Guidance responses can be modulated by other effectors within the growth cone. b, These guidance cues and their receptors have been highly conserved through evolution, and homologues of each are found in organisms as diverse as *C. elegans, Drosophila,* and vertebrates. EGF, epidermal growth factor repeat; Ig, immunoglobulin domain; FN, fibronectin type III domain; TK, tyrosine kinase domain; GPI, glycophosphatidylinositol linkage; Sema, Semaphorin domain.

Goodman, 1996). In vertebrates, two members were initially identified in chick (netrin-1 and netrin-2) and orthologues of netrin-1 have been reported in the mouse, human, frog, and zebra fish (reviewed in Meyerhardt *et al.*, 1999).

The netrin-1 receptors were also initially characterized in *C. elegans* by studying worm mutants with axon guidance defects. These proteins, UNC-40 and UNC-5, are transmembrane proteins that mediate different responses to netrin-1 *in vivo*. UNC-40, whose vertebrate homologue is DCC (deleted in colorectal cancer), mediates attraction. In some populations of axons, netrin-1s can also act as a repellent. The UNC-5 receptor, either on its own or in conjunction with UNC-40, mediates these effects (Chan *et al.*, 1996). This is important, as it shows that netrin-1s, though primarily attractants, can also be repulsive. Such bifunctionality is one

way in which neural complexity is created during nervous system development.

1.12.3.2.2 Semaphorins The first axonal chemorepellents to be thoroughly characterized were members of the Semaphorin (Sema) family. The identification of these proteins was the culmination of a study investigating a potent growth cone collapse-inducing molecule from the adult chick brain and another study in which the fascicle-specific expression of proteins was examined in the adult grasshopper CNS (Kolodkin *et al.*, 1992; Luo *et al.*, 1993). Many more Sema proteins have since been isolated, and members of this family are present in vertebrates, invertebrates, and even viruses. Although the Sema proteins form a large and heterogeneous group, they all share a common ~420 amino acid region at their NH_2-termini, called the Sema domain. Insects and nematodes share a small number of semaphorins that are divided in two subfamilies, transmembrane Semas (class 1) and secreted Semas (class 2). In contrast, vertebrates contain a large number of semaphorins that are divided into five subfamilies based on their common structure. An interesting point is that vertebrate semaphorins are not strict orthologues of invertebrate semaphorins, probably due to the existence of a duplication and a divergence of the semaphorin genes across evolution (reviewed in Chisholm and Tessier-Lavigne, 1999).

How does the growth cone respond to Sema proteins in the extracellular environment? Sema proteins bind to multimeric receptor complexes on the surface of the axonal growth cone. Although the exact makeup of these receptor complexes is in many cases unclear, they will often contain a transmembrane plexin protein. Another well-characterized component of the Sema receptor complex are the neuropilins, which act as receptors for class 3 Semas. Thus, Sema proteins in *Drosophila* and vertebrates generally act as repellents, with their spatiotemporal expression being used to channel axons through repulsive corridors or to prevent them from entering inappropriate areas, so enabling axons to navigate correctly within the developing CNS (see Section 1.12.4).

Although studies of netrin-1s have shown the remarkable molecular and functional conservation of guidance molecules in axon pathfinding, studies on the semaphorin receptors provide an example of a strong evolutionary divergence. Indeed, the neuropilin receptors are not conserved across species as they have not been found in *C. elegans* and *Drosophila*. It is thought that in invertebrates, instead of the neuropilin receptor, the plexin protein mediates the repulsive action of semaphorins. However, the reason why, during evolution, the plexin proteins present in invertebrates have been replaced by the neuropilin receptors functioning in vertebrates remains a mystery (reviewed in Chisholm and Tessier-Lavigne, 1999).

1.12.3.2.3 Slits and their receptors Another highly conserved family of guidance molecules are the Slits. In a screen for cuticular defects in *Drosophila* conducted over 20 years ago, one of the many mutations identified was dubbed *Slit*. In this mutation the longitudinal axon scaffold of the ventral midline collapsed into a single, fused tract, suggesting the absence of an unknown midline repellent in the mutant flies. This repellent was identified as the product of the *Slit* gene, a large, secreted protein with multiple protein–protein-binding motifs (Kidd *et al.*, 1999). Slit has three vertebrate homologues, which also primarily act as repellents during nervous system development. The Slits signal via interactions with the Roundabout (Robo) family of transmembrane receptors (in *Drosophila* Robo mutants, axons recross the midline multiple times, forming loops, hence Roundabout), which are expressed on the surface of the axon and growth cone. Like netrin-1s, Slits have been implicated in guiding axons from many developing neuronal populations. However, two developmental systems in particular have provided insights into the mechanisms underlying Slit-induced repulsion: the *Drosophila* ventral midline and the vertebrate optic chiasm (see Section 1.12.4).

1.12.3.2.4 Ephrins and Eph receptors Eph receptors were first found in a screen for tyrosine kinases in a cultured cell line, as their name reflects (erythropoietin-producing hematoma cell line). The ligands for these receptors, named ephrins, were identified using the extracellular domain of the Eph receptor for affinity chromatography, screening expression libraries, and in a search for axon guidance cues in the tectum. Based on their amino acid sequence similarities, both Eph receptors and ephrins are grouped into two subclasses: A- and B-type. There are eight EphA receptors (EphA1–EphA8) and six EphB receptors (EphB1–EphB6). The ephrinAs (ephrinA1–ephrinA6) are glycosylphosphatidyl (GPI)-anchored and ephrinBs are transmembrane-anchored proteins. Eph receptors and ephrins have been found in several species. The highest diversity of expression is found in chick, mouse, and human, followed by *Xenopus* and zebra fish, although family members have also

been identified in both flies and worms, indicating that the ephrins and their receptors are evolutionarily ancient. Indeed, the large diversity of vertebrate ephrins and their receptors and the high sequence homology of these proteins within vertebrate phyla suggest a major expansion of the Ephs and ephrins has occurred. This may have accompanied the evolution of vertebrates, which might have been critical in the construction of increasingly complex brains (reviewed in Flanagan and Vanderhaeghen, 1998; Wilkinson, 2000).

High-affinity binding usually occurs between ligands and receptors of the same subtype (ephrinA activating EphA, ephrinB activating EphB), although there is increasing evidence for a cross activation between subclasses (for example, EphA4 activated by ephrinB2, and recently it was found that ephrinA5 can activate EphB2; Himanen *et al.*, 2004). Ligand binding results in a clustering of the receptors, autophosphorylation, and activation of the intracellular pathways, finally leading to re-arrangements of the cytoskeleton. Both ligands and receptors are enriched in lipid rafts providing a platform for clustering and signaling. One interesting aspect of the signaling is the finding that both A- and B-type ephrins and Ephs can act as receptors and ligands, a fact described as bidirectional signaling. This signaling can occur in the forward (ephrin) or reverse (Eph) direction.

The most prominent role of this family is its role in cell contact repulsion. Eph receptors and their ligands act as stop signals at boundaries to prevent overshooting specific targets, or to channel axon growth. They are also involved in the formation of topographic maps in the retinotectal projection and in other pathfinding events, such as in the vomeronasal projection (critical for pheromone detection in rodents), in the hippocamposeptal projection (involved in learning and memory), and in the connection of motor neurons with their muscle targets.

1.12.4 The Retinal Projection and the Midline Choice Point: Model Systems to Study Axon Pathfinding

During the past decade, our understanding of axon-pathfinding mechanisms has been advanced through the use of invertebrate and vertebrate model systems. Two well-known model systems have provided many insights into the processes of axon pathfinding and we will confine our discussion to these examples: the vertebrate retinal projection and the midline of the developing CNS. Importantly, many of the guidance cues described in the previous section play integral roles in mediating axon guidance during development of these systems. Moreover, these two systems provide an introduction to the general processes that underlie axon guidance in other neuronal populations.

1.12.4.1 The Retinal Projection as a Model System

The retinal projection is the axonal pathway linking retinal ganglion cells (RGCs) of the eye to their primary target in the brain, the optic tectum (in lower vertebrates) or superior colliculus (in mammals) (Figures 6a–6d). The eye is a highly complex light-capturing organ which generates neuronal signals corresponding to external visual information and conveys them via the retinal projection to visual centers in the brain. To ensure that visual information is correctly relayed to the CNS, axons of embryonic RGCs must find their way from the eye to their CNS targets. The number of RGC axons that must navigate correctly from the eye is impressive. In mice, for example, more than 50 000 RGC axons exit from each developing eye, while in humans, over a million RGC axons must navigate accurately during embryonic development.

In this section, we will describe the different decisions that RGC axons make at various choice points during pathfinding to their targets in the brain. Although much remains unknown, it is clear that attractive and repulsive cues in the developing brain act in concert to guide RGC axons correctly. An important concept, shared by navigating axonal populations across a wide evolutionary distance, is that the retinal pathway is broken into smaller segments with distinct molecular characteristics. The subdivision into shorter distinct segments is used to simplify navigation, as well as allowing multiple regulatory check points.

1.12.4.1.1 General organization of the retinal pathway The first step in the journey of a RGC axon is to extend across the (vitreal) surface of the retina toward the optic nerve head (ONH), or future optic disk, where they form a bundle of axons that will exit the eye. Axons extend through the ONH toward the back of the eye (pigmented epithelium) where they emerge as a tightly fasciculated bundle to form the optic nerve. The optic nerve enters the brain at the ventral diencephalon and axons grow toward the optic chiasm at the midline. The chiasm is a key choice point as axons may cross to the other

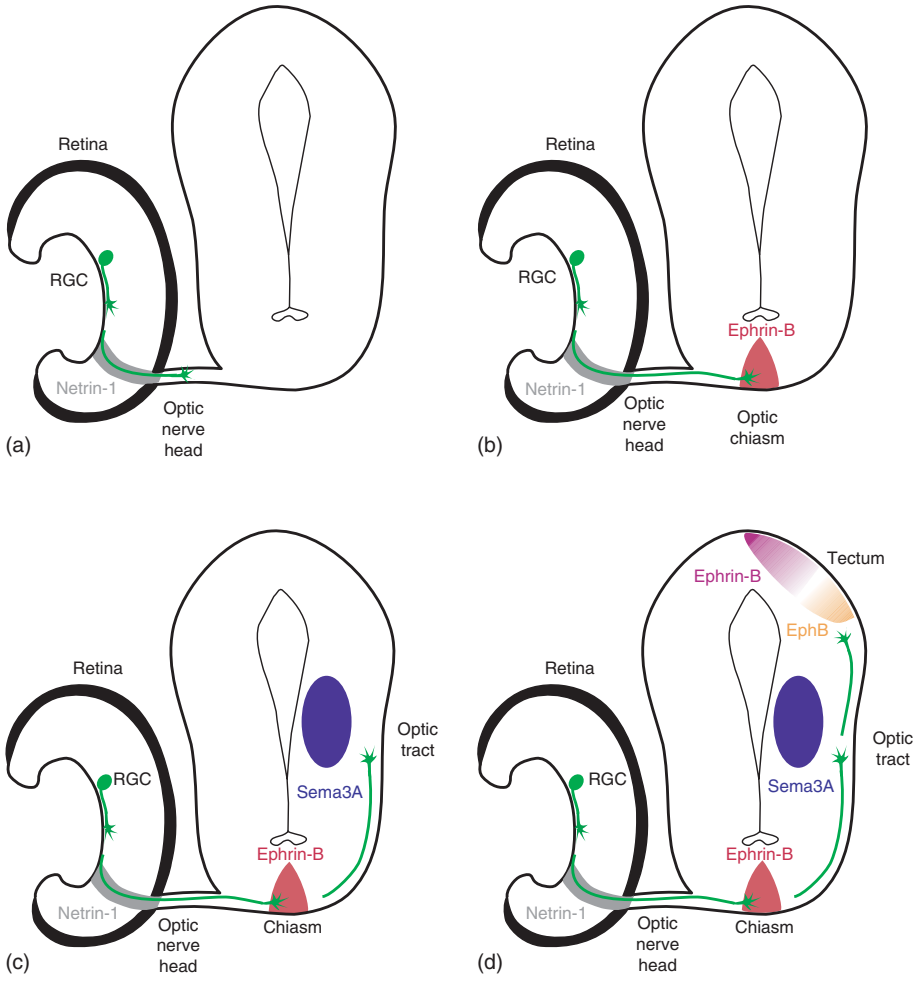


Figure 6 *Xenopus* retinotectal projection. In the *Xenopus* retinotectal projection, RGC axons navigate from the retina to the optic tectum in the brain. Guidance molecules are expressed at key choice points to ensure fidelity of pathfinding. Some of the major pathfinding decisions are made at: a, the optic nerve head; b, the optic chiasm; c, the optic tract; and d, the tectum itself (see text for details).

side of the brain or remain ipsilateral. After passing through the optic chiasm, RGC axons form the optic tract which courses dorsally along the lateral surface of the diencephalon. The final step in the retinal pathway involves the departure of RGC axons from the optic tract and entry into the main synaptic target, the optic tectum in lower vertebrates, the superior colliculus in mammals, in the midbrain. Within the target area, retinal axons terminate next to their retinal neighbors, thus forming a precise topographic map of visual space (Figures 6a–6d).

1.12.4.1.2 Pathfinding across and out of the retina In the first step of the retinal pathway, axons converge on the ONH. Remarkably, axons arising from all points of origin across the retinal surface orient immediately toward the ONH, suggesting that they receive directional information early in axonogenesis (reviewed in Oster *et al.*, 2004). The mechanisms responsible for this directed intraretinal growth, although not well understood, are beginning to be uncovered (reviewed in Mann *et al.*, 2004). For example, experimental studies that perturb the function of cell adhesion molecules (CAMs), such as L1, neural CAM (NCAM), and neurolin, that are expressed on both RGC axons and their substrate neuroepithelial cells, show that these molecules play a role in intraretinal growth (Brittis *et al.*, 1992; Ott *et al.*, 1998). In addition, chondroitin sulfate proteoglycans (CSPGs) have been proposed to play a role in directing axons toward the ONH. These extracellular matrix proteins, known to inhibit axon growth *in vitro* (Snow *et al.*, 1991), are expressed in a receding ring in the developing rat retina just peripheral to differentiating RGCs. Enzymatic removal of CSPGs causes defects in directional guidance with some axons

erroneously heading peripherally (Brittis *et al.*, 1992), suggesting that the ring of CSPG may serve to push growing axons centrally. However, a CSPG ring has only been observed in the rat retina, indicating that it may not represent a common guidance mechanism. Another class of molecule implicated in intraretinal guidance is the B-type family of Eph tyrosine kinases. Some members, such as EphB2, are expressed in a ventral-to-dorsal gradient in the retina and mutant mice lacking EphB2 and EphB3 display pathfinding errors within the retina (Birgbauer *et al.*, 2000).

When axons reach the ONH they make a sharp change in direction (approximately 90°) to enter the ONH, marking this as a critical decision point. This behavior is mediated by the chemoattractant netrin-1, which is localized to the ONH (de la Torre *et al.*, 1997; Deiner *et al.*, 1997). In netrin-1 and DCC-deficient mice, axon growth across the retinal surface to the ONH is unaffected, but many axons fail to enter the ONH, resulting in optic nerve hypoplasia (Deiner *et al.*, 1997). In this context, netrin-1 appears to be acting as a short-range attractant, guiding RGC axons that have arrived at the ONH into the optic nerve itself. This contrasts with its well-characterized long-range role in guiding commissural axons to the floorplate of the ventral spinal cord (Tessier-Lavigne *et al.*, 1988). Within the optic nerve, axons fasciculate into bundles through the action of homophilic binding of axonally expressed adhesion molecules. Sema5A, which is known to repel retinal axons *in vitro*, helps to confine the axons to the nerve bundle, as it is expressed as an inhibitory sheath around the nerve, preventing them from wandering off the defined path (Goldberg *et al.*, 2004).

1.12.4.1.3 Axon divergence at the chiasm From the optic nerve, RGC axons enter the brain and approach the midline, a key intermediate target. At this point, axons have to make the critical decision of whether to cross the CNS midline at the optic chiasm. At a functional level, the decision for RGC axons to cross or not cross at the chiasm correlates with the degree of binocular overlap. In species with eyes placed laterally there is no binocular overlap and the retinal projections are completely crossed (i.e., contralateral). Thus, the visual information from the left and the right eyes is processed independently. In species with forward-facing eyes and, hence, binocular vision, the visual fields of both eyes overlap to some extent. For example, a subset of RGCs in the left eye will receive stimuli that overlap with those in the right eye. To process such shared visual information, some RGC axons from the region of binocular overlap project ipsilaterally while others project contralaterally. This arrangement enables information about the same point in visual space from the two eyes to be brought together in the brain. Thus, in vertebrates with binocular vision, the ability to integrate shared visual information relies on the decision of RGC axons to cross or not to cross at the chiasm.

The first insights into the molecular regulation of crossing came from studies in the amphibian, *Xenopus*. In the filter-feeding tadpoles the eyes are placed laterally, so there is no binocular overlap as all the visual projections are crossed. During metamorphosis, the eyes migrate dorsally to the top of the head, creating binocular vision essential for the frog's new prey-catching lifestyle. Beginning at metamorphosis, axons arising from RGCs in the ventrotemporal binocular part of the retina extend ipsilaterally. What makes these axons alter their behavior? It turns out that ephrin-B expression is switched on during metamorphosis at the midline at the optic chiasm (Nakagawa *et al.*, 2000). Ephrin-B is the ligand for EphB receptors (Figure 5) and the ventrotemporal axons express high levels of EphB receptors. Since ephrin-B acts repulsively, the current working model is that ventrotemporal axons are deflected into the ipsilateral optic tract by the ephrin-B signal encountered at the midline chiasm in metamorphosing and postmetamorphic frogs. The same mechanism seems to play a role in other vertebrate species such as mice (reviewed in Mann *et al.*, 2004). Significantly, animals without binocular vision, such as zebra fish and chick, lack ephrin-B expression at the chiasm, suggesting that this mechanism has arisen during evolution to accommodate the development of binocular vision in vertebrates.

The determinant of regionalized EphB expression in the retina appears to be the transcription factor Zic-2, which is exclusively expressed in the ventrotemporal retina after the onset of metamorphosis in *Xenopus* and in the temporal retina in embryonic mice (Herrera *et al.*, 2003). As with ephrin-B expression at the chiasm, Zic-2 is not expressed in the retina of organisms without visual field overlap, such as the chick (Herrera *et al.*, 2003). In addition, the transcription factor Isl-2 is expressed only by RGCs that cross the chiasm and has been shown to repress the expression of Zic-2, preventing them projecting ipsilaterally (Pak *et al.*, 2004). Therefore, Zic-2 and Isl-2 transcription factors are expressed in mutually exclusive areas of the retina and are key regulators of an RGC's sensitivity to directional signals at the midline.

Finally, gene knockout studies in mice have shown that Slit1 and Slit2 are involved in the correct development of the chiasm. Mice deficient for both genes exhibit multiple retinal axon-pathfinding errors, including the formation of an ectopic chiasm (Plump *et al.*, 2002). Slit1 and Slit2 are expressed in complementary domains surrounding the path of the growing retinal axons, creating a repulsive barrier around the chiasm, which acts to channel the axons into a narrow corridor across the ventral diencephalon. Slits, therefore, unlike ephrin-B, are not involved in the decision to cross the midline but rather in the anterior–posterior positioning of the chiasm. Together these two complementary molecular systems help to determine the exact path of axons across the midline.

1.12.4.1.4 Pathfinding in the optic tract In the last segment of their journey, RGC axons have to elongate along the optic tract in the diencephalon and perform a 45° turn posteriorly in the mid-diencephalon to reach the anterior border of the tectum. Again, a variety of different cues work together to guide axons in the optic tract, and the expression of repulsive molecules stops them from leaving the optic tract and innervating inappropriate territories.

In vitro studies performed in *Xenopus* have highlighted a role for the netrin-1 and Sema3A cues in RGC axon pathfinding in the optic tract. In addition to its function at the ONH, netrin-1 is also expressed in the dorsal diencephalon in an area that is nonoverlapping with, but adjacent to, growing retinal axons, suggesting that it might act as a repellent cue to prevent RGC axons from leaving the optic tract (Shewan *et al.*, 2002). Sema3A is expressed along the boundary of a segment of the optic tract and may act as a repulsive cue forcing RGC axons to turn posteriorly (Campbell *et al.*, 2001).

A zebra fish mutant, called astray, corresponding to a mutation in the Robo2 gene has provided evidence that an interaction between Slit and Robo is essential for axon pathfinding in the optic tract. The astray mutant phenotype exhibits severe retinal axon-pathfinding defects, including defasciculation of axons in the optic tract and widespread invasion of inappropriate regions of the brain (Fricke *et al.*, 2001; Hutson and Chien, 2002). This suggests that Slit/Robo signaling may be required to prevent axons from leaving the optic tract. These results are also consistent with the finding that Slit2 controls RGC axon pathfinding and targeting *in vivo* within the rat diencephalon (Ringstedt *et al.*, 2000).

Slit/Robo signaling also seems to be implicated in the topographic sorting of axons in the optic tract. In this process, axons from the dorsal part of the retina are sorted into the ventral brachium of the optic tract, and axons from the ventral part of the retina axons are sorted into the dorsal brachium of the optic tract in fish (Scholes, 1979; Stuermer, 1988). In a screen for zebra fish mutants with retinal axon-pathfinding defects, two mutants were isolated, exhibiting optic tract-sorting defects: boxer (box), dackel (dak). Boxer and dackel were identified to encode for genes involved in heparan sulfate proteoglycan (HSPG) biosynthesis and therefore in both mutants the level of HSPG is dramatically reduced (Lee *et al.*, 2004). Remarkably, the dak box double mutant exhibits a severe phenotype similar to the astray zebra fish mutant, indicating that HSPG biosynthesis regulates Slit function. Indeed, there is accumulating evidence that heparan sulfate is essential for the function of Slit (Liang *et al.*, 1999; Hu, 2001; Ronca *et al.*, 2001). However, exactly how the HSPGs interact with Slit and regulate its function remains unknown.

Further evidence supports an essential role for HSPGs and CSPGs in axon navigation within the optic tract. Both are highly enriched in the *Xenopus* optic tract and enzymatic removal of HSPG during development results in abnormally short retinal projections, suggesting that HSPG plays a role in promoting growth. Addition of an HSPG-binding growth factor, FGF2, to such HSPG-free brains enables axons to continue growing but they then follow extremely aberrant trajectories, suggesting that HSPG is dually involved in guidance and growth (Walz *et al.*, 1997). Exogenous HSPG added *in vivo* to the embryonic diencephalon causes a highly penetrant mistargeting phenotype in which retinal axons avoid entering the tectum, suggesting that HSPG plays an important role in target recognition (Walz *et al.*, 1997). Exogenously applied CSPGs lead to defasciculation of the optic tract and axons invade inappropriate territories (Walz *et al.*, 2002). In addition, it has been found recently that both HSPGs and CSPGs interact with the guidance cue Sema5A and differentially modulate its action on axons in the developing rat brain (Kantor *et al.*, 2004).

At the end of their journey, RGC axons enter their target area, and are topographically sorted to ensure correct mapping of the retinal image. This topographic mapping mechanism involves the A- and B-type of ephrin guidance cues and their Eph receptors. Since mapping is not strictly part of the axon-pathfinding process, it is not included in this article (but is reviewed in McLaughlin *et al.*, 2003).

In conclusion, we have seen that a combination of molecules act as attractive and/or repulsive cues to guide RGC axons during their journey to innervate

their appropriate target in the brain. However, only a small part of this complex process has been elucidated so far, and we are just beginning to understand the mechanisms and the molecular determinants involved in each step of the retinal pathway.

1.12.4.2 The Midline Choice Point as a Model System

1.12.4.2.1 The *Drosophila* ventral nerve cord A second model system that has provided many important insights into the mechanisms underlying axon guidance has been the ventral nerve cord of *Drosophila*. Many neurons from this population are interneurons, that is, neurons that connect with other neurons. As with many other regions in the developing CNS of bilaterally symmetrical organisms, most (but not all) of these interneurons extend axons toward the midline. A brief synopsis of the development of the *Drosophila* ventral nerve cord is outlined in Figure 7a. In each segment of the ventral nerve cord, interneuron axons cross the midline at either of two defined points, known as the anterior and posterior commissures, and then project parallel to the midline in distinct longitudinal fascicles. Importantly, those axons that have crossed the midline never do so again in the wild-type situation.

Thus, the ventral midline represents an important choice point for interneuron growth cones. Decisions that need to be correctly made include: (1) whether to remain ipsilateral or to cross the midline and project contralaterally; (2) which commissure to cross in; and (3) which longitudinal fascicle to extend in postcrossing. How is this accomplished? A number of genetic and biochemical studies have led to the identification of many factors responsible for these decisions. Firstly, crossing axons are attracted to the midline by netrin-1-A and netrin-1-B expressed by midline cells (Harris *et al.*, 1996; Mitchell *et al.*, 1996). These axons must then leave the attractive midline to continue toward their targets.

Large-scale screens for *Drosophila* mutants that display guidance defects at the midline have isolated three key players involved in expelling crossing axons from the midline: Commissureless (Comm), Robo, and Slit (reviewed in Kaprielian *et al.*, 2001). Comm mutants lack all commissural tracts. Robo mutants exhibit a thickened midline, a result of ipsilateral axons crossing the midline and contralateral axons recrossing the midline several times. Slit mutants show a thickened midline, resulting from all spinal axons collapsing onto the midline. These observations led to the following model for axon guidance at the *Drosophila* ventral midline: Slit secreted by the midline glial cells acts as a short-range repellent. Axons that extend contralaterally repress the surface expression of Robo until past the midline, after which it is upregulated at the growth cone surface to ensure exit from the midline and to prevent recrossing. Axons that remain ipsilateral express Robo from the outset and so are repelled from the midline by Slit. A combination of Robo and the other two Slit receptors, Robo2 and Robo3, is then used to specify lateral positions within the longitudinal axon bundles (Rajagopalan *et al.*, 2000; Simpson *et al.*, 2000; Figure 7).

Comm regulates the surface expression of Robo, therefore being the switch mechanism between the contra- and ipsilateral decision (Keleman *et al.*, 2002). A recent study has resolved how this occurs (Keleman *et al.*, 2005). In commissural axons expressing Comm, Robo is sorted to the degradation machinery, so that no Robo reaches the growth cone surface and these axons can cross the midline. In postcrossing axons and ipsilateral neurons, which lack Comm, Robo is targeted to the growth cone membrane; therefore, these axons are repelled by the midline repellent Slit.

1.12.4.2.2 The vertebrate spinal cord The vertebrate spinal cord is another key model system for axon guidance and a comprehensive review of this area can be found elsewhere (reviewed in Kaprielian *et al.*, 2001). Importantly, the role of many of the molecules that regulate midline crossing has been strongly conserved during evolution. In the developing vertebrate CNS, commissural interneurons in the dorsal regions of the spinal cord extend axons ventrally toward the spinal cord floorplate (Figure 7b). This specialized area, considered to be functionally equivalent to the midline of the *Drosophila* ventral nerve cord, is where commissural axons cross the midline, and constitutes an important intermediate choice point for axon guidance. The way commissural axons are initially attracted to the floorplate is analogous to the situation in *Drosophila*. Both *in vitro* and *in vivo* studies have shown that netrin-1, expressed by the floorplate cells, attracts these axons to the midline (Kennedy *et al.*, 1994; Serafini *et al.*, 1994). The way in which these axons are compelled to leave the floorplate is also similar. Midline cells also express homologues of the Slit gene, namely Slit1, Slit2, and Slit3. After crossing, commissural axons gain sensitivity to these repellents, and exit the midline. Removal of all three Slit genes results in guidance errors indicative of axons no longer being repelled from the floorplate postcrossing (Long *et al.*, 2004).

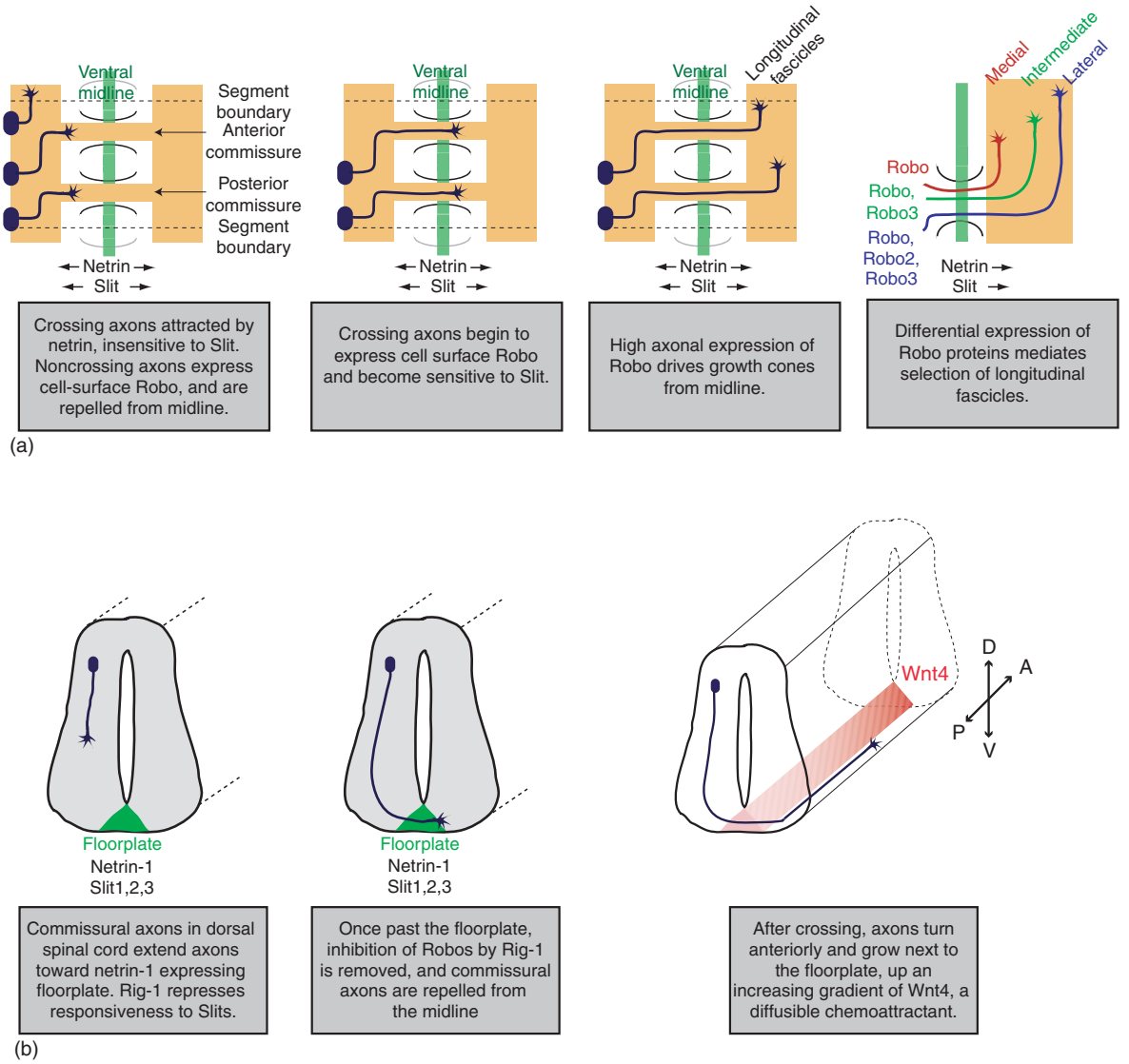


Figure 7 Axon navigation in the ventral nerve cord of *Drosophila* and in the vertebrate spinal cord. a, The ventral nerve cord of *Drosophila*. Within each segment of the *Drosophila* body, axons of the ventral nerve cord that project contralaterally cross the midline in either the anterior or posterior commissure. Expression of netrin and Slit by cells at the ventral midline mediates axonal navigation through the commissures. Precrossing axons are attracted by netrin-1. The insensitivity of these precrossing axons to Slit is mediated by Commissureless, a protein that sorts Robo away from the cell surface. As axons reach the midline, this repression is removed, and Robo is transported to the cell surface, rendering axons sensitive to the midline-derived Slit. Slit also controls selection of longitudinal fascicles once axons have exited the midline. b, The vertebrate spinal cord. In the developing vertebrate CNS, commissural interneurons in the dorsal regions of the spinal cord extend axons ventrally toward the netrin-1-expressing floorplate. The protein Rig-1 represses Slit responsiveness in precrossing axons. Once past the floorplate, the Slit inhibition is removed and axons are repelled from the midline. The chemoattractant Wnt4 is responsible for the anterior turn of axons which then grow next to the floorplate.

Interestingly, although precrossing commissural axons in the developing vertebrate spinal cord are not responsive to the Slit proteins, a Comm-independent mechanism seems to underlie this. Indeed, to date no Comm homologues have been identified in those vertebrate genomes sequenced. Instead, a divergent Robo homologue, Rig-1, has been shown to repress Slit responsiveness in precrossing axons (Sabatier *et al.*, 2004). How this occurs remains unclear, but it provides an illuminating example of evolution providing two different solutions to the problem of inhibiting repulsion of precrossing commissural axons.

After the midline, axons have another decision to make, namely whether to extend anteriorly or posteriorly parallel to the midline. Recently, another protein family was found to be involved in this aspect of axon guidance, the wingless proteins (Wnts) and their Frizzled (frz) receptors. The Wnts were originally identified as morphogens, which normally induce transcriptional changes by acting

inside the nucleus to pattern tissue and instructing cells to choose distinct cell fates. In contrast, axon guidance cues are defined to influence migration of motile cells or growth cones, primarily by inducing cytoskeletal changes and membrane dynamics. A surprising recent finding has been that the classical morphogens such as Wnts, Hedgehog (Hhs) and bone morphogenetic proteins (BMPs) can also act as guidance molecules in this context (reviewed in Schnorrer and Dickson, 2004). Indeed, in the rat spinal cord, a diffusible attractive gradient in the anterior–posterior direction was found to influence the guidance decision of commissural axons after crossing the midline to turn anteriorly. A search for candidates identified Wnt4. Application of Wnt function blocking SFRPs (secreted Frizzled-related proteins) resulted in stalling of axons after midline crossing. When Wnt4-expressing cell aggregates were ectopically positioned at a posterior position, these explants were able to reorient axon growth into a posterior turn. In fzd3 knockout mice, which lack the Wnt receptor, postcrossing axons exhibit random turning into anterior and posterior direction, suggesting that the signaling responsible for the anterior turn is impaired (Lyuksyutova *et al.*, 2003). Therefore, it has become clear that the Wnt family plays an important role in axon pathfinding and can also act as guidance cues to initiate signaling pathways involved in growth cone navigation.

1.12.5 Modulation of Axon Guidance

While additional axon guidance cues are known, and more surely remain to be identified, there is still only a small suite of factors in comparison to the high degree of complexity found in the nervous system. Importantly, members of the Slit, semaphoring, and netrin-1s families are multifunctional; their role in attraction or repulsion is defined by the axon population involved and developmental context in which pathfinding occurs. Indeed, some axons can switch responsiveness to a single cue dramatically over time. How is this plasticity achieved? Recently, a number of studies have begun to address how diverse outcomes are generated using the limited repertoire of guidance cues. It is becoming clear that both extrinsic and intrinsic factors modulate the behavior of developing axons, and that integration of signals from multiple guidance pathways is essential to coordinate directional pathfinding.

1.12.5.1 Regulated Gene Expression

Axon guidance cues and their receptors are dynamically expressed in the embryonic nervous system. By controlling when and where transcription occurs, axon guidance can be coordinated in a precise spatiotemporal fashion. Visual system development in the frog *Xenopus* provides one example. We have seen that in the tadpole, which has laterally placed eyes, all axons cross to the contralateral side of the brain at the optic chiasm. However, after metamorphosis, medialward movement of the eyes results in their visual fields having a degree of overlap. To integrate information from the overlapping visual field, some axons must project to the ipsilateral side of the brain. This is achieved by the expression of repulsive ephrin-B at the chiasm during metamorphosis. Thus, late-born ventrotemporal axons expressing the Eph-B receptor are repelled from the chiasm, and project ipsilaterally (Figure 8a).

1.12.5.2 Post-Transcriptional Control

The activity of guidance ligands and receptors is also controlled at the post-transcriptional level. For example, Slit2 can bind extracellular proteins including laminin and various proteoglycans, which may modulate its activity and presentation. Other instances of post-transcriptional control include alternative splicing, proteolytic cleavage and receptor shedding, receptor inactivation, and selective trafficking.

Selective trafficking of Robo at the midline crossing in *Drosophila* commissural axons provides a good example for the post-transcriptional control. As we have already mentioned, though these neurons express Robo from an early stage, it is not present on the surface of precrossing axons because of the presence of the Comm, an intracellular sorting receptor, that traffics Robo to lysosomes (Figure 8b; Keleman *et al.*, 2005).

1.12.5.3 Local Translational Control

The discovery of polyribosomes located beneath postsynaptic sites on dendrites (Steward and Levy, 1982; Steward and Fass, 1983) and the identification of various mRNA species specifically localized within dendrites (Garner *et al.*, 1988) suggest that compartmentalized synthesis of protein occurs in neurons. Altough the existence of local translation in dendrites has been widely accepted, the question of whether or not translation occurs in axons has remained more controversial. In invertebrate axons such as the squid giant axon and the marine gastropod *Aplysia*, all of the necessary components for RNA translation and a variety of mRNA species have been identified (reviewed in Alvarez *et al.*, 2000). *In vitro* experiments using fractions isolated from squid axons (Ingoglia *et al.*, 1983) and synaptosomes (Ingoglia *et al.*, 1983) have demonstrated that axonal protein can occur in axons. In vertebrates, ribosomes have

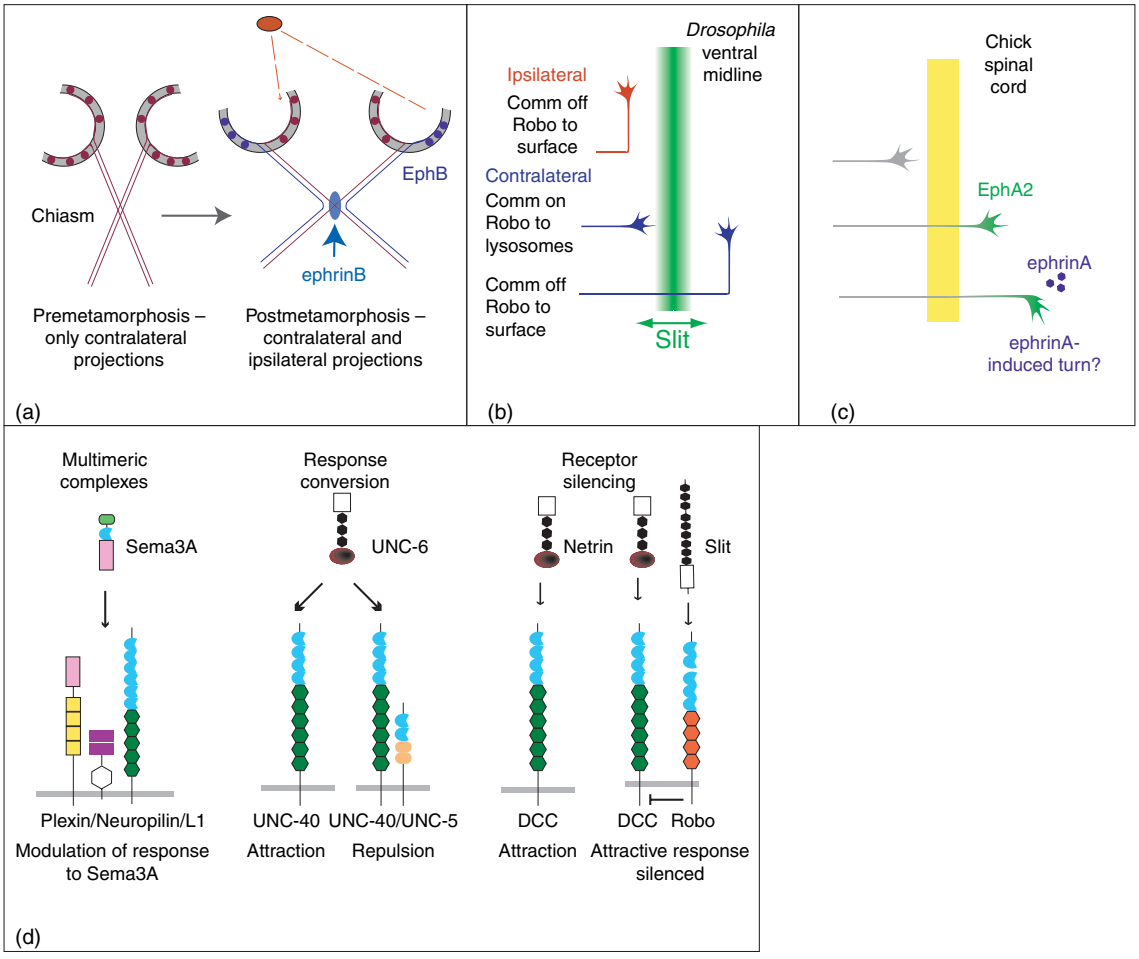


Figure 8 Modulators of axon navigation. Examples of ways in which responses of axons can be modulated during development (see text for description of each example): a, regulated gene expression; b, post-transcriptional control; c, local translation; and d, receptor interaction. Modulation of axon guidance using such methods allows for the generation of diverse outcomes using a small suite of guidance cues.

been identified in the initial axon segment (Steward and Ribak, 1986) and intermittently along the axon shaft (Pannese and Ledda, 1991; Koenig *et al.*, 2000) using both biochemical and immunohistochemical methods (Bassell *et al.*, 1998; Campbell and Holt, 2001). Other components necessary for translation have also been identified in vertebrate axons (reviewed in Alvarez *et al.*, 2000).

What is the role of protein synthesis in axons and does it play a role in aspects of axon guidance? A key finding with respect to these questions is that axon guidance molecules, like netrin-1 and semaphorin 3A, can trigger protein synthesis in retinal growth cones isolated from their cell bodies within 5–10 min. Moreover, inhibition of translation blocks the chemotropic responses of retinal growth cones to netrin-1 and Sema3A *in vitro* (Campbell *et al.*, 2001) and local protein synthesis is necessary for the normal turning responses of *Xenopus* retinal and spinal axons toward gradients of attractants *in vitro* (Ming *et al.*, 2002). By synthesizing specific receptor/effector proteins locally, an axon can potentially alter its responsiveness to previously encountered signals or gain responsiveness to cues expressed on its subsequent trajectory. Such a mechanism may be used by chick commissural axons, which express high levels of EphA2 on the postcrossing region of the axon. By linking mRNA for the marker protein GFP to the 3′ untranslated region (3′UTR) of EphA2 mRNA and driving its expression in commissural neurons, it has been shown that commissural axons specifically translate GFP only in the region extending beyond the midline. This suggests that a region in the EphA2 3′UTR promotes selective translation of EphA2 in distal axonal segments. EphA2 may be used later in guidance, conferring sensitivity to ephrinA ligands, though this remains to be shown (Brittis *et al.*, 2002). The key questions in all of these studies are: which proteins are translated in response to various axon guidance cues and how does their translation control the chemotactic turning behavior of a

growth cone? A local contact with a guidance cue, for example, through a single filopodium, may induce fast and specific change in protein levels (synthesis and/or degradation) within the growth cone, leading to asymmetric cytoskeletal rearrangements and turning, as has been shown in dendrites (Steward and Schuman, 2003). However, the role of local protein synthesis stimulated by guidance cues in the growth cone is still largely unknown (Figure 8c; reviewed in van Horck *et al.*, 2004).

1.12.5.4 Local Protein Degradation and Endocytosis at the Growth Cone

Xenopus retinal growth cones contain the machinery for proteosomal degradation (proteasome proteins, ubiquitin, and ubiquitinating enzymes). Moreover, inhibitors of proteasome function can block the chemotropic response of growth cone to guidance cues, such as netrin-1 and lysophosphatidic acid (LPA) (Campbell and Holt, 2001). An increase in ubiquitin-conjugated proteins within 5 min is induced by these guidance cues, indicating that they rapidly stimulate the degradation pathway. In addition, the protein caspase-3, a marker of the apoptotic pathway, has been found to be involved in chemotropic guidance in retinal axons (Campbell and Holt, 2003). Of interest is that one of the known cleavage targets of caspase-3 is the translation initiation factor eIF4G (Clemens *et al.*, 1998), raising the possibility that guidance cues, such as netrin-1 and brain-derived neurotrophic factor, which simultaneously activate both translation and caspase pathways, can downregulate the synthesis of proteins that they stimulate using a negative-feedback loop. Furthermore, in *Drosophila*, the pruning of the γ neuron axonal projections requires protein degradation. Indeed, mutations of an ubiquitin-activating enzyme or proteasome subunits prevent normal pruning (Watts *et al.*, 2003).

Another mechanism involved in growth cone guidance is the removal and/or inactivation of activated receptors. To advance along their way, growth cones have to break previous interactions with the surface. Several mechanisms are involved in this process, including proteolytic cleavage (Hattori *et al.*, 2000), transreceptor silencing (Stein and Tessier-Lavigne, 2001), and endocytosis. Recently, it has been shown that metalloproteases regulate *in vivo* the growth and guidance of retinal growth cones (Webber *et al.*, 2002). Moreover, it has been shown that endocytosis of functional ephrinB/EphB complexes promote cell detachment of the interacting cells *in vitro* (Marston *et al.*, 2003; Zimmer *et al.*, 2003). Together, these data suggest that endocytosis might be a fast mechanism for ending the adhesive contacts between growth cones and neighboring cells.

1.12.5.5 Receptor Interaction

Interaction between receptor proteins at the surface of the growth cone has been shown to be a remarkably efficient means by which the complexity of axonal responses to guidance cues can be magnified. The combinatorial assembly of heteromeric receptor complexes has a number of potential benefits, such as receptors with distinct signaling properties being used to potentiate the activity of other guidance ligands. The ability to modulate receptor activity via the expression of coreceptors is evident in instances of Semaphorin signaling. Sema receptors are often complexes of different proteins, and this confers the potential to modulate responses to a small number of cues with great subtlety. For example, the responses of dorsal root ganglion (DRG) neurons to Sema3A are modulated by the CAM, L1-CAM, which is also expressed on DRG axons (Castellani *et al.*, 2000).

As well as modulating axonal responses, coreceptor expression can directly convert responses to guidance cues. As mentioned previously, netrin-1s are bifunctional. When signaling via UNC40/DCC, netrin-1s act as attractants. However, when axons express UNC-5, this response is converted to repulsion. Netrin-1 can interact with UNC-5 directly to mediate repulsion, and UNC-5 can also bind DCC though its cytoplasmic domain, which essentially silences DCC-mediated attraction.

As a growth cone navigates through the terrain of the developing nervous system, it undoubtedly encounters multiple guidance cues simultaneously. At the choice point of the spinal cord floorplate, for example, a commissural growth cone will 'see' both netrin-1 and Slit, each of which is promoting a completely opposite reaction. How is such information successfully processed within the axon? Here too, interactions between receptors may be pivotal, as *in vitro* experiments with *Xenopus* spinal neurons suggest. Axons from these neurons turn and grow toward a local source of netrin-1 in culture. Application of Slit blocks netrin-1-mediated turning, yet axon growth mediated by netrin-1 is not affected. Silencing of attraction occurs via a direct interaction between the cytoplasmic domains of DCC and Robo. This specific silencing of netrin-1-mediated attraction by the Robo receptor is an elegant example of signal integration within the growth cone, demonstrating how encounters with concurrent signals may be ordered and prioritized to generate a directional response (Figure 8d; Stein and Tessier-Lavigne, 2001).

1.12.5.6 Implications for Axon Guidance

The degree of plasticity endowed by the above mechanisms enables developing axons to regulate their sensitivity to extrinsic guidance cues in a highly specific manner. Thus, in addition to responding in a stereotyped fashion to attractants or repellents, growth cones can navigate in environments which may not intuitively appear to be conducive for guidance, such as extending toward repulsive targets (e.g., Slit-expressing midline) or moving on from attractive intermediate targets (e.g., netrin-1-expressing ONH). Such changes in axon sensitivity to guidance cues have been described in many different neuronal populations (see above). Modulation of axonal responses has been particularly well documented for netrin-1. For instance, commissural axons from the rodent metencephalon lose responsiveness to this chemoattractant after crossing the midline (Shirasaki *et al.*, 1998), and *Xenopus* retinal axons advancing along the visual pathway switch netrin-1-responsiveness from attraction to repulsion over time (Shewan *et al.*, 2002).

As well as gaining (or losing) responsiveness to guidance molecules, growth cones can also adjust their sensitivity to changing concentrations of such cues. This process, known as adaptation, enables growth cones to navigate in gradients of chemotropic molecules. Adaptation has been described in *Xenopus* retinal (Piper *et al.*, 2005) and spinal (Ming *et al.*, 2002) growth cones *in vitro*, where exposure to a low level of a guidance cue elicits an initial desensitization to additional exposure to the cue, subsequently followed by resensitization and a resumption of responsiveness. Growth cone adaptation is thought to increase the sensitivity of axons when in gradients of guidance cues, allowing them to respond to subtle differences in the environmental concentration of the cue as they proceed. For example, retinal axons *in vitro* are able to grow further up a gradient of a repulsive guidance cue when initially exposed to the cue as compared to those axons not exposed to the cue at the start of the assay (Rosentreter *et al.*, 1998).

In summary, axon guidance cues can be modulated by a variety of intrinsic and extrinsic factors that ultimately regulate growth cone sensitivity and adaptation during development. Thus, by controlling the response of growth cones in a precise spatiotemporal fashion and by integrating and prioritizing coincidently encountered signals, the incredibly complex connections of the nervous system can be generated using a small repertoire of guidance molecules.

1.12.5.7 Axon Guidance and Evolution

In recent years, our understanding of the development and function of the CNS has expanded significantly. With respect to axon guidance, one of the most important advances in our knowledge has been the identification of the main molecular families responsible for navigation, patterning, and target innervation: the netrin-1s, semaphorins, Slits and ephrins. Remarkably, despite millions of years of evolutionary divergence, most of the key molecules that mediate axon guidance during formation of the nervous system in vertebrates and invertebrates are highly conserved, as are the intracellular signaling pathways they activate. For example, the netrins act as critical determinants controlling attraction of commissural axons toward the midline in both flies and mammals, despite an evolutionary separation exceeding 600 million years. The way in which pathfinding axons navigate to distant targets by sensing the molecular characteristics of the local environment, thereby breaking their journey into small segments, is also very similar across a broad range of species. For instance, to find their correct target, the pioneer Ti1 axons of the grasshopper limb are guided by local cues or guidepost cells, that are spaced short distances apart, while many developing vertebrate axon tracts, such as the retinal pathway, have multiple points at which axons receive directional information to simplify their navigation en route to their target. Such pathway subdivision is an extremely efficient method of enabling axons to navigate in an error-free way to their targets, and also endows the pathway with multiple regulatory checkpoints that can be altered during development to enable differing guidance decisions to be made over time.

However, there are a number of features of axon guidance that do reflect the evolutionary divergence of the vertebrate and invertebrate lineages. One example of this lies in the number of ligand and receptor molecules acting to control axon navigation. Vertebrates generally possess a greater number of axon guidance molecules, perhaps due to the genome and chromosomal duplications that have occurred in the vertebrate lineage. For instance, the Eph and ephrins form large, highly homologous families within the vertebrate phyla, suggesting that a major expansion of these genes has occurred. This expansion may have occurred in tandem with the evolution of the vertebrate lineage, and could have been a critical factor facilitating the construction of increasingly complex brains. The broad range of molecular components that can comprise semaphorin receptor complexes also provides an

example of strong evolutionary divergence, as these complexes differ widely between different species.

1.12.6 Concluding Remarks

In summary, we have presented a broad overview of the process of axon guidance, describing the fundamental tenets of axonal navigation shared by disparate species, as well as giving an insight into how different evolutionary advances, such as the development of more complex brains, has been accomplished using a limited suite of axon guidance cues. The growth cone, the tip of the growing axons, is the key structure in sensing and integrating the information provided by guidance cues within the environment. We have discussed some historical axon-pathfinding concepts and experimental techniques that provide insight in both the identification of guidance molecules and mechanisms. Two extensively studied model systems, the retinotectal projection and the midline choice point, have been used to identify the guidance cues and to study the mechanisms of attraction and repulsion induced by them. Finally, several mechanisms modulate the axonal responses at different levels of the signaling pathways, including receptor silencing, protein synthesis, degradation, and endocytosis. In the long term, understanding the mechanisms that underlie axon pathfinding during development will surely help us to determine the basis of many human syndromes with neurological deficits, and perhaps help us to elucidate the cause of neurodegenerative disorders. Encouragingly, studies of neural development have indeed begun to provide insight into neurological diseases and may eventually culminate in strategies for restoring neural connectivity following injury.

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1.13 Evolution of the Action Potential

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Glossary

A-type current — A transient outward K^+ current that activates and inactivates rapidly. A-type currents are coded for by the Kv4 potassium channel gene subfamily. In addition, Kv1.4 and other Kv1 proteins can form A-type currents when complexed with accessory cytoplasmic proteins (e.g., β-subunits). A-type currents contribute to the resting potential, participate in repetitive firing and spike repolarization, and prevent back-propagation of dendritic APs.

absolute refractory period — The time interval during which a second AP cannot be elicited, regardless of stimuli intensity.

afterhyperpolarization (mV) — A temporal slow hyperpolarization observed after a train of APs. Often plays a role in the regulation of the neuron's firing rate.

amplitude (mV) — Property of an AP that refers to the mV difference between the RMP and peak.

anode break excitation — Generation of an AP at the end of a long-duration depolarization or hyperpolarizing pulse.

conductance — Property of a channel that characterizes its ability to conduct current.

delayed rectifier — Outward K^+ currents that activate slowly (delayed with respect to activation of voltage-gated sodium current). These channels show little, if any, inactivation in the range of seconds. These channels play a predominant role in AP repolarization.

depolarization — Change that results in a more positive (less negative) MP.

duration (ms) — A measure of the time that depolarization lasts during an AP (e.g., interval between threshold on the rising phase and recovering half of the AP amplitude on the falling phase).

hyperpolarization (mV) — Change that results in a more negative (less positive) MP.

inactivation — A process that leads to a nonconducting channel state that does not respond to depolarization by opening of the channel.

inward rectifier — Also known as anomalous rectifiers (as opposed to normal outward rectification), these potassium channels pass inward currents at potentials more negative than the K^+ equilibrium potential of (E_K). The Kir subfamily of voltage-gated ion channels (VGICs) codes for inward rectifiers. Inward rectifiers are strongly regulated by intracellular factors and second messengers and contribute to maintenance of the resting potential and K^+ homeostasis.

ionic dependence — Property of an AP that refers to the principal ion driving the depolarization.

outward rectifier — A channel that allows current to flow in an outward direction more easily than an inward direction.

peak (mV) — The voltage at which the maximum AP amplitude is reached.

permeability — Ion channel property that determines the rate at which ions pass through the pore.

potassium current (I_K) — Ion flux through a potassium-selective ion channel.

rate of fall ($mV\ ms^{-1}$) — Change in MP per unit time during the falling (repolarizing) phase of the AP. Used as an indirect measure of potassium current density.

rate of rise ($mV\ ms^{-1}$) — Change in MP per time unit during the rising (depolarizing) phase of the AP. Used as an indirect measure of sodium current density.

rectifier — In electronics, a circuit that converts bidirectional current (AC) to unidirectional current (DC). In physiology, a nonlinear I/V relationship produced when a membrane conductance is dependent upon the direction of traffic of the permeant ion. As a result, a rectifying current preferentially flows in one direction and not the other.

refractory period (ms) — A primary determinant of the maximal rate of firing of a neuron.

relative refractory period — The briefest interval after which a second AP can be elicited, albeit with diminished amplitude.

repolarization — Change that brings the MP back towards the resting value.

rheobase (nA) — The minimum amount of current needed to generate at least one AP.

sodium current (I_{Na}) — Ion flux through a sodium-selective ion channel.

threshold (mV) — The minimum value of the MP at which an AP is initiated. Threshold can also be defined as the absolute depolarization magnitude from the RP required to initiate an AP.

1.13.1 Introduction

The nervous system collects, coordinates, integrates, and disseminates diverse types of information regarding both the external and internal environments. Processing of this information leads to appropriate physiological and/or behavioral responses. In order for stimuli to produce accurate descriptions of the environment, a variety of neural codes and operations arose during evolution that allow information exchange within and between neurons (for review, see Perkel and Bullock, 1968; Gerstner *et al.*, 1997). One mechanism, the action potential (AP), arose early during evolution and is essential for rapid signaling in the nervous system.

In this article, we focus on how embryonic neurons acquire the ability to fire APs. AP generation represents a significant challenge because of the requirement for function of several different membrane proteins. Further, there are several examples of neurons that fire APs with developmentally regulated properties. Consequently, the roles of APs in emerging nervous systems are not static and depend upon developmental stage. We review mechanisms that lead to the developmental regulation of excitability. We conclude by identifying key issues that remain unresolved and warrant being the focus of future study (see Neuronal Migration, Axon Pathfinding, A Tale of Two CPGs: Phylogenetically Polymorphic Networks).

1.13.2 What Are Action Potentials?

Electrically excitable cells share in common the ability to generate APs. During an AP, the membrane potential (MP) of an electrically excitable cell displays dramatic, stereotypic, and rapid changes. In

muscle cells, AP generation quickly leads to muscle contraction. In neurons, APs allow rapid intra- and intercellular communication.

The classic work of Hodgkin and Huxley (1952; Hodgkin, 1958, 1964) demonstrated that time-dependent changes in the membrane conductance to specific ions underlie the generation of APs. For more detailed treatment of the ionic basis of the MP and AP, we refer the reader to any of several excellent books (Jack *et al.*, 1988; Johnston and Wu, 1994; Kandel *et al.*, 2000; Hille, 2001; Nicholls *et al.*, 2001). Below, we briefly review key aspects of AP generation. We have defined important terms in the glossary for readers who wish to read the primary literature.

Typically, all cells display an electrical difference across their membrane. This electrical difference, known as the MP, has values in the mV range. By convention, the inside of the cell is negative with respect to the outside. The predominant value of the MP is referred to as the resting MP (RMP).

Excitable cells generate APs (also referred to as spikes or impulses) in response to stimuli that bring the MP to a new, less negative value, known as threshold. Threshold values are not fixed and vary among excitable cells. Moreover, for any given cell, the value of threshold can change over time as a result of developmental regulation, activity, or cell–cell interactions. Stimuli that bring the MP to values less positive than threshold do not lead to AP generation and instead generate subthreshold responses. Thus, APs are not generated in a graded manner but rather as an all-or-none response of the membrane to stimuli of sufficient intensity (Hodgkin and Huxley, 1952).

Once threshold is achieved, the conductance to sodium and/or calcium ions increases, allowing rapid entry of positively charged sodium and/or calcium ions. The influx of positive charge results in a more positive MP. With further depolarization, more sodium channels open, resulting in greater sodium influx and even more positive MP values. When depolarization occurs, the membrane conductance to potassium ions also increases, but with a delay. The increased potassium conductance and inactivation of sodium channels repolarize the membrane to its original negative resting value, thereby terminating the AP.

In neurons, AP generation initiates near the cell body at a site known as the axon hillock (Coombs *et al.*, 1957a, 1957b). Subsequently, APs propagate down the axon at constant velocity and amplitude. The ability to propagate APs without diminution in amplitude is an essential feature of neuronal cell–cell communication and guarantees that signals will be transmitted faithfully without failure.

1.13.2.1 APs are Neuronal Signatures

Figure 1 presents an example of an AP fired by a mature neuron. Typically, adult neurons fire APs that are brief in duration and rely upon voltage-gated sodium channels for generation. However, APs fired by mature neurons vary significantly. Further, some neurons respond to stimulation by firing a single AP, while others fire multiple impulses in a characteristic pattern. Consequently, the type of AP or AP train fired in response to stimulation can serve as a signature of neuronal identity (for review, see Contreras, 2004).

1.13.2.2 AP Waveform Properties

The plot of the MP as a function of time during an AP is known as the AP waveform (Figure 1). Characterization of APs involves analysis of specific properties of the waveform that, in turn, reflect the complement of voltage-gated channels expressed by the cell (see 'Glossary').

During the AP, the MP achieves positive values near the equilibrium potential for sodium. The most positive MP achieved during an AP is known as the peak. The rapid depolarization leading to the peak reflects the activity of voltage-gated sodium channels. Thus, measuring the rate of rise provides an indication of sodium current density. In contrast, the activity of voltage-gated potassium channels contributes to the subsequent repolarization and the rate of fall.

After repolarization, the MP often becomes slightly more negative than the standard RMP,

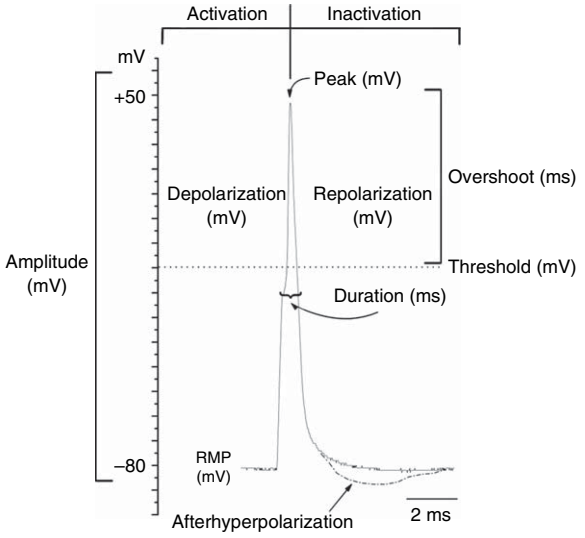

Figure 1 Stereotypic AP of a mature neuron: an AP recorded from a Rohon–Beard cell of a 2-day-old zebra fish embryo is shown (Pineda and Ribera, unpublished data). Several AP properties that are defined in 'Glossary' are illustrated in the figure.

resulting in an afterhyperpolarization. Afterhyperpolarizations play important roles in determining the frequency of AP firing. Because information is often encoded by AP frequency, afterhyperpolarizations can greatly influence processing of information in the nervous system.

One property of APs that varies greatly between excitable cells is the duration. In some neurons, the AP duration is extremely brief and barely 1 ms (Storm, 1987). In contrast, skeletal muscle fibers fire APs that have slightly longer durations (~5–10 ms; Kuriyama *et al.*, 1970). Cardiac myocytes fire strikingly different impulses with durations that are often as long as 500 ms (Cavalié *et al.*, 1985; Hume and Uehara, 1985). As discussed further below, the duration of neuronal APs often undergoes substantial developmental regulation.

1.13.3 Molecular Determinants of APs

The membrane conductances that underlie the AP reflect the activities of several members of the voltage-gated ion channel (VGIC) superfamily of membrane proteins (Figure 2). VGICs respond to membrane depolarization with conformational changes that reveal an ion-selective pore through which specific ions pass in a diffusion-limited manner. Members of the VGIC superfamily have a positively charged transmembrane domain known as the S4 helix, the structure of which depends upon the transmembrane voltage (for review, see Gandhi and Isacoff, 2002).

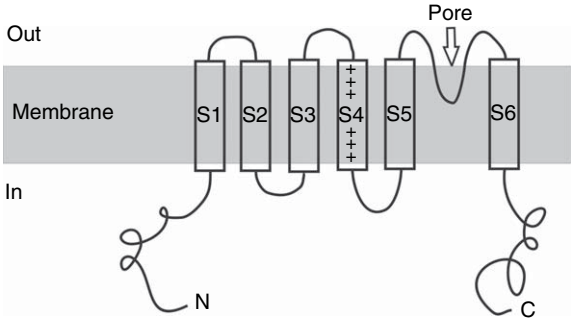

Figure 2 Proposed membrane topography of a voltage-gated potassium channel: the proposed disposition of a single voltage-gated potassium channel α-subunit in the membrane is shown. The S4 transmembrane domain, thought to be critical for voltage-dependent gating, is indicated. Similar S4 domains are also found in voltage-gated sodium and potassium channels and are a hallmark of the VGIC superfamily. 'N' and 'C' indicate the amino- and carboxyl-termini, respectively. Reprinted by permission from Macmillan Publishers Ltd: *Nature* (Yellen, G. 2002. The voltage-gated potassium channels and their relatives. *Nature* 419, 35–42), copyright (2002).

VGICs exist in species throughout the animal and plant kingdoms, including prokaryotes, protozoa, yeast, vascular plants, coelenterates, nematodes, arthropods, mollusks, teleosts, and tetrapods (for review, see Hille, 2001). Not surprisingly, APs have been recorded from cells in a range of species spanning the animal and plant kingdoms (for review, see Hille, 2001). During the last 20 years, many genes and transcripts for a large variety of VGIC proteins have been cloned and characterized. Noda *et al.* (1984) reported the cloning of a voltage-gated sodium channel from the electric organ of the electric eel, *Electrophorus electricus*. The cloning of a voltage-gated calcium channel from rabbit skeletal muscle followed in 1987 (Tanabe *et al.*, 1987). That same year, several groups reported cloning of the *Drosophila Shaker* potassium channel gene (Kamb *et al.*, 1987; Papazian *et al.*, 1987; Pongs *et al.*, 1988; Schwartz *et al.*, 1988). Comparisons of the primary sequences of cloned VGIC genes have revealed a high degree of conservation among species that are distantly related (for review, see Jan and Jan, 1990; Coetzee *et al.*, 1999; Moreno-Davila, 1999; Goldin, 2001; Yu and Catterall, 2003). Such findings suggest that the key structural and functional properties of VGICs have been conserved during evolution.

Molecular cloning has revealed an unexpectedly large number of VGIC genes, many more than might have been expected on the basis of physiological recording. Current research seeks to identify the specific roles of the many VGIC genes that have been identified. A common finding has been that VGIC gene subfamilies may have multiple members in vertebrates (e.g., Kv1 family: Kv1.1–Kv1.9; Nav1 family: Nav1.1–Nav1.9, respectively) but only a single orthologous gene in invertebrates (e.g., *Drosophila Shaker*; *Drosophila para*, respectively).

Phylogenetic analyses suggest that VGICs evolved from an ancestral voltage-gated potassium channel (for review, see Hille, 2001; Yu *et al.*, 2005). Further, voltage-gated calcium, but not sodium, channels have been detected in unicellular organisms (DeHertogh *et al.*, 2002). APs recorded from species lacking voltage-gated sodium channels rely upon voltage-gated calcium channels for their initiation and typically are much longer-lasting events that signal via changes in intracellular calcium ion concentrations.

Phylogenetic analyses indicate that voltage-gated sodium channels evolved later than did voltage-gated potassium and calcium channels. Voltage-gated sodium channel function also enlisted a sodium pump that establishes a transmembrane

sodium gradient (Stein, 2002). Voltage-gated sodium channels underlie the ability of the AP to be a rapid spike, occur in bursts, and propagate rapidly. Thus, the later evolution of voltage-gated sodium channels introduced important changes into the AP waveform, allowing it to be a rapid spike. Further, voltage-gated sodium channels allow APs to occur repetitively at high frequencies. Thus, voltage-gated sodium channels significantly expanded the roles that APs can play in information processing and behavior.

1.13.4 Roles in Information Coding

During development, the roles that APs play in the nervous system vary substantially (Figure 3; see below). Interestingly, many neurons fire APs prior to synapse formation. Several lines of evidence indicate that early-appearing APs play a developmental role. After synapse formation, APs take part in mechanisms that select and/or eliminate specific connections. In the mature nervous system, APs contribute to plasticity mechanisms in addition to being essential for the rapid processing of information.

1.13.4.1 Prior to Synapse Formation

Many aspects of neuronal differentiation begin prior to synapse formation. Importantly, many neurons acquire electrical excitability prior to synapse formation, thus allowing neuronal activity to influence subsequent aspects of differentiation (Holliday and Spitzer, 1990). For example, soon after neurons exit the cell cycle and initiate postmitotic differentiation, they often migrate long distances and take up residence at distant sites. Blocking electrical activity can affect migratory patterns of embryonic neurons for both invertebrates and vertebrates (Komuro and Rakic, 1992; Tam *et al.*, 2000). Neurons differentiate biochemically and often synthesize and secrete neurotransmitter prior to synapse formation. Perturbations of excitability alter biochemical differentiation of neurons, resulting in inappropriate neurotransmitter synthesis (Gu and Spitzer, 1995; Borodinsky *et al.*, 2004). Morphological differentiation also occurs during this period, notably axon outgrowth and initial contact of targets. Blockade of activity also perturbs this important aspect of neuronal differentiation (Cohan and Kater, 1986; Gu *et al.*, 1994). As discussed further below, electrical membrane properties are also developmentally regulated. Patterns of early activity influence acquisition of mature channel properties (Desarmenien and Spitzer, 1991; Gomez and Spitzer, 1999).

A common finding for effects of activity prior to synapse formation concerns its dependence on calcium ions. The majority of studies indicate that calcium ions act as intracellular messengers and participate in mechanisms that translate patterns of activity into developmental programs (for review, see Spitzer *et al.*, 2004). As we discuss below, many neurons fire long-duration calcium-dependent APs prior to synapse formation, thus accounting, at least in part, for the calcium dependence.

1.13.4.2 During Synapse Formation and Early Circuit Activity

Synapses can form in the absence of neural impulses (Verhage *et al.*, 2000; Trachtenberg *et al.*, 2002; De Paola *et al.*, 2003). However, the maintenance of synapses requires transmitter secretion that normally depends upon impulse propagation (for review, see Sanes and Lichtman, 2001). Moreover, in several instances, after synapses initially form,

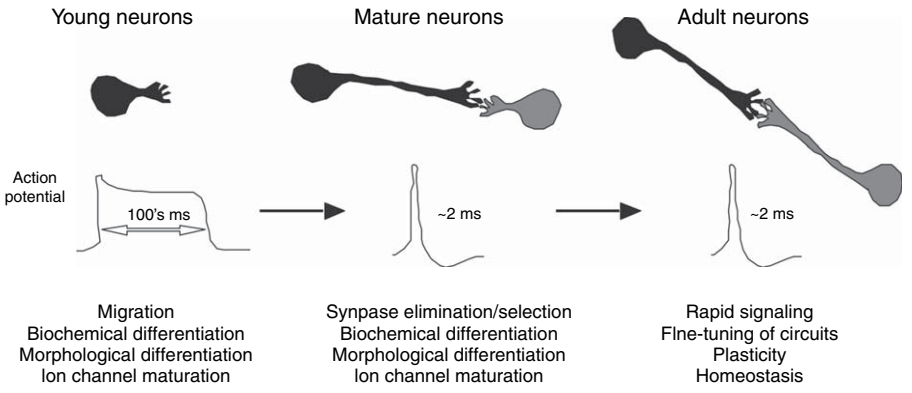

Figure 3 AP roles change during development. In neurons, APs play several roles in addition to rapid processing of information. Prior to synapse formation, APs play developmental roles. During the early stages of synapse formation, APs function in mechanisms that eliminate or stabilize specific connections. In the adult nervous system, APs contribute to plasticity mechanisms.

there is a period of pruning or synapse elimination. For example, at the neuromuscular junction, muscle fibers that receive inputs from multiple axons become singly innervated (for review, see Colman and Lichtman, 1993). Also, in the cerebellum, Purkinje cells are initially innervated by more than one climbing fiber but later respond to inputs from only one (Mariani and Changeux, 1981). Similar observations have been made for developing synapses in the visual, auditory, and autonomic nervous systems (Lichtman, 1977; Shatz and Stryker, 1988).

During development, activity regulates neuronal arbor growth by regulating branch lifetime in retinal ganglion cell axons and tectal dendrites of *Xenopus* and zebra fish larvae (Rajan and Cline, 1998; Rajan *et al.*, 1999; Lohmann *et al.*, 2002; Schmidt, 2004). Activity-dependent expression of structural proteins may also be involved in synapse stabilization and dendritic branch formation (Ziv and Smith, 1996; Lichtman, 2000; Star *et al.*, 2002; Fukazawa *et al.*, 2003; for review, see Steward and Schuman, 2001; Hua and Smith, 2004). Moreover, activity promotes secretion of neurotrophins that have multiple effects on synaptic development and function as well as ion channels (Nick and Ribera, 2000; for review, see Poo, 2001; Lu and Je, 2003; Figure 4).

1.13.4.3 Mature Nervous System

In the mature nervous system, a principal role of the AP is transduction, conduction, and processing of information (Eggermont, 1998; Sanger, 2003). However, even at these stages, AP generation contributes to mechanisms that alter the nervous system both structurally and functionally. For example, activity sculpts the time course of elimination of Rohon–Beard cells from the spinal cord of larval zebra fish (Svoboda *et al.*, 2001). Neural activity also promotes both short- and long-term synaptic changes and affects the maintenance, synthesis, and release of neurotrophins (Madison *et al.*, 1991; Poo, 2001; Lu and Je, 2003).

1.13.5 Regulation of Excitability during Embryogenesis

Developmental regulation of excitability occurs throughout embryogenesis. In the early 1970s, Takahashi and co-workers recorded the changes in electrical excitability that occur in the tunicate *Halocynthia roretzi* as it develops from an unfertilized egg to an adult with differentiated tissues (Takahashi, 1979; for review, see Takahashi and Okamura, 1998). Their results indicated that, in both the unfertilized and the newly fertilized egg, APs were generated. Further, AP generation required the function of voltage-gated sodium and/or calcium channels. In contrast, differentiated muscle fibers showed a predominantly calcium-dependent AP. These observations suggested that, during early embryonic differentiation: (1) some types of ion channels are eliminated; (2) some types of ion channels increase in density; and (3) new channel types appear.

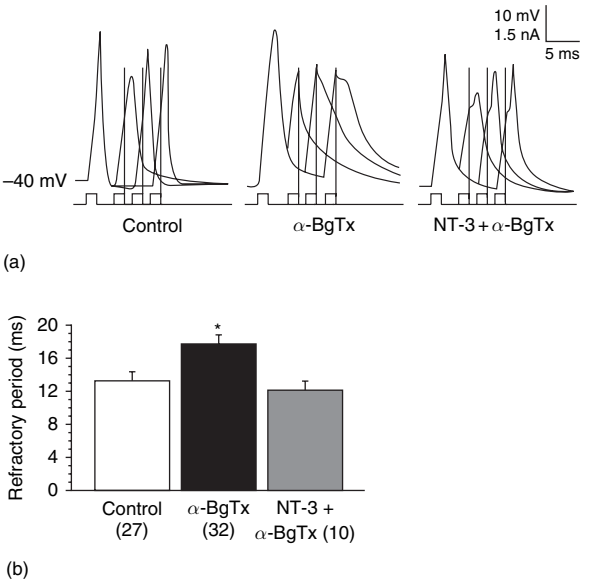

Figure 4 Synaptic activity regulates presynaptic excitablility. Muscle activation leads to release of retrograde factors that modulate excitability of the presynaptic neurons. These effects are mediated by the neurotrophin, NT-3. a, The refractory period was measured using a two-pulse protocol with a longer interpulse interval for each successive trial. Three trials are superimposed for each condition. In the presence of α-BgTX, APs did not overshoot the stimulus artifact. b, Co-application of NT-3 with α-BgTX rescued the effects of the toxin. Reprinted by permission from Macmillan Publishers Ltd: *Nat. Neurosci.* (Nick, T. A. and Ribera, A. B. 2000. Synaptic activity modulates presynaptic excitability. *Nat. Neurosci.* 3, 142–149), copyright (2000).

1.13.6 Developmental Regulation of the AP

The results of several studies reveal three general developmental patterns of APs (Figure 5). The first two patterns, classes 1 and 2, have been studied more frequently and are relevant to the vast majority of neurons that continue to fire APs in the nervous system. These two patterns are distinguished on the basis of the ionic dependence of the impulse initially fired by a differentiating excitable cell (for review, see

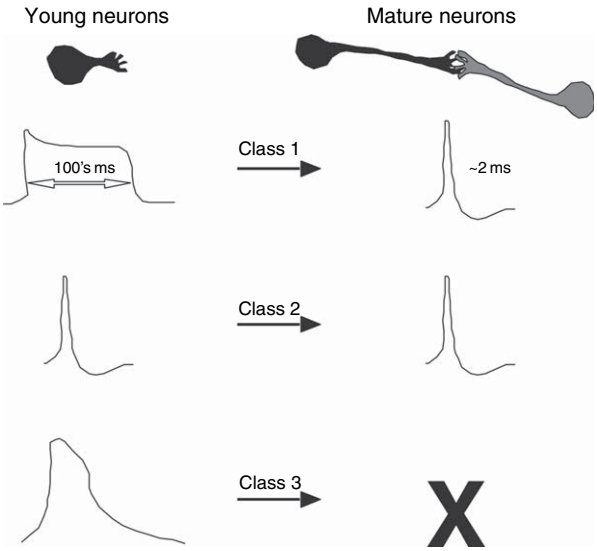

Figure 5 Patterns of AP development. Three patterns have been described. In the first, APs are of long duration when first present, but mature in time to the more stereotypic brief spikes, characteristic of mature neurons. The second pattern consists of APs that show little developmental regulation and are brief and sodium-dependent from the time of first appearance. The third pattern, characteristic of some sensory neurons, consists of APs at early stages followed by the loss of AP generation. At adult stages, neurons of the class 3 pattern respond with graded depolarization or hyperpolarizations to sensory stimuli.

Spitzer, 1991; Ribera and Spitzer, 1992; Spitzer *et al.*, 2000). We discuss these two patterns in more detail below.

The third developmental pattern consists of an early period of electrical activity followed by loss of AP generation. This pattern appears to be unique to sensory cells and consists of an early transient period of excitability followed by the generation of graded potentials in response to sensory stimuli (Beutner and Moser, 2001). Interestingly, the available evidence implicates the transiently appearing APs in proper development of sensory neurons (Beutner and Moser, 2001; Brandt *et al.*, 2003).

1.13.6.1 Class 1 Pattern of AP Development

The class 1 pattern of AP development consists of APs that are initially of long duration (5–500 ms; Spitzer and Lamborghini, 1976; Baccaglini and Spitzer, 1977). The early-appearing APs can be generated in the absence of extracellular sodium or in the presence of the sodium channel blocker tetrodotoxin and are eliminated by substitution of calcium in the extracellular media. Hence, these APs are described as calcium-dependent. In this respect, the class 1 pattern is similar to the third pattern mentioned above: the early APs that are transiently expressed by sensory neurons are calcium-dependent (Beutner and Moser, 2001).

As development proceeds, the AP duration becomes progressively briefer. In addition, the AP acquires sensitivity to blockers of voltage-gated sodium channels (e.g., tetrodotoxin) and insensitivity to calcium channel blockers (e.g., cobalt). The later-appearing APs are hence considered to be sodium-dependent. Examples of excitable cells displaying the class 1 pattern of development include amphibian primary spinal neurons, neurons of the rat dorsal nucleus of the vagus, chick motor neurons, ferret lateral geniculate neurons, and rat nucleus accumbens neurons (Spitzer, 1976; Spitzer and Lamborghini, 1976; Baccaglini and Spitzer, 1977; McCobb *et al.*, 1990; Ramoa and McCormick, 1994; Belleau and Warren, 2000; for review, see Spitzer, 1991; Moody, 1995).

1.13.6.2 Class 2 Pattern of AP Development

The class 2 pattern of AP development consists of APs that have brief durations from the time of their first appearance. The APs initially expressed are and remain sodium-dependent as differentiation proceeds. Further, the AP duration does not change significantly. Examples of cells displaying the class 2 pattern include chick ciliary ganglion neurons, quail mesencephalic neural crest cells, rat spinal and phrenic neurons, grasshopper interneurons, and amphibian myocytes (Goodman and Spitzer, 1981; Bader *et al.*, 1983, 1985; DeCino and Kidokoro, 1985; Henderson and Spitzer, 1986; Krieger and Sears, 1988; Ziskind-Conhaim, 1988a, 1988b).

1.13.6.3 General Principles

Even though several different types of ion channel underlie AP generation, the principal difference between the three patterns of AP development concerns developmental regulation of potassium current (Barish, 1986; Krieger and Sears, 1988; O'Dowd *et al.*, 1988; McCobb *et al.*, 1989; Nerbonne and Gurney, 1989; Ribera and Spitzer, 1989, 1990). The class 1 pattern reflects a program of ion channel regulation in which voltage-gated potassium channels are present at low density and have slow activation properties when APs are initially expressed and of long duration. The subsequent developmental shortening of the AP duration is due to a progressive increase in potassium channel density with concomitant changes in channel activation properties (Barish, 1986; O'Dowd *et al.*, 1988; Ribera and Spitzer, 1989; Lockery and Spitzer, 1992; Harris *et al.*, 1998).

Computer reconstructions of the APs recorded from *Xenopus* spinal neurons support the view that the delayed rectifier potassium current plays the predominant role during AP maturation in amphibian spinal neurons (Barish, 1986; Lockery and Spitzer, 1992).

Regardless of the developmental pattern, calcium and sodium currents appear early in neuronal differentiation (O'Dowd *et al.*, 1988; Alzheimer *et al.*, 1993; Albrieux *et al.*, 2004). Once present, calcium currents may increase in density but often remain stable (Barish, 1986; Gottmann *et al.*, 1988; McCobb *et al.*, 1989). In contrast, sodium currents typically increase in density and undergo kinetic changes (Huguenard *et al.*, 1988; O'Dowd *et al.*, 1988; McCobb *et al.*, 1990; Alzheimer *et al.*, 1993; Pineda *et al.*, 2005).

1.13.7 Myelination

In the nervous system, developmental regulation of ion channels is not unique to neurons. Glial cells also display developmentally regulated properties of excitability (Sontheimer *et al.*, 1992; Kressin *et al.*, 1995; Bordey and Sontheimer, 1997; Maric *et al.*, 1998; Bringmann *et al.*, 2000; Pannicke *et al.*, 2002; for review, see Waxman *et al.*, 1993). The distribution of specific sodium and potassium channel isoforms in myelinated axons provides one of the most interesting examples of developmental regulation of ion channels. Recent studies have revealed that interactions between axons and glia during development play key roles in sculpting the differential localization of VGICs in the axonal membrane.

1.13.7.1 Function

Glial cells wrap around axons and form several layers of membrane known as myelin (for review, see Sherman and Brophy, 2005). The identities of the glial cells that form myelin differ in the peripheral versus central nervous systems. In the central nervous system, oligodendrocytes form myelin. Schwann cells are the relevant glia for the peripheral nervous system.

Myelination of axons in both the peripheral and central nervous systems underlies the amazing ability of axons to propagate APs rapidly (for review, see Sherman and Brophy, 2005). Myelin provides insulation to the axon and current flow is restricted to nonmyelinated areas, known as nodes of Ranvier. Consequently, APs need not be conducted down the entire length of the axon but only to successive nodes of Ranvier, thereby accelerating AP conduction velocities. Further, the metabolic demands of transmitting APs over long distances are diminished because impulses are only generated at nodes and not throughout the entire length of the axon.

Morphological studies indicate that myelin is present in jawed vertebrates but not lamprey or hagfish (Bullock *et al.*, 1984). In addition, a few copepod crustacean species that display rapid escape behaviors necessary for life in predator-rich ocean waters also have myelin (Davis *et al.*, 1999). The copepods that display rapid behaviors and myelin-like sheaths are thought to have evolved later than other members of their species. Thus, myelin represents a relatively recent evolutionary adaptation that results in rapid behavioral responses.

1.13.7.2 Distribution of Ion Channels during Developmental Myelination

Toxin labeling studies indicated that axons have an overall low density of sodium channels, even though they can propagate APs (Waxman *et al.*, 1989). These studies raised the possibility that, in addition to myelin, the distributions of VGICs may be optimized for generation of APs at nodes of Ranvier. More recent immunocytochemical studies have demonstrated that sodium channels are maintained at high densities at nodes of Ranvier (for review, see Rasband and Trimmer, 2001; Figure 6). In contrast, specific potassium channel isotypes are maintained at high densities in nearby, non-nodal regions known as the juxtaparanodes. Moreover, the specific locations of the different ion channel types are specified during developmental myelination and require interactions between the axon and glia (Wu and Barish, 1994; Demerens *et al.*, 1996; Stevens *et al.*, 1998; Stevens and Fields, 2000).

In both the peripheral and central nervous systems, one sodium channel isoform, Nav1.2, appears early in unmyelinated zones (Westenbroek *et al.*, 1992; Gong *et al.*, 1999; Boiko *et al.*, 2001; for review, see Rasband and Trimmer, 2001). As nodes form, another isoform, Nav1.6, becomes the dominant sodium channel type (Caldwell *et al.*, 2000; Boiko *et al.*, 2001; for review, see Rasband and Trimmer, 2001).

Potassium channels also display stereotypic distributions in myelinated axons. Electrophysiological studies demonstrated that potassium current densities were not constant across the length of myelinated axons, suggesting nonhomogeneous, optimized distributions of potassium channels (Chiu and Ritchie, 1980; Chiu and Wilson, 1989; Roper and Schwarz, 1989). Immunocytochemical results have directly demonstrated the locations of specific potassium channel subtypes in myelinated axons. In myelinated axons, potassium channel

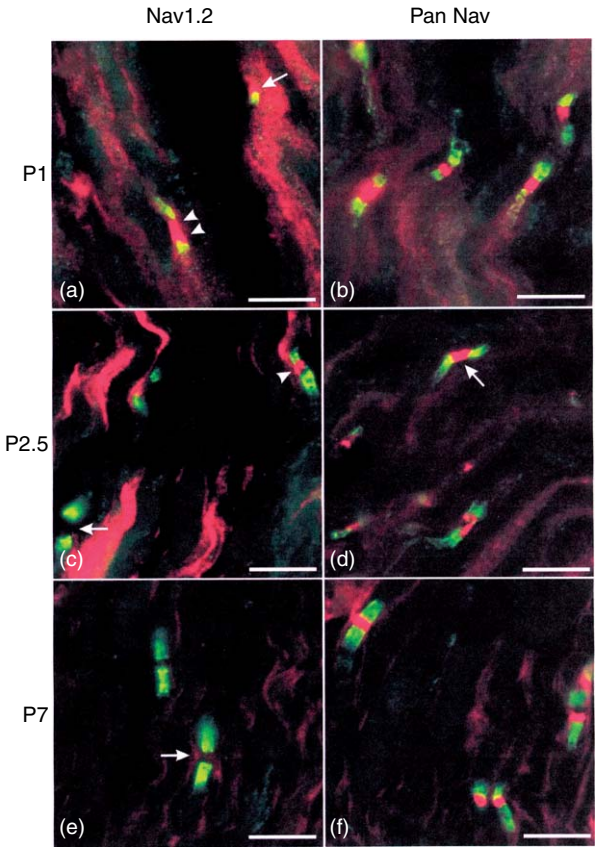

Figure 6 VGIC distributions in axons. At nodes of Ranvier, the identity of the sodium channel α-subunit changes during development in rat sciatic nerve. Panels (a), (c), and (e) present Nav1.2 immunoreactivity (red) data, whereas panels (b), (d), and (f) present pan-sodium channel immunoreactivity (red) data. Caspr immunoreactivity, a marker of the paranode, is shown in green. At all stages of development, nodes of Ranvier possess high densities of sodium channels (b, d, f). At early (a) but not late (e) stages, the predominant sodium channel isoform is Nav1.2. Scale bar: 10 μm. Reproduced from Rasband, M. N. and Trimmer, J. S. 2001. Developmental clustering of ion channels at and near the node of Ranvier. *Dev. Biol.* 236, 5–16, with permission from Elsevier.

densities are especially high in the juxtaparanodal regions (Wang *et al.*, 1993; Arroyo *et al.*, 1999; for review, see Rasband and Trimmer, 2001).

In sum, recent studies are revealing the molecular organization of nodal, paranodal, and juxtaparanodal regions of myelinated axons. It is clear that interactions between the axon and myelinating glia are required to form and maintain these regions and the concomitant distributions of ion channels. A better understanding of the underlying mechanisms will provide insights about how ion channel expression and distributions are regulated during development. Moreover, the information could potentially be useful for intervention and treatment of conditions associated with demyelination or axon regeneration.

1.13.8 Mechanisms of Developmental Regulation

Even though developmental changes in potassium currents typically drive maturation of the AP, most VGICs show developmental regulation. Analyses of mRNA and protein indicate that regulation of ion channel expression occurs across both temporal and spatial domains (Table 1). Electrophysiological analyses further indicate that the properties of voltage-gated currents expressed by a given cell are not constant over time but change during differentiation and in response to activity (Table 2).

Developmental regulation of channel function could occur at transcriptional, post-transcriptional, translational, and post-translational levels. These levels of control are not mutually exclusive and several could be operating simultaneously (Giraud *et al.*, 1998; Blaine *et al.*, 2004).

In some cases, developmental regulation of ion currents occurs in the absence of interactions with other cells or factors (Henderson and Spitzer, 1986). Additionally, cell–cell interactions, often apparent at the onset of synaptogenesis, play important roles (Okado and Takahashi, 1990a, 1990b; Okamura *et al.*, 1994; Subramony *et al.*, 1996; Bahls *et al.*, 1998; Nick and Ribera, 2000; Martin-Caraballo and Dryer, 2002a). Growth factors and neurotrophins often mediate the effects of activity or cell–cell interactions (Subramony *et al.*, 1996; Rothe *et al.*, 1999; Martin-Caraballo and Dryer, 2002a; for review, see Dryer *et al.*, 2003).

Further, extrinsic cues affect functional expression of VGICs differently depending upon the neuron type. For example, growth factors regulate expression of currents post-translationally in ciliary ganglion neurons but their effects require new protein synthesis in lumbar motor neurons (Subramony *et al.*, 1996; Martin-Caraballo and Dryer, 2002a). Thus, both intrinsic and extrinsic cues operate to regulate ion currents during development (for review, see Dryer, 1998; Ribera, 1998).

In Table 1, we review the molecular bases of developmental regulation of ion channel expression and function in the developing nervous system.

1.13.8.1 Transcriptional Regulation

Numerous stimuli, such as injury, electrical activity, growth factors, and development, modulate VGIC gene transcription (Beckh *et al.*, 1989; Ribera and Nguyen, 1993; Toledo-Aral *et al.*, 1995; Burger and Ribera, 1996; Gurantz *et al.*, 1996; Villeneuve *et al.*, 2000; Vega *et al.*, 2003; for review, see Levitan and Takimoto, 1998; Sashihara *et al.*, 1998; Black and Grabowski, 2003). Recent

Table 1 Developmentally and spatially specific expression of ion channels

VGIC type	*Molecular identity*	*Regulation*	*Neuron type*	*References*
Sodium	Nav1.3	Expression of Nav1.3 was linked to acquisition of electrical excitability.	Murine preplate and Cajal–Retzius cells (E12–13)	Albrieux *et al.* (2004)
Sodium and potassium	Nav1.1, Nav1.2, Nav1.3, Nav1.6; Kv1.3, Kv1.4, Kv1.6; Kv2.1; Kv3.1, Kv3.3; Kv4.2, Kv4.3	RT-PCR analyses indicated that full-length variants of Nav1.1 and Nav1.3 were expressed. In contrast, variants of Nav1.2 and Nav1.6 coding for truncated subunits were expressed. Several potassium channel subunits were already expressed at these early stages of differentiation.	Rat spinal motor neurons *in vitro*	Alessandri-Haber *et al.* (2002)
Sodium	Nav1.1, Nav1.2, Nav1.3	*In situ* hybridization studies showed that: (1) Nav1.1 was predominant at late postnatal stages; (2) Nav1.2 was expressed at all stages studied with regional variability; and (3) Nav1.3 was predominantly expressed at fetal and early postnatal stages.	Rat CNS, E10–P90	Beckh *et al.* (1989)
Potassium	Kv 1.1, Kv2.1, Kv2.2	*In situ* hybridization studies revealed Kv2.2 and Kv1.1 mRNA in ventral and dorsal spinal cord, respectively. Both Kv2 genes were detected in RNA of developing embryos by RNAase protection assays.	*Xenopus* spinal cord, 1–3 days	Burger and Ribera (1996)
Potassium	Kvβ1	*In situ* hybridization studies revealed developmentally and spatially regulated expression of the auxiliary subunit.	Mouse brain, E16–P7	Butler *et al.* (1998)
Potassium	Kvβ1, Kvβ2, Kvβ3	Kvβ1 expression was high at birth in all brain regions examined and decreased with age. Kvβ2 expression was low at birth and increased with age, reaching adult levels by the third postnatal week.	Mouse CNS, spinal cord and dorsal root ganglia, E16–adult	Downen *et al.* (1999)
Calcium	Calcium channel isotypes A, B, and E	Expression of Ca^{2+} channel transcripts are developmentally regulated *in vitro* and can be influenced differentially by transmembrane signaling via chronic depolarization and Ca^{2+} entry.	Rat cerebellar cortex	Falk *et al.* (1999)
Potassium	Kv3.1	Kv3.1 transcripts were upregulated *in vivo* and *in vitro* during the period of maturation of I_{Kv}.	*Xenopus* spinal neurons, 1–3 days	Gurantz *et al.* (1996)
Potassium	xKvβ2, xKvβ4	mRNA of both subunits is expressed during the period of impulse maturation in different neuronal populations.	*Xenopus* spinal neurons, 1–3 days	Lazaroff *et al.* (1999)
Sodium	TuNa I and TuNa II	Regional specific expression. Gene transcription is dependent on specific cellular contacts.	Ascidian embryo and neural cells (*Halocynthia roretzi*)	Okamura *et al.* (1997)
Calcium	N and L type	Immunocytochemical and physiological analyses demonstrated developmentally regulated expression of calcium channel isotypes.	Rat hippocampal neurons, *in vitro*	Pravettoni *et al.* (2000)
Potassium	XSha2 (Kv1.2)	RNAase protection assays demonstrated that Kv1.2 expression is neural-specific. Further, Kv1.2 transcripts were first detected at the time of neural induction.	*Xenopus* spinal neurons *in vivo* and *in vitro*	Ribera (1990)

(Continued)

Table 1 (Continued)

VGIC type	*Molecular identity*	*Regulation*	*Neuron type*	*References*
Potassium	XSha1	mRNA detection in neural crest derivatives and in both CNS and PNS glia.	*Xenopus* embryo, 1–3 days	Ribera and Nguyen (1993)
Sodium	Navβ3	*In situ* hyridization studies revealed developmentally regulated expression of Navβ3.	Rat CNS, E10–P14 and adult	Shah *et al.* (2001)
Potassium, sodium	Kv1.1, Kv1.2, Kvβ2; Pan Nav1	Immunocytochemical analyses revealed progressive clustering of Kv subunits in axonal juxtaparanodes during developmental myelination. Nav1 subunits were in nodes of Ranvier.	Rat sciatic nerve, P3–21	Vabnick *et al.* (1996)
Calcium	P/Q, N, and R	Immunocytochemical analyses revealed cell-specific patterns of expression for calcium channel isotypes A, B, C, D, and E.	Adult rat spinal motor neurons, interneurons, and nerve terminals	Westenbroek *et al.* (1998)

RT-PCR, reverse transcriptase polymerase chain reaction; CNS, central nervous system; PNS, peripheral nervous system.

Table 2 Development and activity regulate VGIC properties

VGIC type	*VGIC molecular identity*	*Regulation*	*Neuron type*	*References*
Sodium	Nav1.3	Expression of Nav1.3 was linked to acquisition of electrical excitability.	Murine preplate and Cajal–Retzius cells (E12–13)	Albrieux *et al.* (2004)
Sodium	Persistent current	Persistent current density was upregulated during development.	Rat sensorimotor cortex, P2–21	Alzheimer *et al.* (1993)
Calcium, sodium, and potassium	ND	Potassium and sodium currents were detected at early stages. Calcium currents developed later. All were subsequently upregulated.	Quail embryonic mesencephalic neural crest cells *in vitro*	Bader *et al.* (1983)
Calcium, sodium, and potassium	ND	At the time of neurite appearance, functional Na^+, Ca^{2+}, and voltage-gated K^+ channels were present. However, I_{Kv} amplitude increased during neural development. Changes in kinetic parameters were observed. I_{Na} and I_{Ca} amplitudes were also increasing to a lesser extent during differentiation.	Amblystoma spinal neurons	Barish (1986)
Hyperpolarization-activated	HCN1, HCN2, HCN4	Febrile seizures differentially altered expression patterns of several HCN channel genes and proteins.	Rat hippocampus, E10–11	Brewster *et al.* (2002)
Potassium	Large-conductance K channels	Electrical recordings demonstrated developmental changes in calcium and voltage sensitivities.	Rat embryonic rat telencephalon cortical slices, E12–14 and E21	Bulan *et al.* (1994)
Potassium	A-type	Spontaneous electrical activity, but not target tissues, regulated the normal developmental increase in potassium current density.	Chick lumbar motoneurons, E6 and E11	Casavant *et al.* (2004)
Potassium	Large-conductance Ca-activated K channels	The developmental expression of functional K_{Ca} channels was regulated differentially in choroids versus ciliary cells.	Chick choroid and ciliary ganglion neurons, E9–13	Cameron and Dryer (2000)
Whole-cell conductance	ND	Development and hypergravity altered electrophysiological properties of hair cells.	Rat utricular hair cells, P0–8	Chabbert *et al.* (2003)

(Continued)

Table 2 (Continued)

VGIC type	*VGIC molecular identity*	*Regulation*	*Neuron type*	*References*
Calcium	LVA and HVA calcium channels	Large modifications in the expression of voltage-dependent calcium channels occurred during a developmental period associated with neuronal growth and the beginning of synaptogenesis.	Mouse vestibular ganglia, E14–17	Chambard *et al.* (1999)
Calcium and potassium	Calcium-dependent K current; inward rectifier; voltage-gated K current; Ca current	Spontaneous activity regulated functional expression of calcium-dependent K current. The effects were calcium-dependent and required *de novo* transcription.	Ascidian embryonic muscle	Dallman *et al.* (1998)
Potassium	Delayed rectifier	Calcium influx through voltage-dependent channels during early developmental stages regulated the differentiation of potassium current kinetics and modulated the ionic dependence of APs via a PKC-dependent pathway.	*Xenopus* embryonic spinal neurons *in vitro*	Desarmenien and Spitzer (1991)
Calcium	Calcium channel isotypes A, B, and E	Expression of Ca channel transcripts were developmentally regulated *in vitro* and modulated differentially by transmembrane signaling via chronic depolarization and calcium entry.	Rat embryonic cerebellar cortical neurons *in vitro*	Falk *et al.* (1999)
Calcium	LVA and HVA	Patterns of activity differentially regulated densities of LVA and HVA calcium currents.	Mouse dorsal root ganglion neurons *in vitro*	Li *et al.* (1996)
Calcium and potassium	Calcium-activated potassium current	A calcium-activated potassium current was not present in the cochlea of the chick embryo, although it is present at adult stages.	Chick cochlea, E14	Fuchs and Sokolowski (1990)
Several VGICs	Sodium, potassium, calcium, calcium-dependent potassium current	Developmental changes in APs waveforms and the onset of repetitive firing correlate with increase in the current density of existing VGICs.	Rat spinal motor neurons, E15–16 and P1–3	Gao and Ziskind-Conhaim (1998)
Potassium	Kv1 subfamily	Activity regulated the expression of some, but not all, Kv1 channel genes.	Mouse hippocampus *in vivo* and *in vitro*, E17, P2–6 and adult	Grosse *et al.* (2000)
Potassium	Delayed rectifier (single channels)	Single-channel recordings revealed three different potassium single-channel types. One type showed developmental changes in kinetic properties.	*Xenopus* spinal neurons *in vitro*	Harris *et al.* (1988)
Sodium	ND	Developmental changes in sodium current densities but not kinetic properties were observed.	Rat sensorimotor cortical neurons, E16–P50	Huguenard *et al.* (1988)
Potassium	BK (large-conductance) calcium-activated potassium (single-channel analysis)	Single channel analysis of large conductance calcium-activated potassium channels in neocortical pyramidal neurons indicated the presence of both fast- and slow-gating channels between P0 and P5. However, at later stages, only slow-gating channel was detected.	Neocortical pyramidal neurons, P1–28	Kang *et al.* (1996)
Sodium	Nav1.3, 1.8, and 1.9	Changes in neuronal activity altered the expression of sodium channel genes in a subtype-specific manner, via an NGF-independent mechanism.	Mice dorsal root ganglion neurons, E13.5 *in vitro*	Klein *et al.* (2003)

(Continued)

Table 2 (Continued)

VGIC type	*VGIC molecular identity*	*Regulation*	*Neuron type*	*References*
Potassium	ND	Developmental upregulation of potassium current occurred during acquisition of hearing.	Mouse hair cells	Kros *et al.* (1998)
Potassium	Kv1.5, Kv1.4, and Kv2.1	Membrane depolarization specifically inhibited Kv1.5 channel gene transcription.	Rat pituitary cell line	Levitan *et al.* (1995)
Potassium	Kv3.1	Elevated potassium induced increased Kv3.1 mRNA levels. The effect of the block was prevented by the addition of calcium channel blockers.	Rat inferior colliculus neurons, P3–30	Liu and Kaczmarek (1998)
Potassium	Kv1.1, Kv3.1	Perturbations of activity in the auditory system regulated the levels of Kv1.1 and K3.1 channel proteins in the nucleus magnocellularis. However, alterations in channel proteins did not linearly predict changes in current densities.	Chick auditory system neurons, P1–2 (electrophysiology) and P5–10 (immunocytochemistry)	Lu *et al.* (2004)
Sodium, calcium, and potassium	Sodium, calcium, and potassium	Developmental changes in voltage-gated currents led to the class 3 pattern of AP development. Initially, hair cells fired APs. However, at late stages, hair cells responded to stimulation with graded potentials.	Mouse cochlear inner hair cells, E14.5–P12	Marcotti and Kros (1999); Marcotti *et al.* (2003)
Potassium	Ca(v)1.3 and SK2	The appearance of the SK current coincided with their becoming responsive to acetylcholine. The transiently expressed SK channel was activated by Ca^{2+} influx through both $Ca_v1.3$ channels and nicotinic receptors.	Mouse inner and outer hair cells, E14.5–P18	Marcotti *et al.* (2004)
Potassium	ND	During early stages of neuronal differentiation, potentiometric dye studies revealed developmentally regulated changes in RMP.	Rat cortical cells, E11–22	Maric *et al.* (1998)
Potassium	Large-conductance Ca-activated K (BK); delayed rectifier; inactivating A-type	Two BK channel variants were described. One variant was preferentially expressed during embryonic development. In addition, there were quantitative changes in I_K expression, that underlied the overall increase in excitability of differentiating cells.	Embryonic rat telencephalic neuroepithelium, E12–21	Mienville and Barker (1996, 1997)
Potassium	rSlo and Kv3.1 transcripts; single-channel analysis	Molecular analyses revealed developmental upregulation of rSlo transcripts but uniform expression of Kv3.1. Upregulation required depolarization and calcium and occurred at the transcriptional level.	Rat cerebellum, E20 *in vitro* and P2–21 *in vivo*	Muller *et al.* (1998)
Calcium	ND	The density of HVA calcium current increases after cell death, during the period of synapse elimination.	Chick motor neurons	Mynlieff and Beam (1992)

(Continued)

Table 2 (Continued)

VGIC type	*VGIC molecular identity*	*Regulation*	*Neuron type*	*References*
Sodium and potassium	ND	Development of excitability in presynaptic motor neurons required synaptic activation of the postsynaptic muscle cells. The data suggested that muscle released a retrograde factor, perhaps NT-3.	*Xenopus* embryo spinal motor neurons *in vitro*	Nick and Ribera (2000)
Sodium, calcium, and potassium		During development, no changes in peak density or kinetics of I_{Ca} were noted. I_{Na} showed a twofold increase in its density with subtle changes in kinetics. In contrast, I_{Kv} increased threefold in density and displayed changes in kinetic properties.	*Xenopus* spinal neurons *in vitro*	O'Dowd *et al.* (1988)
Sodium	ND	During this early developmental period, most outer hair cells expressed sodium current, in contrast to the situation in the adult.	Rat outer hair cells, P0–11	Oliver *et al.* (1997)
Sodium and potassium	ND	During late embryonic and early postnatal periods, I_{Na} density was upregulated in cortical plate neurons. I_K density showed subtle changes. The AP acquired a larger amplitude, shorter duration, and more negative value of threshold.	Mouse cerebral cortex – intermediate zone and cortical plate, E14–P17	Picken-Bahrey and Moody (2003)
Sodium and potassium	Nav1.1 and Nav1.6	Sodium current was developmentally upregulated. Morpholino antisense knockdown indicated that Nav1.1 and channels were more prevalent at early and late stages, respec-tively. Action potentials acquired larger overshoots and briefer dura-tions as both sodium and potassium currents increased in density.	Zebra fish Rohon–Beard neurons, 16–48 h postfertilization	Ribera and Nüsslein-Volhard (1998); Pineda *et al.* (2005)
Potassium	Delayed rectifier and A-type currents	A critical period for the maturation of the delayed rectifier current required mRNA synthesis during a 9 h critical period. After transcriptional blockade, A-current development recovered but delayed rectified current did not.	*Xenopus* spinal neurons, *in vitro*	Ribera and Spitzer (1989)
Potassium	A-current	Maturation of A-current extended to times later than that for other voltage-dependent currents.	*Xenopus* spinal neurons, *in vitro*	Ribera and Spitzer (1990)
Potassium	ND	Extensive upregulation of I_K current density occurred with changes in activation kinetics.	*Xenopus* embryonic myocytes, *in vitro*	Ribera and Spitzer (1991)
Sodium	ND	Changes in sodium channel mRNA and protein levels were followed, beginning during embryonic development. Although there were dramatic increases in sodium channel mRNA levels postnatally, the increase did not fully account for protein levels, suggesting other levels of regulation (translational or post-translational). Increased gene transcription and channel mRNA. Kinetic analysis suggests a requirement for a developmentally regulated translational or post-translational step in brain sodium channel expression.	Rat forebrain, E16–adult	Scheinman *et al.* (1989)

(Continued)

Table 2 (Continued)

VGIC type	*VGIC molecular identity*	*Regulation*	*Neuron type*	*References*
Sodium	ND	During development, I_{Na} increased in density and displayed kinetic changes. These developmental changes were largely restricted to the period of axon ingrowth (E30–38).	Cat retinal ganglion cells, E30–E55	Skaliora *et al.* (1993)
Calcium	LVA and HVA	The density of LVA Ca^{2+} currents decreased during early stages of postnatal development, while the density of HVA increased.	Rat visual cortical neurons, P2–12	Tarasenko *et al.* (1998)

HVA, high-voltage-activated; LVA, low-voltage-activated; ND, not determined; E, embryonic; P, postnatal.

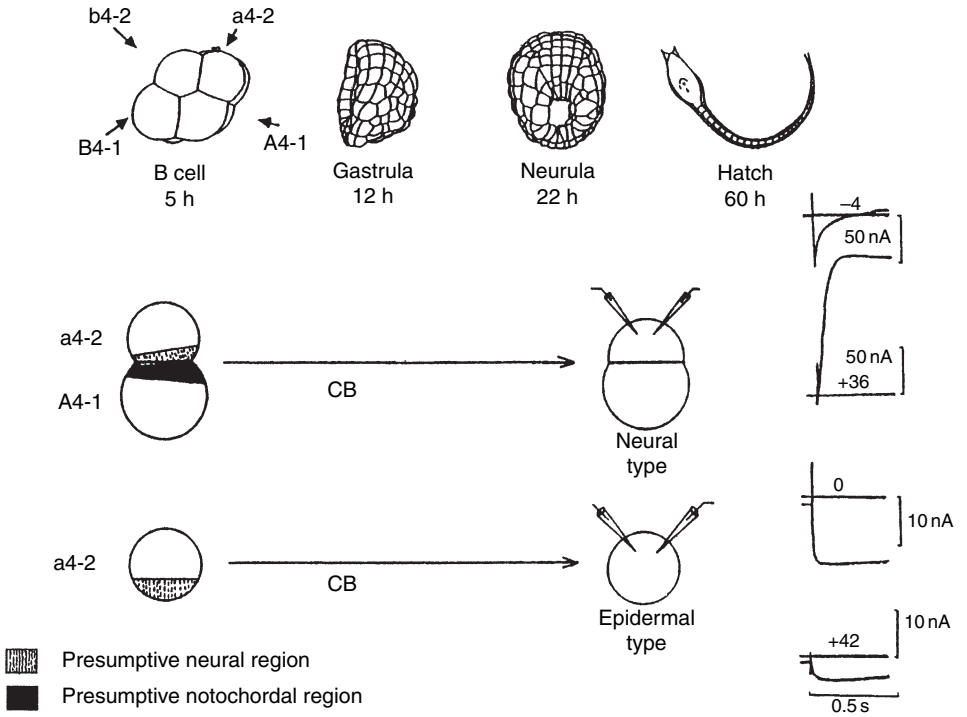

Figure 7 Neural induction activates VGIC gene transcription. Cell–cell interactions between appropriate blastomeres of the tunicate *H. auratum* induce expression of voltage-gated sodium current. When anterior animal blastomeres (a4-2) are cultured with anterior vegetal blastomeres (A4-1) as a two-cell system, the a4-2 blastomere develops normally and expresses sodium channels and tetraethylammonium (TEA)-sensitive delay-rectifier potassium channels. In contrast, when a4-2 blastomeres are cultured in isolation, each cell autonomously develops long-duration calcium-dependent APs. Reproduced from Takahashi, K. and Okamura, Y. 1998. Ion channels and early development of neural cells. *Physiol. Rev.* 78, 307–337, used with permission from The American Physiological Society.

studies are providing insights into how these stimuli are transduced into signals that regulate VGIC gene transcription (Mori *et al.*, 1993; Dolmetsch *et al.*, 2001; Tao *et al.*, 2002; Chen *et al.*, 2003). Less is known about the promoter regions that control VGIC transcription.

A series of interesting studies using cleavage-arrested blastomeres of the tunicate *Halocynthia roretzi* indicated that neural induction leads to the appearance of voltage-gated sodium current in the cell that adopts a neural fate (Takahashi and Yoshii, 1981; Takahashi and Okamura, 1998; for review, see Okamura *et al.*, 1993; Figure 7). More recent studies have characterized the effects at a molecular level and demonstrated that neural induction leads to the expression of a specific voltage-gated sodium channel isotype, TuNa1 (Okamura *et al.*, 1997). Transcription of TuNa1 is activated during neural induction by interactions between specific blastomeres of the animal and vegetal poles during a critical period of time (Okado and Takahashi, 1988, 1990a, 1990b; Okamura *et al.*, 1994).

Inappropriate cell contacts lead to differentiation of alternate cell types and the expression of different ion channels (Okado and Takahashi, 1990b). Similarly, a specific potassium channel transcript begins expression at the time of neural induction in embryonic spinal neurons of the frog *Xenopus laevis* (Ribera, 1990).

While it is clear that cell–cell interactions during development can activate transcription of specific ion channel genes, the underlying mechanisms are poorly understood. Moreover, depending upon its identity, a neuron will express a specific repertoire of ion channels. It is possible to accelerate the expression of one type of channel by premature expression of another (Linsdell and Moody, 1994). Future work needs to address how transcription of the subset of ion channel genes expressed in any individual neuron is coordinated. For example, co-regulation of transient potassium and hyperpolarization-activated inward currents has been observed in lobster stomatogastric ganglion neurons (Maclean *et al.*, 2003).

In comparison to the vast number of ion channel genes that have been identified by molecular cloning, little is known about their DNA regulatory elements or the transcription factors that regulate transcription. The best-studied transcription factor for an ion channel gene is REST, or repressor element silencing transcription factor (also known as neuron-restrictive silencing factor or NRSF, Kraner *et al.*, 1992; Mori *et al.*, 1992; Schoenherr and Anderson, 1995a, 1995b). REST specifically controls expression of the voltage-gated sodium channel α-subunit Nav1.2 (Chong *et al.*, 1995; Schade and Brown, 2000; Dallman *et al.*, 2004). REST binds to RE-1, a DNA element found in the regulatory region of many neuronal vertebrate genes (Kraner *et al.*, 1992; Schoenherr and Anderson, 1995b). Most non-neuronal tissues express REST. Overexpression of REST recombinant protein in neuronal cells prevents Nav1.2 expression (Huang *et al.*, 1999; Nadeau and Lester, 2002). Conversely, expression of a dominant negative REST in non-neuronal cells results in Nav1.2 transcription (Chong *et al.*, 1995). Thus, REST functions in a negative pathway and suppresses expression of Nav1.2 in non-neuronal cells.

Future work also needs to address what role transcriptional mechanisms play in the developmental upregulation of ion channels. For example, *Xenopus* spinal neurons display a threefold increase in potassium current density that underlies the developmental shortening of the AP duration (O'Dowd *et al.*, 1988; Lockery and Spitzer, 1992). A critical period of RNA synthesis is required for maturation of I_{Kv} (Ribera and Spitzer, 1989; Figure 8). Transient inhibition of RNA synthesis during a 9 h period prevents the normal threefold increase in density of I_{Kv}, even when a 48 h recovery period is allowed. Conversely, increasing the levels of potassium channel RNAs (e.g., Kv1.1 or Kv2.2) leads to premature maturation of I_{Kv} (Jones and Ribera, 1994; Blaine *et al.*, 2004). These findings are consistent with the notion that the critical RNA synthesized during the 9 h period codes for a potassium channel subunit.

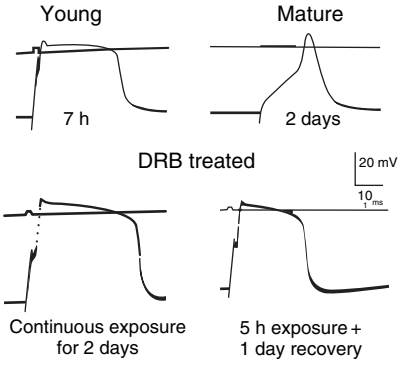

Figure 8 AP development requires a critical period of transcription. Blockade of transcription with an inhibitor of the RNA synthesis (DRB, 5,6-dichlorobenzimidazole 1-β-D-ribofuranoside) during a critical period of development prevents maturation of the AP and a voltage-gated potassium current. Reproduced from Ribera, A. B. and Spitzer, N. C. 1989. A critical period of transcription required for differentiation of the action potential of spinal neurons. *Neuron* 2, 1055–1062, with permission from Elsevier.

1.13.8.2 Post-Transcriptional

Post-transcriptional mechanisms, such as alternative splicing, editing, mRNA stability, and localization, influence ion channel function. Neural activity can activate post-transcriptional effects, as is the case for stabilization of transcripts coding for specific calcium channel isoforms (Schorge *et al.*, 1999). Moreover, recent studies indicate that post-transcriptional control of ion channel function occurs throughout development of the nervous system.

1.13.8.2.1 Alternative splicing Alternative splicing influences translational efficiency as well as protein stability, transport, and localization (Black, 2003; Stamm *et al.*, 2005). Alternative exon usage constitutes a major mechanism to increase functional diversity of VGICs. Almost all aspects of VGIC function can be affected by alternative splicing: channel activation and inactivation, gating, kinetic properties, and sensitivity to blockers and

modulators (Iverson *et al.*, 1997; Chemin *et al.*, 2001; Decher *et al.*, 2001; Tian *et al.*, 2001a, 2001b). The effects range from subtle ones to complete loss of function.

Alternative exon usage can change dynamically in response to diverse stimuli, including growth factors, pH, and neural activity. Of particular interest, development influences alternative exon usage (Kaufer *et al.*, 1998; Oh and Waxman, 1998; Stamm *et al.*, 2005). For example, multiple sites for alternative splicing usage have been identified in voltage-gated potassium and sodium channels. In *Drosophila*, the major class of sodium channel is encoded by a single gene known as *para*. Alternative splicing of *para* generates several different channel isoforms. The isoforms display temporally and spatially distinct expression patterns (Thackeray and Ganetzky, 1994; Lee *et al.*, 2002). Alternative splicing also regulates sodium channels in the cockroach and results in mRNA variants coding for proteins with different activation, inactivation, and gating characteristics (Song *et al.*, 2004). Further, several of the isoforms display tissue-specific distribution and developmental specificity. Similar results have been obtained by the study of *Drosophila* genes coding for voltage-gated (i.e., *Shaker*) or calcium-dependent (i.e., *Slo*) potassium channels (Atkinson *et al.*, 1991; Adelman *et al.*, 1992; Butler *et al.*, 1993).

Ion channel gene families that are represented by a single gene in invertebrates often have undergone duplications during evolution and exist as multigene families in vertebrates (i.e., *Shaker* gene vs. Kv1 gene family; *para* gene vs. Nav1 gene family). Despite the increased functional diversity created by gene duplication, alternative splicing still operates to create molecularly diverse channel transcripts. For example, the mammalian sodium channel α-subunit genes, Nav1.1, Nav1.2, Nav1.3, Nav1.6, and Nav1.9, are alternatively spliced (Sarao *et al.*, 1991; Gustafson *et al.*, 1993; Plummer *et al.*, 1997; Lu and Brown, 1998; Jeong *et al.*, 2000). Moreover, expression of specific splice variants is developmentally regulated. In *Xenopus*, alternative spicing of the *slo* (xSlo) gene generates variants that differ in their tissue and developmental expression patterns (Kukuljan *et al.*, 2003; Figure 9). Additionally, the variants code for channels that differ with respect to both voltage and calcium sensitivities.

Nav1.1, Nav1.2, and Nav1.6 genes display developmentally regulated patterns of alternative splicing (Sarao *et al.*, 1991; Plummer *et al.*, 1997; Alessandri-Haber *et al.*, 2002). Interestingly, the coding regions of neonatal variants of Nav1.2 and Nav1.6 genes predict truncated proteins. Whether or not the predicted truncated proteins are expressed and what their potential roles are remain unknown. However, the existence of these splice variants that are developmentally regulated raises the interesting possibility of negative-feedback mechanisms implemented by alternative splicing. Developmentally regulated variants of calcium channels that code for truncated ion channel proteins have also been observed (Okagaki *et al.*, 2001; Figure 10).

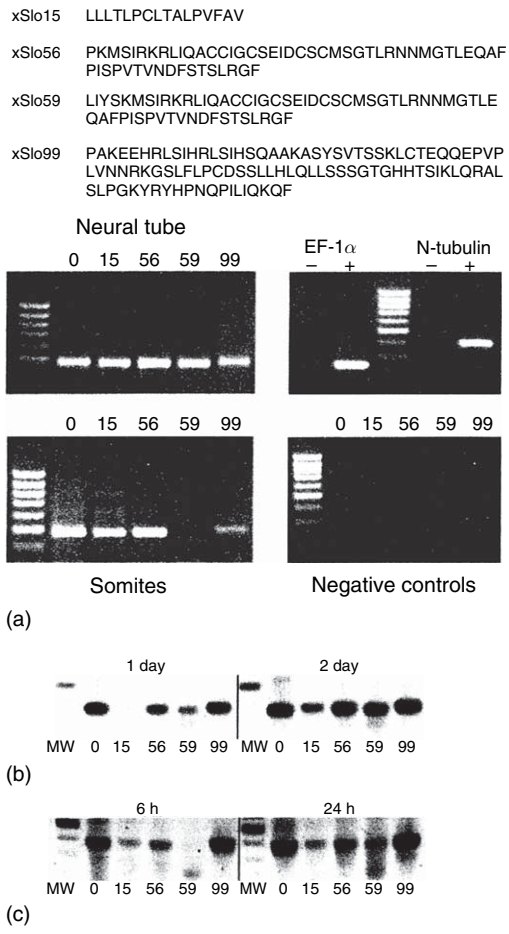

Figure 9 Development regulation of splicing of xSlo transcripts. The xSlo gene of *X. laevis* displays both tissue-specific and developmentally regulated splice variants. a, Four alternative splice variants – xSlo15, xSlo56, xSlo59, and xSlo99 – were detected by reverse transcriptase polymerase chain reaction (RT-PCR) analysis of RNA isolated from 2-day-old *Xenopus* embryos. Three of the alternatively spliced variants were expressed in both embryonic spinal cord and somites. One variant, xSLO59, was only detected in spinal cord. Constitutively expressed EF-1α and N-tubulin served as positive controls. Examination of 1- or 2-day-old embryo RNA indicated that the xSLO59 variant was developmentally upregulated (b) *in vivo* and (c) *in vitro*. Reproduced from Kukuljan, M., Taylor, A., Chouinard, H., Olguin, P., Rojas, C. V., and Ribera, A. B. 2003. Selective regulation of xSlo splice variants during *Xenopus* embryogenesis. *J. Neurophysiol.* 90, 3352–3360, used with permission from The American Physiological Society.

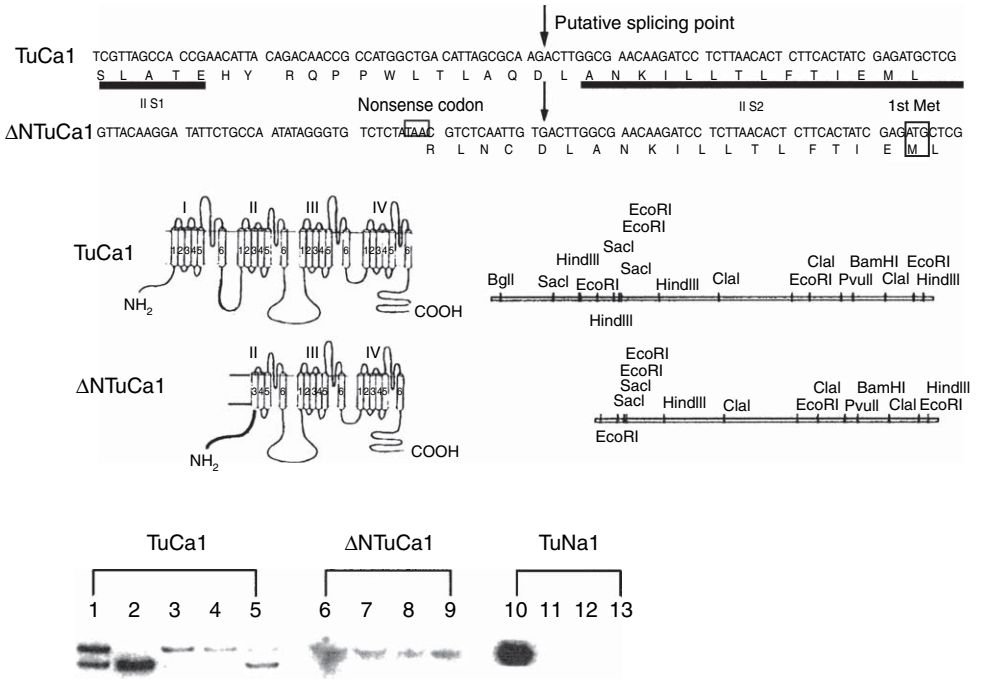

Figure 10 Alternative splicing generates transcripts for a voltage-gated calcium channel that code for truncated proteins. RT-PCR analyses indicate that the tunicate calcium channel gene expresses splice variants. One variant, TuCa1, codes for a full-length calcium channel α-subunit. In contrast, the other variant, ΔTuCa1, codes for a truncated subunit that lacks a substantial portion of the N-terminal region. Okagaki *et al.* (2001) demonstrate that the truncated subunit acts in a dominant-negative manner and inhibits function of the full-length protein. Lane 1 is a positive control with whole-tadpole RNA. RT-PCR analyses for a sodium channel gene TuNa1 are shown for comparison. Lanes 2, 6, and 10 present analyses done using neuronal RNA. Lanes 3, 7, and 11 present analyses done using RNA from epidermal (non-neuronal) cells. Lanes 4, 8, and 12 present analyses done using RNA from cells giving rise to motor neurons. Lanes 5, 9, and 11 present analyses done using muscle cell RNA. Reproduced from Okagaki, R., Izumi, H., Okada, T., Nagahora, H., Nakajo, K., and Okamura, Y. 2001. The maternal transcript for truncated voltage-dependent Ca^{2+} channels in the ascidian embryo: A potential suppressive role in Ca^{2+} channel expression. *Dev. Biol.* 230, 258–277, with permission from Elsevier.

1.13.8.2.2 Editing RNA editing leads to specific alterations in single nucleotides of mRNA transcripts (for review, see Simpson and Emerson, 1996). Selective mRNA editing of VGIC transcripts during development has been observed for sodium channels in invertebrates (Hanrahan *et al.*, 2000; Song *et al.*, 2004). The *Drosophila para* gene, in addition to being alternatively spliced, shows developmentally regulated patterns of RNA editing (Hanrahan *et al.*, 2000). In the German cockroach, editing of the sodium gene BgNav transcripts occurs in a developmentally specific manner (Song *et al.*, 2004). Furthermore, editing resulted in transcripts that coded for proteins that varied significantly in voltage-dependent activation and inactivation properties.

1.13.8.3 Translational/Post-Translational

Ion channels display many different types of post-translational modifications that are linked to specific functional consequences. Here, we summarize examples of developmentally regulated post-translational modifications. For many cases, however, the potential physiological roles of post-translational modifications during development are poorly understood (but see Misonou *et al.*, 2004). Post-translation mechanisms might be involved in setting current density levels in mature neurons (Blaine *et al.*, 2004; Figure 11).

1.13.8.3.1 Surface membrane insertion One of the best-studied examples of post-translational control of excitability concerns regulation of large-conductance calcium-activated potassium (K_{Ca}) channels of chick ciliary ganglion neurons (for review, see Dryer, 1998; Dryer *et al.*, 2003). Normal developmental upregulation of K_{Ca} channels requires interactions with both targets (iris) and inputs (afferent innervation provided by the Edinger–Westphal nucleus).

Transforming growth factor-β1 (TGF-β1) and β-neuregulin-1 mediate the effects of cell–cell interactions with targets and inputs, respectively, on K_{Ca} channels both *in vitro* and *in vivo* (Subramony *et al.*, 1996; Cameron *et al.*, 1998, 2001).

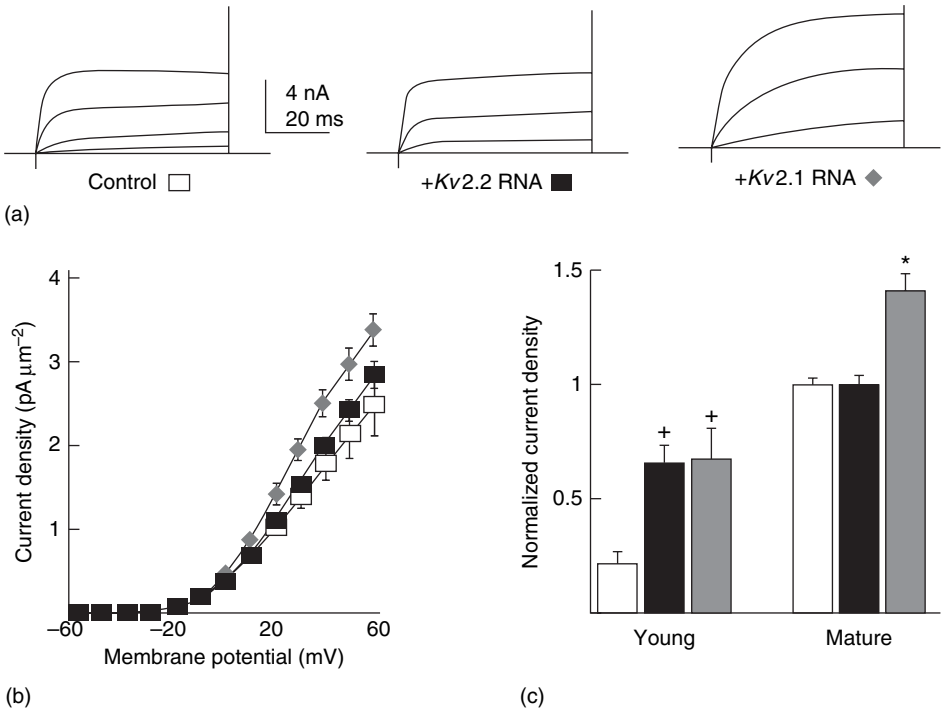

Figure 11 Post-translational control of potassium current density. a, Potassium current density of mature neurons can be increased by excess Kv2.1 mRNA but not by excess RNA from another Kv2 isoform, Kv2.2. b, The mean I_{Kv} densities of +Kv2.1 but not +Kv2.2 neurons were significantly larger than those of control neurons. c, Overexpression of Kv2.2 RNA increased current density in young but not in mature neurons. In contrast, overexpression of Kv2.1 RNA increased current density in both young and mature neurons. Blaine *et al.* (2004) demonstrated that regulation of potassium channel density induced by excess Kv2.2 RNA required a proximal region of the carboxyl terminal, known as *proxC*. These studies indicate that post-translational mechanisms can regulate VGIC current density in neurons. Reproduced from Blaine, J. T., Taylor, A. D., and Ribera, A. B. 2004. Carboxyl tail region of the Kv2.2 subunit mediates novel developmental regulation of channel density. *J. Neurophysiol.* 92, 3446–3454, used with permission from The American Physiological Society.

Importantly, the effects of TGF-β1 and β-neuregulin-1 persist in the presence of protein synthesis inhibitors (Subramony *et al.*, 1996). These studies indicate that new protein synthesis is not required for the developmental upregulation of K_{Ca} channel expression mediated by TGF-β1 and β-neuregulin-1 in chick ciliary neurons. Interestingly, the effects of extrinsic factors on developmental regulation of current in chick lumbar motor neurons do require new protein synthesis (Martin-Caraballo and Dryer, 2002a, 2002b).

More recent studies suggest that the effects of TGF-β1 on ciliary ganglion neurons lead to surface membrane insertion of presynthesized K_{Ca} channels (Lhuillier and Dryer, 2002). This finding is especially significant because large intracellular pools of several different types of VGICs have been observed. Thus, for several different types of ion channels, post-translational control of surface membrane insertion would be an effective and possible way to regulate functional expression of current (for review, see Misonou and Trimmer, 2004).

Conversely, post-translational regulation of ion channels in the surface membrane could result in their removal. For example, activation of sodium channels and sodium influx leads to removal of these channels from the surface membrane (Dargent and Couraud, 1990; Dargent *et al.*, 1994). This type of regulation might function after circuit formation as a homeostatic mechanism to keep channel densities and electrical activity in an optimal range (for review, see Turrigiano and Nelson, 2004).

1.13.8.3.2 Glycosylation In the adult nervous system, glycosylation promotes proper protein folding, function, stability, intracellular sorting, and membrane targeting (Bar-Sagi and Prives, 1983; West, 1986; Marban *et al.*, 1998; Tyrrell *et al.*, 2001). For some plasma membrane sodium channels, removal of glycosylation leads to changes in the voltage dependence of gating (Recio-Pinto *et al.*, 1990; Bennett *et al.*, 1997; Zhang *et al.*, 2003). Notably, depolarizing shifts in the steady-state activation and inactivation curves are produced (Recio-Pinto *et al.*,

1990; Bennett *et al.*, 1997; Zhang *et al.*, 2003). Of particular interest is the finding that the glycosylation levels of voltage-gated sodium channels are developmentally regulated and linked to modulation of voltage-dependent properties (Tyrrell *et al.*, 2001). Further, glycosylation of sodium channels has been linked to developmentally regulated changes in single-channel conductance and steady-state activation (Castillo *et al.*, 1997, 2003). In comparison to sodium channels, much less is known about developmental regulation of glycosylation for other VGICs.

1.13.8.3.3 Phosphorylation Protein phosphorylation and dephosphorylation underlie modulation of the activity of ion channels and modulate neuronal excitability (for review, see Levitan, 1999). VGIC α-subunits are common substrates for phosphorylation mediated by the major protein kinases, including cyclic adenosine monophosphate-dependent kinase (PKA), protein kinase C (PKC), calcium calmodulin kinase II (CAM kinase II), and tyrosine kinase. Moreover, electrophysiological studies indicate that key properties of VGIC can be modified by phosphorylation, at least in the mature nervous system (Brum *et al.*, 1983; Flockerzi *et al.*, 1983; Emerick and Agnew, 1989; Perozo and Bezanilla, 1990, 1991; Armstrong *et al.*, 1991; Hoger *et al.*, 1991; Murphy and Catterall, 1992; Covarrubias *et al.*, 1994; Cohen, 1996; Cohen *et al.*, 1996; Roeper *et al.*, 1997; Beck *et al.*, 1998). Comparatively little is known about the role of phosphorylation in developmental regulation of ion channel function. However, expression of kinases, such as CAM kinase II, is developmentally regulated (Hanson and Schulman, 1992; Menegon *et al.*, 2002). Further, this kinase participates in developmental regulation of synapse formation (Zou and Cline, 1996; Wu and Cline, 1998). These findings motivate further study of the potential role of phosphorylation in the developmental regulation of VGIC function.

1.13.8.3.4 Auxiliary subunits Auxiliary (also known as accessory) subunits form complexes with the pore-forming subunits of VGICs. Pore-forming subunits are typically designated as α whereas auxiliary ones are referred to by another Greek letter, such as β. Auxiliary subunits influence the kinetic properties and the voltage dependence of VGIC activation and inactivation (Isom *et al.*, 1992; Patton *et al.*, 1994; Rettig *et al.*, 1994; Morales *et al.*, 1995; Heinemann *et al.*, 1996) without major effects on ion conductance (for review, see Trimmer, 1998; Hanlon and Wallace, 2002). Further, auxiliary subunits have effects on the assembly and expression of VGICs (Patton *et al.*, 1994; Isom *et al.*, 1995a, 1995b; Qu *et al.*, 1995; Shi *et al.*, 1996; for review, see Isom *et al.*, 1994; Striessnig, 1999; Goldin, 2001). Because many of these properties are developmentally regulated, the role of auxiliary subunits during differentiation of excitability is of interest.

Spatial and developmental regulation of VGIC auxiliary subunit expression has been observed in several types of excitable tissue, including brain, muscle, and heart (Butler *et al.*, 1998; Downen *et al.*, 1999; Lazaroff *et al.*, 1999; Franco *et al.*, 2001; Falk *et al.*, 2003; Grande *et al.*, 2003). Very little evidence exists linking auxiliary subunits to developmental regulation of excitability (but see Falk *et al.*, 2003).

1.13.9 Future Studies

Electrophysiological analyses have indicated three general patterns of development of excitability in neurons. Each pattern is associated with specifc programs of development for underlying voltage-gated currents. Because ion channel genes have been and are being cloned, analyses of mechanisms can be approached at the molecular level. Regulation of excitability is complex and involves control of multiple VGIC genes at multiple levels.

Important issues for future research will be to identify transcription factors and DNA regulatory regions involved in the development control of VGIC gene transcription. It is likely that many VGIC genes will be coordinately controlled. At the other end of the spectrum, recent research suggests that an important post-translational mechanism for control of VGIC density involves regulation of plasma membrane insertion. This mechanism is not unique to VGICs as it is also crucial for regulation of postsynaptic function following periods of activity (for review, see Malinow, 2003).

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1.14 Genetic Analysis of Neural and Non-Neural Co-Evolution

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Glossary

agnathan Jawless vertebrates (also cyclostomes); living agnathans are lampreys and hagfish.

amphioxus Common name for the cephalochordate Branchiostoma (also lancelets).

bilaterians Taxa encompassing both the protostomes and deuterostomes.

cell commitment Irreversible restriction in potential, such that a cell will differentiate autonomously when placed into another region of the embryo.

cephalochordates Chordate group that includes genus *Branchiostoma* (also amphioxus or lancelet).

chordates Deuterostomes with a notochord – urochordates, cephalochordates, and vertebrates.

clade A monophyletic set of taxa, all derived from a common ancestor.

competence The ability of a cell to respond to an inductive signal.

deuterostome Coelomates with two mouths – echinoderms, hemichordates, and chordates.

encephalization Expansion of the head anteriorly to form the brain, skull, and sense organs.

floorplate Specialized ventral midline cells of the vertebrate neural tube.

Gastroneuralia Bilaterians with a ventral nerve cord – annelids and arthropods.

gnathostomes Jawed vertebrates.

hemichordates Deuterostomes related to chordates – acorn worms and pterobranchs.

heterochrony Shift in relative timing of developmental processes from one generation to the next.

homeotic gene Also called *Hom* or *Hox*. Genes containing a homeobox sequence involved in DNA binding and anterior–posterior patterning in vertebrates and invertebrates.

hypophysis Pituitary, including an anterior adenohypophysis and a posterior neurohypophysis.

lateral line Sensory system of mechanoreceptors and/or electroreceptors in aquatic vertebrates.

marsupials Metatherian mammals that have pouches – opossums in North America, kangaroos.

monophyletic Group of taxa all derived from a common ancestor.

monorhiny Single midline nostril, as seen in agnathans.

neural crest Cells that arise at dorsolateral edges of the neural tube in vertebrate embryos, adjacent to surface ectoderm. They form sensory neurons, glia, pigment cells, and cranial skeleton among other things.

neural ectoderm Embryonic subdivision that forms neurons and glia of the central nervous system.

non-neural ectoderm Subdivision that forms epidermis and peripheral nervous system.

Notoneuralia	Bilaterians with a dorsal nerve cord, including chordates.
orthologue	Related gene within a gene family between one generation and the next.
outgroup	Closest sister group to a monophyletic clade of interest.
Pedomorphism	Retention of juvenile characters in the adult.
paralogue	Equivalent gene within a duplicated gene cluster in the same organism.
pharyngeal arches	Segmental series of arches in the chordate head – form the jaw and gills in vertebrates (also visceral arches, or branchial arches if referring only to gills).
placode	Focal thickening of ectoderm in the embryonic chordate head that forms special sense organs including the olfactory, lens, hypophyseal, otic, lateral line, and epibranchials.
proprioception	Sense of position of body parts derived from sensory receptors in muscle.
protostome	Coelomates with one mouth – annelids, arthropods.
rhombomeres	Segments along the anterior–posterior axis of the hindbrain (rhombencephalon).
segment	One of a series of repeating morphological units along the anterior–posterior body axis.
specification	Reversible restriction in cell potential, such that the cell will differentiate autonomously when placed into a neutral environment.
tunicate	Common name for a type of urochordate (also sea squirt).
urochordates	Chordate group that includes tunicates (also sea squirt).

1.14.1 Introduction

Formation of the nervous system begins early during embryonic development in cells of the ectoderm. Comparative embryology has revolutionized our understanding of ectodermal evolution. Both protostomes and deuterostomes have a similar basic plan for patterning along the dorsal–ventral (DV) and anterior–posterior (AP) axes, and similar mechanisms of neurogenesis (Arendt and Nubler-Jung, 1999; see Basic Nervous System Types: One or Many?). However, the nervous system has become specialized within each group of animals, such as encephalization in vertebrates, in which the head expands anteriorly to form a large brain and sense organs (see The Evolution of Encephalization). In all cases, coordinated patterning of the neural ectoderm (NE) and non-neural ectoderm (NNE) has been an important developmental constraint during evolution. In this article, I discuss the genetic basis for this constraint, and the evolution of ectodermal cell fates.

Two crucial innovations that distinguish vertebrates from their invertebrate relatives are derived from ectoderm – neural crest and epidermal placodes. These may be key to understanding vertebrate origins and the coordinated development of the brain, skull, and sense organs (Gans and Northcutt, 1983; Northcutt and Gans, 1983; Northcutt, 2005). Neural crest cells form at the dorsolateral edges of the neural tube in the embryo and migrate throughout the body to give rise to neurons and glia, pigment cells, and bones of the skull as well as many other derivatives (LeDouarin and Kalcheim, 1999). Epidermal placodes are thickenings of the surface ectoderm that also migrate in some cases to join the cranial neural crest and form neurons and glia of the peripheral nervous system (PNS). Other placodes contribute to paired sense organs, including the olfactory, lens, pituitary, otic, acoustic, and lateral line (Begbie and Graham, 2001; Bhattacharyya and Bronner-Fraser, 2004; Streit, 2004; Schlosser, 2005). The locations of both neural crest and placodes are highly conserved between species and closely tied to development of the central nervous system (CNS).

Genetic studies in vertebrates have revealed critical pathways that control patterning of the ectoderm. Broader comparative studies across chordates are also leading to new ideas about gene functions and their ancestral roles. Here I consider the roles of genes known to act across both the NE and NNE and to mediate interactions between them during embryogenesis. Gene duplications may have provided some of the raw material for major evolutionary transitions, which I illustrate using several families of transcription factors and their functions in ectoderm.

1.14.2 Defining the Issues

1.14.2.1 Ectodermal Derivatives

Classical embryology identified two major subdivisions of the ectoderm, NE and NNE, in both protostomes and deuterostomes (Figure 1a). NE forms the CNS, and NNE forms an epidermal epithelium that covers the external surface. This so-called non-neural portion of the ectoderm, however, also gives rise to most or all of the PNS. Bilateralian animals can be divided into two large groups based on the location of the NE: (1) Gastroneuralia, such as annelids and arthropods with a ventral nerve cord; and (2) Notoneuralia,

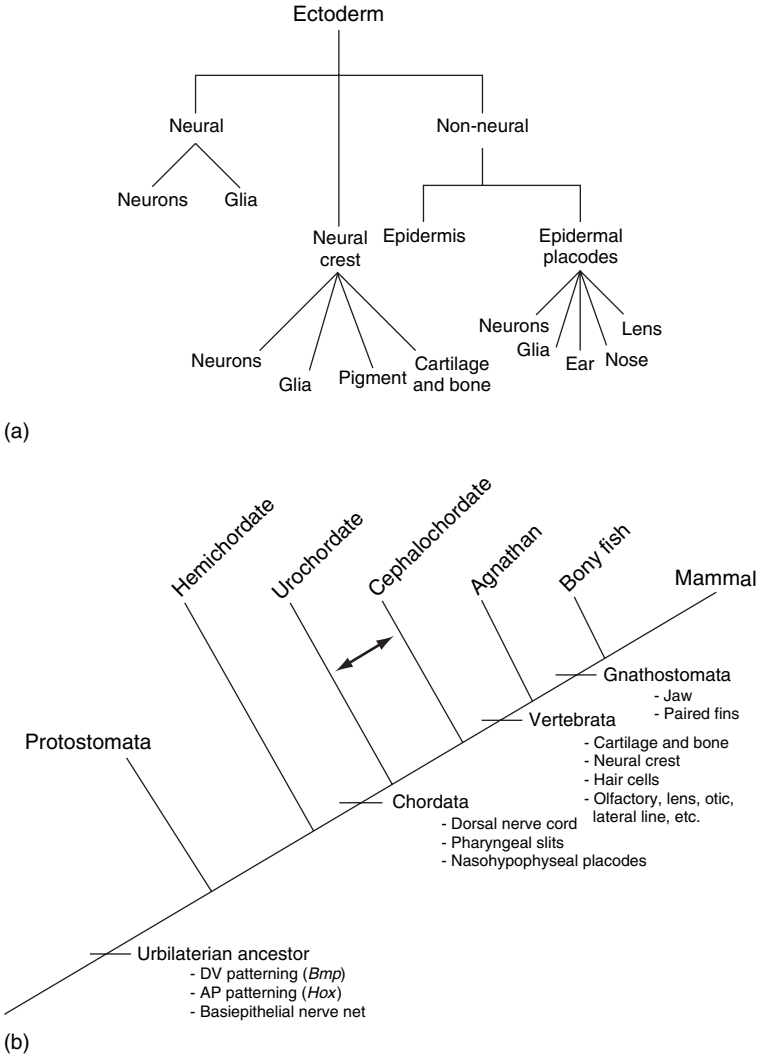

Figure 1 Ectodermal derivatives and their distribution among bilaterians. a, Some representative differentiated cell types of the ectoderm. Cell types are organized according to the ectodermal region from which they arise. b, Cladogram showing major branch points of groups that contain model developmental systems. Chordata comprises the Urochordata, one possible sister group to vertebrates, and the Cephalochordata, which is the other possible sister group. Both are characterized by the morphological features listed. Vertebrata includes the agnathans, sister group to the gnathostomes, characterized most notably by the lack of a jaw and paired appendages. Gnathostomata includes all of the jawed vertebrates.

such as chordates that have a dorsal cord (Hatschek, 1891; Nielsen, 1999). Despite this apparent inversion of the DV axis of the ectoderm, both share basic features of embryonic patterning that are under similar genetic control (Arendt and Nubler-Jung, 1999; Hirth *et al.*, 2003; see Origin and Evolution of the First Nervous System).

The NE in both groups initially consists of multipotent neural precursors that also form glial cells (Doe and Goodman, 1985; Temple and Qian, 1996). These arise in columns stretching the length of the NE and are further subdivided into distinct segments along the AP axis. In *Drosophila*, three bilateral columns of committed neural precursors flank midline glial cells that form the commissures connecting left and right sides (Figure 2e; Jimenez and Campos-Ortega, 1990; Skeath *et al.*, 1994). Similarly, the vertebrate NE thickens during gastrulation to form a neural plate in which neurons form in three longitudinal columns on either side of specialized ventral midline cells called floorplate (Figure 2f; Chitnis *et al.*, 1995; Haddon *et al.*, 1998). These also help guide commissure formation and create a hingepoint around which the neural plate rolls and fuses dorsally to form the neural tube (Schoenwolf and Alverez, 1989). In insects, the NE is more overtly segmented than in vertebrates. Neuroblasts coalesce to form large cephalic

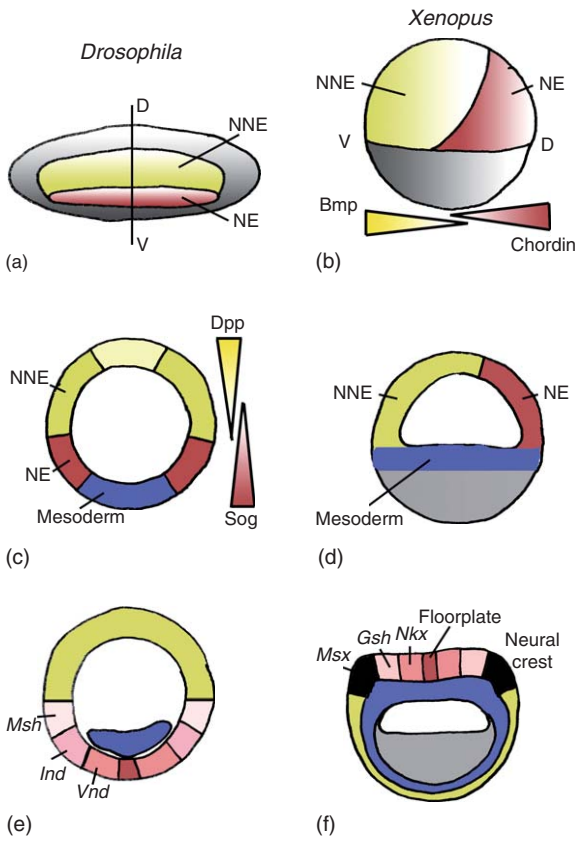

Figure 2 Conserved genetic control of DV patterning among bilaterians and specification of NE and NNE. a, Diagram illustrating a *Drosophila* embryo just prior to gastrulation in lateral view. NNE (yellow) lies dorsal to NE (red) on either side. b, *Xenopus* embryo prior to gastrulation in lateral view. NNE lies ventral to NE. Bone morphogenic proteins (bmps) are thought to form a gradient (triangle) from ventral to dorsal, which is opposed by a gradient of their inhibitor, Chordin. c, Transverse section of *Drosophila* pregastrula, showing DV organization of NNE, NE, and mesoderm and corresponding gradients of Dpp and Sog. d, Sagittal section of *Xenopus* pregastrula embryo. e and f, Transverse sections of *Drosophila* and *Xenopus* embryos after gastrulation. Domains of homeobox gene expression (Vnd, Ind, Msh) within the NE are indicated.

ganglia anteriorly and a ladder-like array of more posterior thoracic and abdominal segments. Segmentation of the vertebrate NE is most evident in the pattern of spinal motor and sensory nerves, as well as rhombomeres of the embryonic hindbrain (Figure 3; Lumsden and Keynes, 1989).

Similarities in the NNE between protostomes and deuterostomes are less clear (Figure 1a). In embryos of both groups NNE spreads to cover the body. However, vertebrate embryos also form neural crest cells at the NE/NNE boundary that are unlike any invertebrate ectodermal cell type (Horstadius, 1950; LeDouarin and Kalcheim, 1999). Neural crest cells have a broad range of possible fates, including cell types not found in invertebrates, such as cartilage and bone (Platt, 1893; Landacre, 1921; Stone, 1929). Neural crest cells also form a majority of the neurons and glia of the vertebrate PNS, unlike invertebrate sensory neurons, which develop within the epidermis (Teillet *et al.*, 1987; LeDouarin and Smith, 1988; Holland, 2005). Their origins from the dorsal neural tube and ability to form neurons suggests that neural crest cells constitute a subset of the NE, but in other respects they are more similar to NNE (Huang and Saint-Jeannet, 2004; Meulemans and Bronner-Fraser, 2004; Luo *et al.*, 2005). Thus, neural crest cells are uniquely poised to coordinate patterning between the CNS and PNS.

Epidermal placodes are also unique features of the vertebrate NNE that form near the NE/NNE boundary (Figure 1a). Placodes are thickenings within the surface ectoderm of the head that contribute to the special sense organs (Van Wijhe, 1883; Von Kupffer, 1891). Some placodes produce sensory neurons and glia within the PNS, similar to neural crest cells. Others form non-neural components of sense organs, such as surface cells of the lens, chemical-sensitive olfactory and taste receptors, motion-sensitive hair cells, and voltage-sensitive electroreceptors. Many of these sense organs connect to the CNS through sensory neurons of the PNS (cranial sensory ganglia) that are themselves descended from neural crest and placodes (Hamburger, 1961; D'Amico-Martel and Noden, 1983). Therefore, unlike invertebrates (Ghysen and Dambly Chaudiere, 1989), in vertebrates connectivity between special sense organs and their central targets in the brain are first established through interactions between NE, neural crest, and epidermal placodes during embryogenesis.

Genetic studies have identified several major steps in ectodermal development, including: (1) specification, in which cells acquire identities as NE or NNE; (2) inductive interactions, in which signals are passed between NE and NNE to coordinate patterning; and (3) circuit formation, when neurons within the CNS (derived from NE) and PNS (derived from NNE) connect to form a functional system. Each of these will be considered in the context of major transitions in ectodermal patterning and vertebrate head development.

1.14.2.2 Conserved Pathways and Evolutionary Transitions in Ectodermal Development

In some basal protostomes and deuterostomes neuronal cells are dispersed throughout the ectoderm in a basiepithelial nerve net, suggesting that this was the case for the common urbilaterian ancestor (Figure 1b). Support for this hypothesis in deuterostomes comes from the fact that echinoderms, a

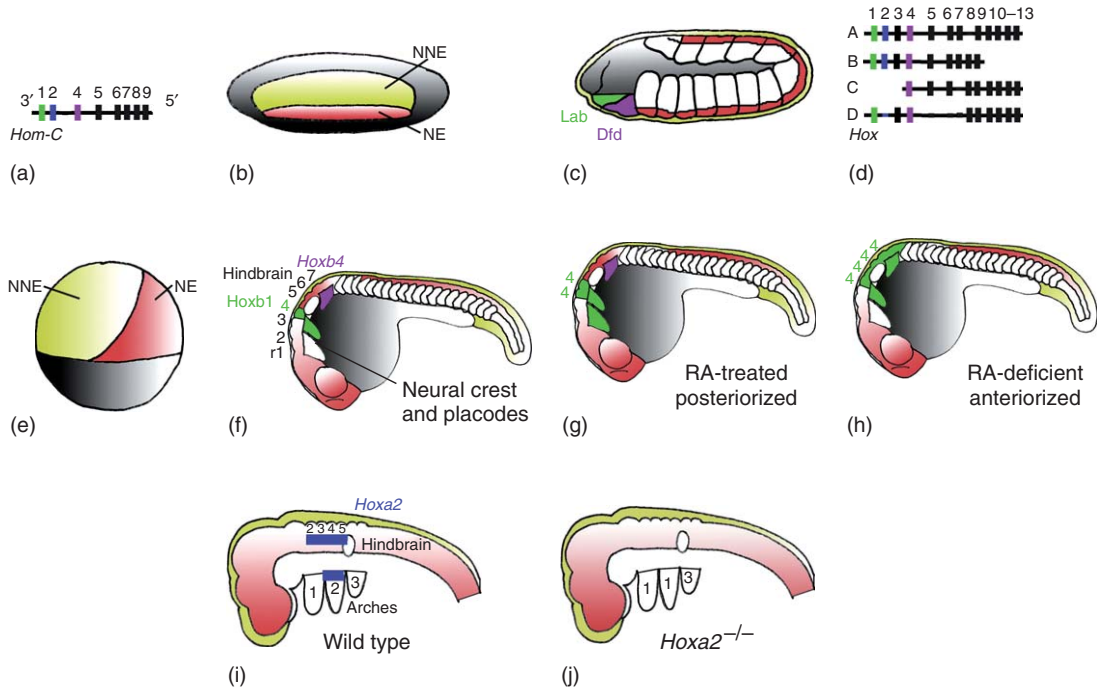

Figure 3 Conserved genetic control of AP patterning among bilaterians and coordinated patterning between NE and NNE. a, Eight genes of the single *Hom-C* cluster in *Drosophila* are numbered. b, Pregastrula fly embryo in lateral view, indicating NNE (yellow) and NE (red). c, Germ band extended-stage fly embryo in lateral view indicating body segments and expression domains of *Hox* group 1 (green) and 4 (purple) genes. d, Thirty-nine genes of the mammalian *Hox* clusters. e, Pregastrula fish/frog embryo. f, Wild-type fish embryo. *Hoxb1a* expression in rhombomere 4 and neural crest of pharyngeal arch 2. g, Fish or frog embryos soaked in RA show expansion of *Hox1* expression anteriorly, in both the NE and NNE. h, In RA-deficient fish, frogs, birds, or mice, *Hox1* expression expands posteriorly, in both the NE and NNE. i, Wild-type expression of *Hoxa2* in the anterior hindbrain (r2–5) and in the neural crest of pharyngeal arch 2. j, *Hoxa2* mutants exhibit a mirror-image duplication of arch 1 in place of arch 2.

chordate outgroup (i.e., their lineages branch at the base of the stem chordate lineage), and hemichordates have nerve nets (Brusca and Brusca, 1990; Adoutte *et al.*, 2000; Lowe *et al.*, 2003). In contrast, two basal chordates form a distinct neural plate. These are the ascidians or tunicates (Urochordata) and amphioxus (Cephalochordata), the closest living outgroups to the vertebrates (Garstang, 1894; Lacalli, 1994; Delsuc *et al.*, 2006). The ancestral chordate shared two major features of ectodermal patterning with its protostome relatives: (1) DV patterning involving transforming growth factor (TGF)-β signaling (Figure 2); and (2) AP patterning involving transcription factors related to the *Drosophila* homeotic (*Hom/Hox*) genes (Figure 3). Thus, the urbilaterian ancestor probably had both DV and AP coordinate systems in place, even if it did not develop distinct NE and NNE components.

1.14.2.2.1 Bmp signaling and DV inversion Dorsal expression of the TGF-β family member Decapentaplegic (Dpp) in *Drosophila* promotes NNE (Figures 2a and 2c). Vertebrate relatives of Dpp, the bone morphogenetic proteins (Bmps), also promote NNE but on the ventral side (Figure 2b), and this has revived the hypothesis that the DV axis is inverted in protostomes versus deuterostomes (Geoffroy-St. Hilaire, 1822; Arendt and Nubler-Jung, 1994; DeRobertis and Sasai, 1996). Ventral inhibition of Dpp in *Drosophila* is controlled by short gastrulation (Sog), which binds the Dpp ligand and prevents interactions with its receptor (Figure 2c). Similarly, in vertebrates Bmp4 is antagonized by inhibitors related to Sog, such as Chordin (Chd), expressed on the dorsal side (Figure 2b; Holley *et al.*, 1995). Vertebrate NE is induced by dorsal mesoderm (neural induction) through inhibition of Dpp/Bmp. Loss of inhibition leads to failure of neural induction and expansion of the NNE domain (Khokha *et al.*, 2005). Many other components of the Dpp/Bmp signal transduction pathway are also conserved in this process (DeRobertis and Kuroda, 2004).

Conservation in DV patterning extends further within the NE to patterning of neuronal fates along the medial–lateral (ML) axis (Figures 2e and 2f; see Basic Nervous System Types: One or Many?). Both *Drosophila* and vertebrates utilize the

transcription factors Vnd (Nkx-2), Ind (Gsh-1), and Msh (Msx) in distinct ML domains of the NE to specify the three longitudinal columns of early neuroblasts (McDonald *et al.*, 1998). These factors are, in turn, regulated by Dpp/Bmp and its inhibitors, thereby translating DV patterning into different ML domains of neurogenesis (Liem *et al.*, 1995). These results suggest that a conserved system of positional information patterns the NE in protostomes and deuterostomes.

In contrast, other upstream factors involved in NE development are less similar. Vertebrate ectoderm acquires the ability (competence) to respond to neuralizing signals by exposure to fibroblast growth factors (Fgfs), which in turn are inhibited by Wnts from the lateral ectoderm (Akai and Storey, 2004; Delaune *et al.*, 2005). Fgf, Bmp, and Wnt signaling also interact to position the NE/NNE boundary region (Streit, 2004; Litsiou *et al.*, 2005). These signals establish specialized precursors from which neural crest and placodes develop (Figure 2f; Ahrens and Schlosser, 2005). Yet, despite these crucial roles in vertebrates, Wnt and Fgf relatives in *Drosophila* do not regulate ectodermal competence or neural induction. Conversely, in flies the expression of Vnd, Ind, and Msh depends on the function of a critical transcription factor, Dorsal, but no such system exists for the vertebrate NE. Other similarities in neurogenesis between protostomes and deuterostomes only become apparent later when cells become neuroblasts (see Basic Nervous System Types: One or Many?).

1.14.2.2.2 *Hox* genes and AP Patterning *Hom/Hox* genes confer segmental identity in the ectoderm in both *Drosophila* and vertebrates (Figure 3). Mutations in many of these genes cause homeosis, in which one segment or structure is replaced by another (Bateson, 1894; Lewis, 1978). *Hox* genes are clustered, with more 3′ genes expressed earlier and further anteriorly than more 5′ genes (Figures 3a and 3d), and the temporal and spatial patterns of *Hox* expression are similar in all major bilaterian groups, including those (e.g., hemichordates) that do not form separate NE and NNE (Harding *et al.*, 1985; Lowe *et al.*, 2003). In some cases human *Hox* genes can functionally compensate for their relatives in flies (Malicki *et al.*, 1992). However, in contrast to the single homeotic complex (*Hom-C*) in *Drosophila* (Figure 3a), mammals have four *Hox* clusters (*HoxA–D*) located on different chromosomes and totaling 39 genes (Figure 3d; McGinnis and Krumlauf, 1992). Each of the clusters consists of up to 13 paralogues (*Hox1–13*), genes located in the same 3′–5′ position in different clusters and more similar to one another than to genes within the same cluster. Double mutant studies in mice have revealed redundancies between paralogues (Rossel and Capecchi, 1999; Greer *et al.*, 2000). Thus, differences in *Hox* gene number and expression, rather than distinct biochemical functions, are thought to underlie many aspects of morphological diversity along the AP axis.

Despite exhaustive studies, however, the tissue-specific roles for many *Hox* genes have remained unclear. In both *Drosophila* and vertebrates, *Hox* expression is generally strongest in the NE and sometimes difficult to detect in NNE and other tissues. Often the AP boundary of expression within the NNE aligns with that of the NE and is under similar genetic control (Couly and LeDouarin, 1990). For example, in vertebrates the vitamin A derivative retinoic acid (RA) promotes expression of *Hox1*-group genes in the posterior hindbrain (Figures 3f–3h; Marshall *et al.*, 1992; Schilling and Knight, 2001). *Hox1* expression shifts anteriorly both in the hindbrain and epidermal placodes upon treatment with RA (Figure 3g; Kolm and Sive, 1995). Conversely, inhibition of RA signaling causes placodes of the lateral line to expand posteriorly, and this coincides with a posterior expansion of the hindbrain rhombomeres that induce these placodes (Figure 3h; Gibbs and Northcutt, 2004). Amphioxus *Hox* genes (1, 3, 4, and 6) are also expressed in distinct AP domains in the NE and NNE that shift in response to changes in RA signaling (Holland and Holland, 1996; Escriva *et al.*, 2002; Schubert *et al.*, 2004). These results suggest that *Hox* genes are under common patterns of regulation in different ectodermal tissues that are highly conserved.

Hox expression is also regulated independently in NE and NNE in some instances. For example, vertebrate *Hoxa2* is expressed in rhombomeres 2–5 (r2–5) of the hindbrain but not in neural crest cells derived from r2–3 that form the first pharyngeal arch (mandibular; Figure 3i; Gavales *et al.*, 1997). Instead, expression is restricted to crest cells of the second pharyngeal arch (hyoid) derived from r4–5 (Hunt *et al.*, 1991), where *Hoxa2* function is required; loss-of-function mutations in *Hoxa2* cause homeotic transformations of the hyoid arch skeleton into a mirror-image duplicate of the mandibular (Figure 3j; Gendron-Maguire *et al.*, 1993; Rijli *et al.*, 1993). Distinct regulatory regions of the *Hoxa2* promoter control hindbrain and neural crest expression in transgenic mice (Maconochie *et al.*, 1999). Several *Hox* genes (*Hoxa2*, *Hoxa3*, *Hoxb4*) are also expressed in the surface ectoderm overlying the pharyngeal arches in avian embryos, and these

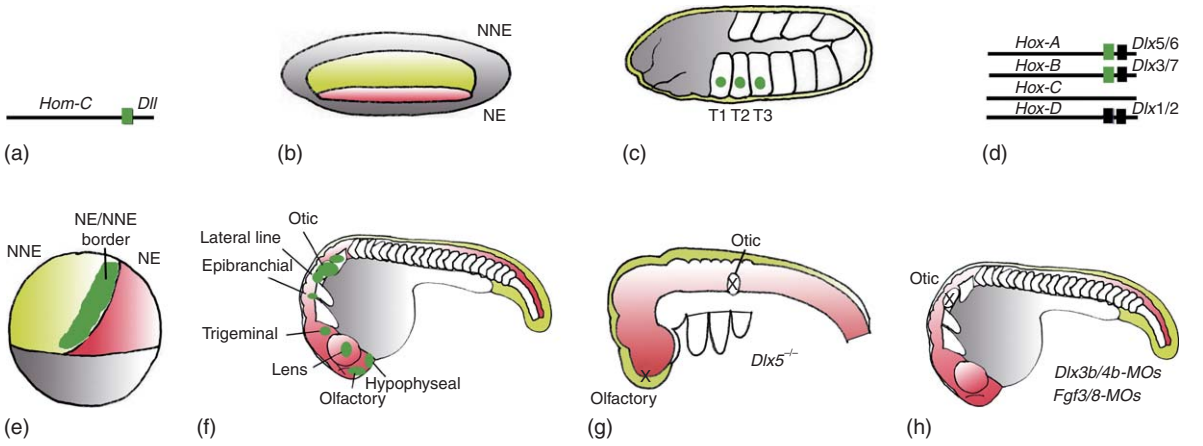

Figure 4 *Dlx* genes and patterning of the NNE. a, Single *Drosophila Dll* gene is linked to the *Hom-C* cluster. b, Pregastrula fly embryo indicating NNE (yellow) and NE (red). c, Germ band extended fly embryo indicating segments and expression of *Dll* (green) in cells of thoracic segments destined to develop legs. d, Three pairs of *Dlx* genes are linked to *Hox* clusters in vertebrates. e, *Dlx3* and/or *Dlx5* expression at the NE/NNE border of vertebrate embryos. f, Lateral view of zebra fish embryo showing positions of epidermal placodes. g, Olfactory and otic placodal defects in *Dlx5* mutant mice. h, Otic placodal defects in *Dlx3/4-* and *Fgf3/8*-deficient zebra fish.

patterns are regulated independently of the NE (Couly *et al.*, 1998). Other *Hox* genes (*Hoxa7*, *Hoxb8*) are expressed in the epidermis but at much later stages of embryogenesis. These results suggest a more complex system in which AP identities of NE and NNE cells can shift independently during evolution.

1.14.2.2.3 *Dlx* genes and non-neural patterning A third conserved system of positional information in the vertebrate ectoderm is encoded by homeobox genes of the *Dlx* family, relatives of *Drosophila Distal-less* (*Dll*; Figure 4a; Cohen *et al.*, 1989). *Dll* is a crucial regulator of appendage development in arthropods. It is only active in the thorax, because its expression is repressed in the abdomen by *Hox* genes such as *Ubx* and *AbdA* (Figure 4c; Vachon *et al.*, 1992). *Drosophila* has a single *Dll* gene, while vertebrates have at least six *Dlx* genes, organized into three pairs, each linked to one of the four *Hox* clusters (Figure 4d; Stock *et al.*, 1996; Panganiban and Rubenstein, 2002). *Dlx3* is expressed broadly throughout the NNE, and humans with *Dlx3* mutations have defects in hair, teeth, and craniofacial skeletal development (Price *et al.*, 1998). *Dlx5* expression in mice is restricted to the NE/NNE boundary and required for the development of olfactory and otic placodes, pharyngeal neural crest, and the dorsal midline of the neural tube (Figures 4e–4g; Acampora *et al.*, 1999; Depew *et al.*, 1999). *Dlx3b* and *Dlx4b* in zebra fish are essential for otic development (Figure 4h; Akimenko *et al.*, 1994; Solomon and Fritz, 2002). In each case, Dlx proteins appear to repress neural differentiation at the NE/NNE boundary, allowing cells to become competent to respond to placode inducers (Feledy *et al.*, 1999; McLarren *et al.*, 2003). This may have been an early ectodermal function for *Dll* that was co-opted during the evolution of the *Dlx* family to new vertebrate-specific functions in neural crest and placodes.

Roles for *Dlx* genes in NNE may have been inherited from a similar function in the protochordate ancestor. *Dlx* genes that have been identified in hemichordates and ascidians are expressed in anterior ectoderm, but not in domains associated with the NE/NNE boundary (Caracciolo *et al.*, 2000; Harada *et al.*, 2001). Amphioxus *Dll* (*AmphiDll*) is expressed throughout the NNE and anterior NE, similar to several *Dlx* family members in vertebrates (Holland *et al.*, 1996). Ectoderm in amphioxus embryos also expresses other relatives of genes characteristic of the NE/NNE boundary in vertebrates (*Pax3/7*, *Msx1/2*, *Zic*, *Slug/Snail*), but does not express transcriptional regulators that specify neural crest cells (*AP-2*, *SoxE*, *FoxD3*, *Id*, *Twist*; Meulemans and Bronner-Fraser, 2002, 2004), as discussed below.

1.14.3 Modularity in Ectodermal Evolution

An important conceptual framework for understanding ectodermal evolution involves the concept of a developmental module (Riedl, 1978; Wagner, 1996). Modules can be morphogenetic fields (i.e., germ layers, tissues, body segments, and organ rudiments), distinct cell lineages, or molecular

pathways. Genes themselves are clearly modular, as are their regulatory elements (promoters and enhancers). If a tissue or gene acquires or loses a module, different morphologies can develop. Modules can be dissociated, duplicated, or co-opted for different purposes at all levels of development (Raff, 1996).

1.14.3.1 Neural/Non-Neural Interactions

Changes in one developmental module will often affect other modules with which it interacts. Prime examples of this are interactions between NE and NNE that induce and pattern the olfactory, lens, otic, and hypophyseal placodes in vertebrates (Figure 5; Baker and Bronner-Fraser, 2001; Streit, 2004; Brugmann and Moody, 2005). Cells of the NNE initially express transcription factors with generic roles in placode formation such as *Six1*, *Eya1*, and *Dlx5/6*, and individual placodal domains are induced within this preplacodal primordium (Schlosser and Ahrens, 2004). Perhaps the best known of these is the induction of the lens by the anterior neural plate, which forms the optic cup (Figure 5a). The optic cup is both necessary and sufficient for lens formation in amphibians, and only certain regions of cranial ectoderm are competent to be induced (Lewis, 1904; Grainger, 1996; Grainger *et al.*, 1997). Both Bmps and Fgfs have been implicated in the inductive signal (Lang, 2004), and competence relies on transcriptional regulators such as *Pax6*, *Otx2*, and *Pitx3*, expressed in the prelens field (Zygar *et al.*, 1998; Khosrowshahian *et al.*, 2005). Lens-inducing signals may be largely permissive, rather than instructive, since in some species of amphibians the lens can develop independently of the optic cup (Balinsky, 1951).

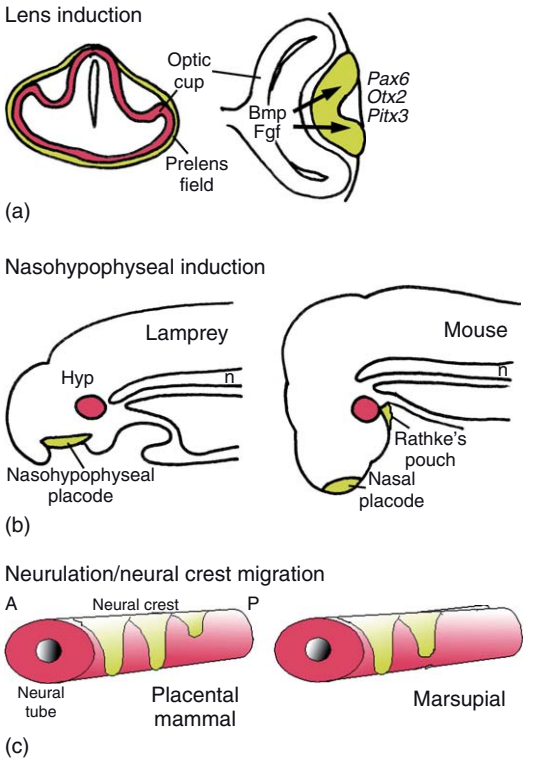

Figure 5 Heterochronic changes in ectodermal development. a, Lens induction. Diagrams illustrating transverse sections through the head of an early amphibian embryo (left panel) and the optic primordium at later stages (right panel). The optic cup secretes Bmp and Fgf that induce lens identities within a prelens field. b, Nasohypophyseal induction in agathans and gnathostomes. Lateral views, anterior to the left. Lampreys form a combined nasohypophyseal placode and a single nostril (left panel). In contrast, nasal placodes separate from Rathke's pouch, which forms the adenohypophysis, through a change in the timing of growth. c, Timing of neurulation and neural crest migration in placental and marsupial mammals. Diagrams illustrate transected segments of neural tube (red) with streams of migrating neural crest (yellow). Anterior to the left. n, notochord.

Similarly, development of the otic placode, which forms the inner ear and vestibuloacoustic ganglion, involves NE–NNE interactions. The otic vesicle is induced by two major steps in urodele amphibians (Yntema, 1933). Recent results in other species suggest that these include early signals from the cranial mesoderm during neurulation and later signals from the posterior hindbrain, both of which involve Fgfs (Ladher *et al.*, 2000, 2005; Barald and Kelley, 2004; Martin and Groves, 2006). The combination of Fgf3 and Fgf8 is required for otic induction in zebra fish (Figure 4h), while Fgf3 and Fgf10 in mice play similar, redundant roles (Solomon *et al.*, 2004). Like the lens placode, competence within the ectoderm to respond to Fgfs correlates with expression of the preplacodal state normally found at the NE/NNE boundary (Martin and Groves, 2006). Interestingly, in mammals and other amniotes with an external ear opening, the whole epidermis is involved, while in amphibians only the interior epidermal cells contribute to the otic vesicle, and no opening forms. Such differences could have evolved through changes in the competence of ectodermal cells to respond to Fgfs.

1.14.3.2 Heterochrony

Developmental modules can also become dissociated during evolution. When this involves changes in the relative timing of developmental events between an ancestor and its descendants, it is known as heterochrony. One tissue or organ system may develop at an accelerated or retarded pace relative to others or there may be a change in developmental sequence (Smith, 2002). For example,

heterochronic shifts in the timing of contact between the NE and adjacent hypophyseal placode underlie species-specific differences in morphology of the pituitary (Figure 5b). Where cells of the ventral hypothalamus contact the hypophyseal placode (i.e., Rathke's pouch) they fuse to form the neurohypophysis and adenohypophysis, respectively. In gnathostomes, the bilaterally symmetric nasal placodes develop adjacent to the hypophyseal placode but quickly become separated by anterior growth of the brain (Couly and LeDouarin, 1985; Glieberman *et al.*, 1999). In contrast, in agnathans such as the lamprey (Figure 1b), the olfactory epithelium and the hypophysis develop as a single median placode, the nasohypophyseal (Figure 5b; Gorbman and Tamarin, 1985; Kuratani *et al.*, 2001), and agnathan adults have a single nasal opening (monorhiny).

Early development of the hypophyseal placode is also intimately tied to the placodes that form the olfactory epithelia and lens, and changes in the timing of inductive signals from the hypothalamus may also play a crucial role in their specification. The secreted signaling molecule Sonic hedgehog (*Shh*), produced in the ventral midline of the neural tube, is both necessary and sufficient for the development of these placodes and may specify distinct placodal fates within a preplacodal field (Figure 5b; Herzog *et al.*, 2003). Recent studies in zebra fish have shown that *Pitx3* and *Dlx3b* define an equivalence domain for the lens and pituitary (Dutta *et al.*, 2005). Targeted gene knockdown studies suggest that *Pitx3* is required to form an early olfactory–lens–hypophyseal preplacode, while *Dlx3b* is required to determine placode size. Misexpression of *Shh* inhibits lens formation and expands the pituitary, but within a placodal domain of normal size. Mutants that reduce *Shh* signaling can, in some cases, result in the formation of a lens in the location of the adenohypophysis (Kondoh *et al.*, 2000). These results exemplify another fundamental feature of developmental modularity, namely, the progressive restriction of developmental potential, and heterochronic changes in the sequence of these restrictions may also underlie many evolutionary transitions in the ectoderm.

A common heterochronic change in both protostomes and deuterostomes has been in the maturation of different body segments along the AP axis. In short-germband insects, such as *Drosophila*, segmental domains are specified more or less simultaneously across a syncytial blastoderm. In contrast, long-germband insects form segments progressively from anterior to posterior, and this is the primitive mode in arthropods. Vertebrate segmentation more closely resembles this mode, with the sequential AP appearance of somites in the mesoderm, neural tube closure, and neural crest migration (Figure 5c). All three are under tightly coordinated genetic control. An interesting case of heterochrony in these processes occurs in marsupial mammals, which have an extremely short period of intrauterine development and are born as altricial neonates that suckle and develop outside the mother (Clark and Smith, 1993). Development of the jaws, tongue, and forelimbs is accelerated relative to other aspects of organogenesis, presumably to allow the neonate to attach and feed. To achieve this, neural crest migration is accelerated dramatically in the head as compared with placental mammals, revealing a shift in timing along the AP axis (Figure 5c; Vaglia and Smith, 2003). Neural crest migration and differentiation are also accelerated relative to closure of the neural tube in these animals. These results demonstrate the modular nature of neural and neural crest development.

Similar changes in the relative timing of neural crest migration may underlie other species-specific differences in vertebrate morphology. For example, neural crest migration in birds occurs largely after the neural tube has closed, while in zebra fish and mammals some cranial neural crest migration precedes tube formation (Nichols, 1981; Schilling and Kimmel, 1994; LeDouarin and Kalcheim, 1999). Thus, the rate of neural crest maturation can be locally regulated along the AP axis through mechanisms that are separate from other modules within the ectoderm.

1.14.3.3 Gene Duplication and Divergence in Ectodermal Patterning

Another fundamental feature of developmental modules is that they can be duplicated, and duplicates can evolve distinct functions. This has occurred at the level of single genes or gene clusters, as we have discussed for the *Hox* and *Dlx* genes (Figures 3 and 4), as well as at the level of gene networks that regulate whole fields of cells, as in the evolution of body segment number. It is now fairly well established that at least one major duplication event occurred on a broad scale in ancestral chordates involving whole-genome duplications, and that this accounts for the larger number of *Hox* clusters in vertebrates (Amores *et al.*, 1998; Holland, 1999). Functional redundancies between duplicates often obscure the roles of individual genes and this has made it difficult to clarify the functions of many Hox proteins. For example, double mutant analyses in mice have revealed

redundancies between *HoxA1* and *B1* in patterning of rhombomeres 4 and 5 (r4 and r5) of the hindbrain, as well as *HoxA3* and *D3* (Rossel and Capecchi, 1999; Greer *et al.*, 2000).

Determination of gene copy number in multiple species, combined with gene expression analysis, can give clues as to their roles in the most recent common ancestor. For example, one or more duplications of *Hox* clusters appears to have accompanied the chordate-to-vertebrate transition, since both ascidians and amphioxus have a single cluster like *Drosophila* (Figure 3; Garcia-Fernandez and Holland, 1994; DiGregorio *et al.*, 1995; Holland and Garcia-Fernandez, 1996; Delsuc *et al.*, 2006). Comparing the *Hox* clusters of chordates, arthropods, and mollusks suggests that there was a common set of seven *Hox* genes in the urbilaterian ancestor. It is attractive to postulate that duplication and divergence of *Hox* genes provided raw material for the more complex development of the brain and sense organs in the vertebrate ectoderm. However, duplications do not necessarily correlate with increased complexity, since additional whole-genome duplications have occurred in the lineages leading to teleost fishes, yielding seven *Hox* clusters, without a concomitant increase in complexity (Amores *et al.*, 1998; Postlethwait *et al.*, 1998).

The *Dlx* gene family has also undergone several rounds of gene duplication and divergence during chordate evolution (Figure 4). This family is represented by a single gene in protostomes, *Dll*, and amphioxus also has a single *Dll* gene, *AmphiDll* (Holland *et al.*, 1996). It appears, however, that the ancestral chordate *Dll* was duplicated in the deuterostome lineage prior to the divergence between urochordates and vertebrates into *DlxA* and *DlxB* (Di Gregorio *et al.*, 1995). Additional duplications of these led to two major classes of *Dlx* genes in gnathostomes, *Dlx-2,3,5* and *Dlx-1,6,7* (Stock *et al.*, 1996; Zerucha and Ekker, 2000; Sumimaya *et al.*, 2002). Since these are organized into three closely linked pairs (*Dlx1/2*, *Dlx5/6*, and *Dlx3/7*) associated with three of four *Hox* clusters in mammals, it seems likely that a pair of *Dlx* genes linked to *HoxC* existed following early whole-genome duplications, and was subsequently lost. Double mutant analyses of mice lacking the functions of linked pairs, *Dlx1* and *Dlx2*, as well as *Dlx5* and *Dlx6*, have revealed redundant functions in neural crest development (Qiu *et al.*, 1997; Depew *et al.*, 1999), as discussed below. In contrast, *Dlx3* and *Dlx5* have evolved distinct roles in placodal development (Price *et al.*, 1998; Acampora *et al.*, 1999; Depew *et al.*, 1999). Similar patterns of gene duplication and divergence have been described for many other vertebrate gene families.

1.14.4 Generating Evolutionary Novelty

1.14.4.1 Neural Crest and Vertebrate Origins

The appearance of neural crest cells was a crucial and seemingly abrupt step in vertebrate evolution (Gans and Northcutt, 1983; Northcutt and Gans, 1983; Northcutt, 2005). The closest chordate relatives do not form neural crest. However, all living vertebrates, including agnathans, have a full complement of neural crest and crest derivatives (Holland and Holland, 2001; Kuratani *et al.*, 2001). In both urochordates and cephalochordates, subsets of ectodermal cells express genes characteristic of the vertebrate NE/NNE boundary region, but these cells do not express relatives of the genes that specify neural crest cells and never migrate (Figure 1b; Meulemans and Bronner-Fraser, 2002, 2004). One exception to this was recently reported for one species of urochordate, in which labeled ectodermal cells were shown to migrate and form pigmented cells (Jeffery *et al.*, 2004). These results suggest that neural crest evolved from modifications of a pre-existing NE/NNE boundary region in an ancestral protochordate.

What cellular and molecular changes at the NE/NNE boundary led to the advent of neural crest? Genes expressed at the boundary (*Pax3/7, Msx1/2, Zic, Slug/Snail* – boundary-specific), and those specific to neural crest (*AP-2*α,β,γ, *Sox9/10, FoxD3, Id, Twist* – crest-specific), are likely candidates (Figure 6; Meulemans and Bronner-Fraser, 2004). Many of these genes are regulated by Bmp signaling in vertebrate DV patterning, which restricts their expression to the NE/NNE boundary. Ectoderm in amphioxus embryos expresses relatives of boundary-specific but not crest-specific genes (Baker and Bronner-Fraser, 1997; Langeland *et al.*, 1998). In larvaceans, which are urochordates, the cranial ectoderm expresses early boundary-specific genes in locations that resemble olfactory and hypophyseal placodes (Figure 4; Bassham and Postlethwait, 2005) and ascidian *Pitx* genes define a clear hypophyseal placode (Boorman and Shimeld, 2002; Christiaen *et al.*, 2002; Manni *et al.*, 2005). Thus, the NE/NNE boundary region may have arisen in ancestral chordates as part of a primitive olfactory/pituitary system (Mazet *et al.*, 2005; Schlosser, 2005).

Among the crest-specific genes, members of the activator protein (*AP-2*) family are notable in that they play ancient roles in NNE development in all

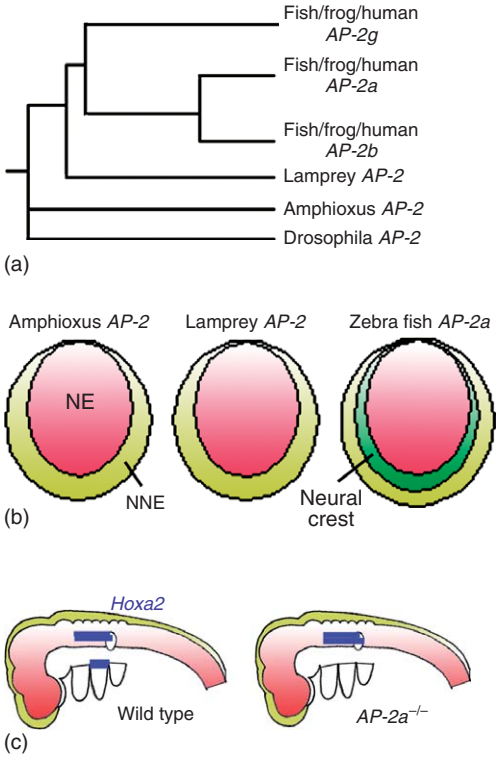

Figure 6 Evolution of the neural plate border, *AP-2* genes, and neural crest origins. a, Molecular phylogeny of AP-2 family members. b, Illustrations of expression of *AP-2* genes in cephalochordates, agnathans, and vertebrates in NNE (yellow) and neural crest (green), but not in NE (red). Views are from the anterior. c, Loss of *Hoxa2* activation in neural crest in *AP-2a* mutants. Lateral views. a, Modified from Meulemans, D. and Bronner-Fraser, M. 2002. Amphioxus and lamprey AP-2 genes: Implications for neural crest evolution and migration patterns. *Development* 129, 4953–4962.

chordates (Figure 6). *AP-2a* and its relatives in mammals (*AP-2b* and *AP-2g*) are among the earliest genes expressed specifically in ectoderm (Figure 6a; Hilger-Eversheim *et al.*, 2000). During gastrulation, AP-2a is expressed throughout the NNE, but is then upregulated in neural crest cells prior to and during their migration away from the NE (Williams *et al.*, 1988; Mitchell *et al.*, 1991). AP-2a is required for neural tube and body wall closure in mice and directly regulates keratin expression, indicating widespread roles in both NE and NNE. It also has specific roles in neural crest development, since null *AP-2a*$^{-/-}$ mutant zebra fish or mice lack neural, skeletal, and pigment cells derived from the cranial neural crest (Schorle *et al.*, 1996; Zhang *et al.*, 1996; Knight *et al.*, 2003, 2004). *AP-2a* is an essential activator of *Hoxa2* expression in neural crest that forms the second pharyngeal arch (Figure 6c), but not in the NE, indicating a direct role for *AP-2* genes in neural crest patterning (Maconochie *et al.*, 1999; Knight *et al.*, 2003). *AP-2b* mutations in humans cause Char syndrome, including craniofacial defects affecting skeletal derivatives of neural crest. Misexpression of *AP-2a* can restore neural crest and epidermal development in *Xenopus* embryos severely deficient in Bmp signaling, suggesting that they function in part as downstream mediators of this pathway (Luo *et al.*, 2002). Thus, multiple AP-2 proteins are thought to play essential roles in both neural crest and epidermal development.

A comparison of the expression patterns and functions of AP-2 proteins in invertebrates and vertebrates suggests that their ancestral roles were in epidermal development and that they were later co-opted in neural crest. This correlates with an increase in the number of *AP-2* genes in vertebrates, similar to the *Hox* and *Dlx* families discussed previously (Figure 6a). A single *Drosophila* AP-2 gene regulates proximal–distal (PD) patterning of appendages (Kerber *et al.*, 2001; Monge *et al.*, 2001). Amphioxus also appears to have a single *AP-2* gene family member, expressed throughout the NNE (Figure 6b; Meulemans and Bronner-Fraser, 2002). In contrast, fish, amphibians, and mammals all have at least three closely related *AP-2* family members (*AP-2a, b*, and *g*; Luo *et al.*, 2003, 2005; Zhang *et al.*, 2006; Javier and Schilling, 2004), which in mice are all expressed in neural crest (Figure 6a). Mammals have two additional family members (*AP-2d* and *e*) that are less well conserved. Zebra fish *AP-2b* is not expressed in neural crest, unlike its relatives in frog and mouse (Luo *et al.*, 2005; Moser *et al.*, 1997), and instead is confined to the pharyngeal ectoderm where it is required to induce skeletogenesis in the cranial neural crest (Knight *et al.*, 2005). This highlights another epithelial–mesenchymal interaction that occurs between the epidermis and underlying neural crest, and the divergence of tissue-specific roles of different members of a closely related family of duplicates.

1.14.4.2 Neural Crest and the Origins of Jaws and Middle-Ear Bones

Another major transition in vertebrate evolution was the appearance of a biting jaw. Ancestral vertebrates, like their chordate relatives, were initially jawless (agnathans) filter feeders with a segmented pharynx composed of gill arches (Figure 1b). Living agnathans, such as the lamprey, lack the distinct upper (proximal) and lower (distal) jaws found in gnathostomes (jawed vertebrates) (Figure 7). In urochordates and cephalochordates arches consist of reiterated folds in the pharyngeal ectoderm and endoderm. In contrast, vertebrate arches have a complex tissue composition, including skeletal

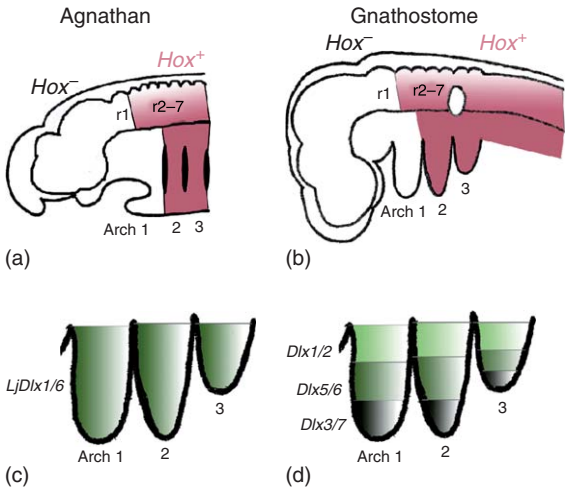

Figure 7 Evolution of pharyngeal arches and the origins of jaws. Left panels, lampreys; right panels, mice. a, Lamprey head showing *Hox*$^+$ region (red) emcompassing rhombomeres 2–7 and arches 2 and 3. b, Mouse head showing similar pattern of *Hox*$^+$ cells. c, Higher-magnification view of arches, lateral view, showing uniform expression of *LjDlx1/6* as compared with d, in which gnathostome *Dlx* genes are expressed in a nested pattern along the PD axis.

elements derived from cranial neural crest as well as muscles and blood vessels derived from mesoderm (Le Lievre, 1974; reviewed by LeDouarin and Kalcheim, 1999). The gnathostome jaw derives from arch 1 (mandibular), and classically has been thought to have evolved from a modified gill arch (Goodrich, 1958; DeBeer, 1937; reviewed in Mallatt, 1996). Comparative studies in lampreys are crucial in this case as they are one of the only living sister groups to the gnathostomes.

One unique molecular feature of the mandibular arch is the absence of *Hox* expression (Figure 7a). Mandibular neural crest cells emanate from *Hox*-negative regions of the neural tube (i.e., the midbrain and anterior hindbrain), while crest cells in more posterior arches are *Hox*-positive; cells in arch 2 (hyoid) express *Hoxa2* and *b2*, whereas cells in arch 3 express *Hoxa3* and *b3* (Hunt *et al.*, 1991). Kontges and Lumsden (1996) showed that *Hox*-positive connective tissue attachments of muscles in arch 2 are derived from neural crest cells that arise from the *Hox*-positive rhombomeres (r2–3) that innervate them. Thus, the skeletal elements, the muscles that move them, and the nerves that innervate the muscles form a developmental module that can be acted upon by evolution. Transformations of arch 2 into a mirror-image duplicate of arch 1 in *Hoxa2* mutants suggest that *Hox* genes function in part to suppress an arch 1 default state (Figure 3; Gendron-Maguire *et al.*, 1993; Rijli *et al.*, 1993). Thus, one attractive evolutionary hypothesis is that the loss of *Hox* expression in the mandibular arch in an ancestral agnathan allowed jaw evolution in gnathostomes. Recent evidence argues against this model, since *Hox* genes that have been described to date in the Japanese lamprey, *Lampetra japonica*, are largely excluded from the mandibular arch (Takio *et al.*, 2004; Kuratani, 2005).

Dlx genes are particularly interesting with regard to jaw evolution because they are expressed in distinct domains along the PD axis within each pharyngeal arch in gnathostomes (Figure 7b). This is reminiscent of *Drosophila* appendages, where *Dll* promotes distal cell identity (Panganiban *et al.*, 1997). All six *Dlx* genes are expressed along the PD axis in the neural crest of the mandibular arch. While *Dlx1* and *Dlx2* are expressed throughout the arch, *Dlx5* and *Dlx6* expression are confined further distally, and *Dlx3/7* expression domains are further distal still (Robinson and Mahon, 1994; Qiu *et al.*, 1995, 1997; Acampora *et al.*, 1999; Depew *et al.*, 1999). In *Dlx5/6* double mutants, distal cells of the lower jaw acquire dorsal characteristics typical of the upper jaw, revealing redundant functions in controlling distal cell identity (Depew *et al.*, 2002, 2005; Robledo *et al.*, 2002). Interestingly, a lamprey relative of *Dlx1* and *Dlx6* (*LjDlx1/6*) is expressed more broadly throughout the arches, and is not restricted to distinct P or D domains (Myojin *et al.*, 2001; Neidert *et al.*, 2001; Shigetani *et al.*, 2002; Kuratani, 2005). Thus, it is possible that a heterotopic (rather than heterochronic) shift in *Dlx1/6* gene regulation facilitated evolution of the lower jaw.

What might have caused such spatial shifts in *Dlx* expression? Epithelial–mesenchymal interactions between the epidermis and underlying neural crest in mice play central roles in *Dlx* regulation and PD patterning in the arches. *Dlx1* expression is induced in proximal neural crest cells by Fgf8, produced in the proximal surface ectoderm of the arch (Barlow and Francis-West, 1997; Neubuser *et al.*, 1997; Tucker *et al.*, 1998; Shigetani *et al.*, 2002), and ectodermal expression of Fgf8 is required for mandibular patterning (Trumpp *et al.*, 1999). *Dlx2* in mice is independently regulated in the arch epithelium and crest-derived mesenchyme (Thomas *et al.*, 2000). Similar to *LjDlx1/6*, a lamprey relative of Fgf8 is expressed widely in the oral ectoderm (Shigetani *et al.*, 2002). These results suggest that the biting jaw evolved from modifications of a preexisting system of interactions between the neural crest and epithelia in the pharyngeal arches of an ancestral agnathan.

PD domains within the arches have also been co-opted for entirely new functions. A famous case is the use of homologues of fish jawbones in the mammalian middle ear (Gould, 1990). Proximal bones of the mandibular and hyoid arches, which support the jaw in fish and attach to the wall of the otic capsule, are homologues of the malleus and incus bones that conduct sound.

1.14.5 Other Developmental Constraints

1.14.5.1 Morphology – Neural Crest and Neural Tube Morphogenesis

As we have seen, one consequence of modular development in the ectoderm is that it limits the possible phenotypes that can be created, and allows changes in some directions more easily than others. One such developmental constraint governs the production of sensory neurons in vertebrates from neural crest and placodes at the NE/NNE boundary. While all sensory neurons of the PNS are derived from these progenitors, a few are also found within the dorsal CNS, and these share many developmental features with neural crest cells (Figure 8a). These include the mesencephalic nucleus of the trigeminal (MesV), found within the midbrain of the CNS near the tectal commissure in all gnathostomes, that carries proprioceptive information from the jaw muscles (Gonzalez and Munoz, 1988). These cells appear to be absent in lampreys (Northcutt, 1979). In addition, Rohon–Beard (RB) neurons of embryonic fish and amphibians are sensory neurons located within the dorsal spinal cord (Figure 8b; Forehand and Farel, 1982). They function as a temporary sensory system in the larva that is subsequently replaced by neural crest-derived neurons of the dorsal root ganglia (DRGs) (Lamborghini, 1987). Both MesV and RB neurons may represent remnants of an earlier evolutionary state, in which such centrally located sensory neurons may have been common.

Sigmund Freud described RB neurons as unmigrated spinal ganglion cells, and recent studies indicate that they bear many similarities to neural crest. Extirpations of crest in urodeles also disrupts RB neurons (DuShane, 1938). In zebra fish, RB precursors share a lineage with neural crest cells, and interactions between these cells determine their fates inside or outside the CNS (Cornell and Eisen, 2000). Neuroblasts that produce RB cells inhibit their neighbors from forming neurons through lateral inhibition involving Delta-Notch

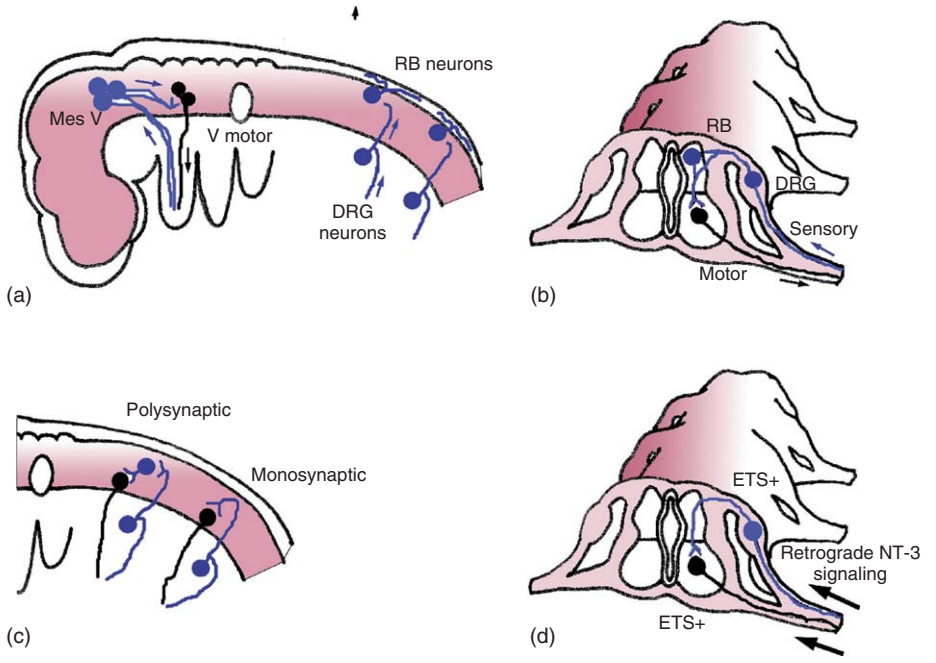

Figure 8 Developmental constraints in the formation of neural circuits. a and b, Morphological: MesV and RB neurons are neural crest cells trapped in the CNS. MesV cells are sensory neurons within the CNS that bring sensory information from the jaw and connect to trigeminal motor neurons (V motor). Similarly, RB neurons are sensory neurons in the trunk. a, Lateral view; b, transverse section. c, Physiological: establishing circuits between CNS and PNS. Polysynaptic and monosynaptic circuits involve information coming to (a) from a common peripheral target. d, NT-3 signals modulating ETS domain transcription factor form the basis of a target-mediated specification of sensory and motor neurons.

signaling, and these neighboring cells give rise to neural crest. Recent studies have also shown that *Dlx* genes in zebra fish are required in the development of RB neurons and in the trigeminal placodes, consistent with their origins at the NE/NNE boundary (Artinger *et al.*, 1999; Kaji and Artinger, 2004). Loss of *Dlx3b* and/or *Dlx4b* expression disrupts Bmp2b expression and this, in turn, regulates cell fate choices between RB neurons and neural crest cells at the boundary.

1.14.5.2 Physiology – Establishing Circuits between Central and Peripheral Neurons

The developing nervous system also has the functional constraint that its sense organs and PNS need ultimately to connect in an appropriate pattern to the CNS to establish circuits. The simplest circuit controls the monosynaptic reflex, in which an incoming axon of the PNS from a muscle receptor (derived from neural crest) enters through the dorsal root and terminates on a motor neuron in the ventral spinal cord (CNS) that innervates that same muscle (Figures 8c and 8d; Ariens Kappers *et al.*, 1960). These connections are established extremely early during embryogenesis. How do the neurons find their targets? Some evidence suggests that the targets of motor neurons are specified before their axons extend into the periphery (Lance-Jones and Landmesser, 1980). Innate neuronal identities are controlled by domains of expression of LIM transcription factors in different populations of motor neuron progenitors (Appel *et al.*, 1995).

Recent evidence suggests that connectivity between motor neurons and sensory neurons within a monosynaptic circuit is controlled by target-derived signals (Figure 8d; Chen *et al.*, 2003; Hippenmeyer *et al.*, 2004). Motor neurons within the ventral horn of the spinal cord receive monosynaptic input from Ia proprioceptive DRG sensory neurons. These two cell types establish peripheral connections with the same muscle targets. Two members of the ETS domain family of transcription factors, Pea3 and Er81 in mice, are expressed by distinct subpopulations of motor and sensory neurons that form such circuits (Lin *et al.*, 1998; Arber *et al.*, 2000; Livet *et al.*, 2002). Onset of Pea3 and Er81 expression coincides with the stages at which the axons of these cells begin to reach their target muscles. In the absence of Pea3 function, motor neurons project to the periphery but fail to innervate their targets. Er81, in contrast, is required in DRG sensory neurons to promote the establishment of axonal projections into the ventral horn of the spinal cord.

What is the nature of the target-derived signals? They must act retrogradely on the cell body to control the acquisition of cell identity and circuit assembly. Interestingly, a phenotype similar to that of Pea3 mutants is observed in mice lacking glial-derived neurotrophic factor (Haase *et al.*, 2002). These mice fail to induce Pea3 expression in motor neurons, and these motor neurons fail to migrate to their normal locations within the spinal cord, possibly due to defects in cell adhesion. Similarly, another neurotrophin called NT-3 induces Er81 expression in proprioceptive afferent neurons (Patel *et al.*, 2003). Thus, neurotrophins play a general role in the development of motor and sensory projections in a manner independent of their better-known roles in neuronal survival.

Generalizing from this example, it seems likely that other more complex neuronal circuits will involve similar processes in which neuronal subtypes are specified prior to the generation of postmitotic neurons. These define cell-autonomous competence to respond to target-derived signals which subsequently alter neuronal identities as their axons reach their targets. Such flexible systems help explain how cells derived from NE and NNE form appropriate connections with one another despite quite different developmental histories. The recent experiments have only begun to shed light on how this fine-tuned interplay between different ectodermal populations controls circuit formation.

1.14.6 Summary and Outlook

The mechanisms that pattern the nervous system in all bilaterian animals begin with signals that induce DV polarity within the ectoderm and combinatorial codes of transcription factors that define neurogenic and non-neurogenic territories. A common trend in ectodermal evolution is the loss of neurogenic potential from the NNE. Genetic studies in mice and zebra fish have begun to uncover mechanisms that coordinate patterning of the NE and NNE in vertebrates. Studies of neural crest and placodal development, in particular, have helped to define roles of Bmp, Hox, Dlx, and AP-2 proteins in ectodermal development. In this article, I have considered this new information in a comparative context to examine how changes in gene functions have co-evolved in the neural and non-neural components of the ectoderm.

All chordates share relatives of these gene families, though the families are typically three- to fourfold larger in vertebrates. Increases in gene copy number like this probably evolved through whole-genome duplications during and after the divergence

of vertebrate and invertebrate chordates, and they complicate comparisons between orthologous genes. However, we can infer ancestral functions for many genes based on their expression in amphioxus and ascidians, since they diverged from the vertebrate lineage prior to these gene duplications. These indicate that the primitive chordate ectoderm contained separate NE and NNE as well as an NE/NNE boundary region with characteristics of the olfactory and hypophyseal placodes in vertebrates. Future studies of the mechanisms controlling formation of this primitive placodal territory, including potential functional studies in ascidians, promise to reveal basic principles underlying specification of ectodermal fates.

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Further Reading

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1.15 The Role of Transient, Exuberant Axonal Structures in the Evolution of Cerebral Cortex

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Glossary

connection In this article, connection is used to refer to situations for which the existence of synapses was demonstrated or appears very probable on grounds of electron microscopic data or light-microscopic data with sufficient resolution.

projection This refers to situations where the demonstration is missing, for example due to the use of retrogradely transported tract-tracers. Notice that the distinction is justified for the developing brain, although it is often unclear whether a certain projection is actually forming synapses. In most of this article, the terms are synonymous.

1.15.1 Introduction

Developmental mechanisms, some of which have needed retuning while others have been maintained, have both permitted and constrained evolution. It is therefore legitimate to ask which developmental mechanisms were modified and which were maintained in the evolution of the cerebral cortex. Comparative studies suggest that two processes dominated the evolution of cerebral cortex: tangential expansion, and increased regional differentiation. The most commonly proposed scenario, accounting for the increased tangential expansion of the cerebral cortex, is an increased period of symmetrical divisions in the proliferative ventricular and subventricular zones. Regional differentiation, however, is multifaceted and the emergence of a new cortical area in the course of evolution must have required the coordination of several different developmental processes.

A primary cause of regional differences in the cortical mantle is probably the differential expressions of genes along tangential gradients. It is unclear, however, how the regionalization of the cortical mantle, presumably caused by gene expression, corresponds to the parcellation of cerebral cortex into structurally and functionally distinct areas, that is, into its arealization. Indeed, the identity of a cortical area is defined by a large set of morphological and functional criteria. The morphological criteria include not only cytoarchitectonics, myeloarchitectonics, and sometimes molecular differences, but also, most importantly, differences in connectivity with thalamic nuclei and with other cortical regions. The functional criteria include the sensory, motor, or cognitive consequences of lesions, the response properties of individual neurons, and the patterns of activation during specific tasks. Assuming that the patterns of genetic expression might be the primary determinants of cortical regionalization, the question is: which other changes in developmental processes were required to achieve the full set of local differentiations that characterize a cortical area?

As mentioned above, connectivity is a central feature in the definition of a cortical area. In addition, however, it also determines some of the other criteria that define a cortical area, in particular its architectonics, the response properties of its neurons, and their participation in specific functional neuronal assemblies and/or processing streams. Thus, the appearance of a new cortical area in

evolution required adjustments of cortical connectivity. These adjustments, in turn, were a major factor in determinning arealization.

This article illustrates how the development of cortical connectivity is based on the following mechanisms: (1) exuberant development, i.e., initial distribution of cortical axons to territories wider than in the adult; (2) selection, based on specific axon/pathway and axon/target recognition mechanisms, as well as on axoaxonal interactions; and (3) selection/validation of the connections by activity.

I claim that the algorithms of connectional development listed above, while maintaining a coherent Bauplan across the mammalian radiation, provided the degree of flexibility required to accommodate genetically based regionalization of the cerebral cortex and thus played a major role in the emergence of new cortical areas. The article is mainly restricted to data and concepts that appeared within the last 10–15 years, since the older literature has been reviewed previously (Innocenti, 1991; O'Leary, 1992; see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates, Cerebral Cortical Folding Patterns in Primates: Why They Vary and What They Signify).

1.15.2 Macroscopic versus Microscopic Exuberance in the Development of Connections

Structural exuberance in development includes the overproduction of neurons and non-neuronal cells, as well as that of cellular components, in particular axons and/or axon collaterals, synaptic boutons, dendritic branches, and spines. The common theme is that a part or all of the juvenile structures are eliminated at later stages of development.

Leaving overproduction of neurons aside, two kinds of developmental exuberance have been described over the last 30 years. Both are involved in the construction of neural circuits.

Macroscopic exuberance refers to the formation of transient projections (and/or connections) between macroscopic partitions of the brain; it includes transient afferent and efferent projections between a cortical site and other macroscopic subdivisions of the brain, such as cerebellum, subcortical nuclei, spinal cord, or cortical areas. Microscopic exuberance refers to the formation, within a restricted cortical territory, of transient structures involved in the communication between neurons; it includes the formation of transient axonal and dendritic branches and/or synapses. Some of the transient structures, but not all, are formed within layers and/or columns where they are no longer found in the adult.

The distinction between the two types of exuberance is not always sharp, and it is essentially based on the methods used. In particular, in studies of synaptic counts, performed at the electron microscopic level, the origin of the supernumerary synapses could not be determined. In some systems of connection there is a smooth transition from macroscopic to microscopic exuberance: the production of exuberant structures becomes progressively more topographically circumscribed, as if the target was reached by progressively refined approximations.

1.15.3 Methodological Issues

Neuronal connections are usually assessed by tracers that are either actively transported or diffuse along axons driven by concentration gradients. Nevertheless, the usage of most tracers in the developing brain can be problematic. Uptake, transport, and diffusion of tracers can vary with age. This is particularly true with the lipophilic tracers of the DiI-DiO family, which tend to mark better the young unmyelinated axons and much less, or not at all, the older and myelinated ones. The existence of tracer-permeable gap junctions and leaky membranes in the young brain raises the possibility of transneuronal diffusion of tracers, producing false-positive results. Finally, tracers tend to be less effectively taken up and/or transported in the young nerve tissue, therefore failing to visualize connections which, if more mature, can be readily visualized. In some of the studies summarized below, particularly for the callosal connections of the cat, the same projections were studied at different ages with different tracers (horseradish peroxidase (HRP), wheat germ agglutinin (WGA)-HRP, fluorescent tracers, including fast blue and diamidino yellow, fluorescent beads, lipophilic tracers, and biocytin). Unfortunately this is not the case for other projections. The possibility that some juvenile connections may have been missed due to insufficiently sensitive tracing conditions must be kept in mind in the interpretation of negative results.

Another difficulty in using tracers in the developing brain is that certain target structures can undergo complex reshaping due to displacement of neuronal populations. Thus, what might appear to be a transient projection could in fact be a projection to the appropriate target, but which has not yet reached its final location. A protection against the latter type of artifact is provided by tracers that can remain in the neurons for a long time, without

being metabolized or eliminated. Tracers of this kind, e.g., fast blue and fluorescent beads, allow us to take snapshots of the state of the same connection at different developmental stages (Innocenti, 1991; O'Leary, 1992). They also permit the differentiation of neuronal death from axonal elimination in the deletion of the transient connections.

While the magnitude of exuberance and elimination can be difficult to estimate, it is clear that some projections are fully eliminated. This is the case, for example, in the corticofugal projections to the spinal cord or to the cerebellum. The most satisfactory quantification, however, comes from electron microscopic studies. These studies have shown a loss of 70% of the callosal axons in both cat and monkey, although this figure might still underestimate the real loss (below).

1.15.4 Exuberant Projections/ Connections in Cortical Development

The first demonstration of macroscopic exuberance in development, i.e., of the formation of long/transient projections, came from the study of connections between the visual areas of the two hemispheres in the cat (Innocenti *et al.*, 1977; reviewed in Innocenti, 1991; O'Leary, 1992). Parts of areas 17 and 18 devoid of callosal connections in the adult exhibited transient projections to the contralateral hemisphere at birth. The elimination of the projections was fast, and was mostly terminated by postnatal day 21, although some elimination might have continued until day 30 and beyond. The elimination was due to selective loss of axons and the neurons, giving rise to the transient callosal projections, forming permanent connections in the ipsilateral hemisphere, by selection of collaterals. These findings were confirmed and extended by the demonstration of exuberant callosal connections between the somatosensory areas, and of projections from cerebral cortex to the spinal cord, cerebellum, and to other cortical areas. Particularly striking among the latter was the discovery of transient projections from the auditory to the visual cortex in both hemispheres in the cat (reviewed in Innocenti, 1991). Equally striking was the more recent demonstration of exuberant projections from the temporal cortex of the monkey into limbic structures (Webster *et al.*, 1991a, 1991b). Finally, the work of several groups focused attention on the microscopic aspects of exuberant connections by showing how elimination of axon collaterals of pyramidal neurons leads to the formation of local, clustered connections. Exuberant intra-areal axons were also described: within sublayers, in the monkey area 17, and as long tangential axons spanning the white matter under area 17 in the cat. In the cat, callosal axons, both those that are maintained and those that are later eliminated, initially form transient branches, first in the subplate and then in the gray matter (Aggoun-Zouaoui *et al.*, 1996; Bressoud and Innocenti, 1999). In addition, the number of synaptic boutons produced in the gray matter overshoots the adult number (Aggoun-Zouaoui *et al.*, 1996; Bressoud and Innocenti, 1999). As mentioned above, it appears that the production of exuberant axonal structures becomes progressively more topographically circumscribed, as if the target were reached by correspondingly more refined approximations. Similar events were described in the striate and extrastriate areas of the cat (Bressoud and Innocenti, 1999).

1.15.5 Exuberance and Selection versus Connectional Specificity

Developmental exuberance in no way excludes the existence of selectivity and order in the formation of the juvenile projections/connections. Both might be the expression of cellular specificities responsible for guiding growing axons along given pathways and determining their choice of a target. Most of the evidence has been gathered in studies of visual callosal connections in the cat.

First, the juvenile corticocortical connections, including the exuberant ones, are topographically organized from their early stages. Thus, injections of tracers spaced in the anteroposterior direction in one hemisphere label correspondingly spaced territories in the other hemisphere. Second, the cortical projections exhibit laminar specificity from the earliest stages in development. Corticocortical axons mainly originate from layers 3 and 6, although the relative contribution of the two layers to a given projection can change in development, due to the elimination of exuberant projections from layer 3. Third, from the earliest stages of their development, cortical axons can be classified into different types based on their pattern of projection to areas in the contralateral hemisphere. Interestingly, this targeting specificity includes axons that establish transient projections. This suggests that the whole projection, including both the transient and the permanent fractions, consists of a mosaic of cell types with different growth/targeting specificities (Bressoud and Innocenti, 1999). Finally, origin-to-target

selectivity is expressed at the time axons grow near, and into their terminal sites. Irrespective of their final fate, both callosal and intrahemispheric axons reach the white matter/gray matter border, which contains a largely transient neuronal population, the subplate, where they branch profusely. Then, the axons to be maintained invade the gray matter, where they develop terminal arbors and synapses, while the transient axons remain mainly in the white matter and are subsequently eliminated.

After entering the gray matter, axons exhibit further specific growth. Axonal branching and the formation of synapses are progressively focused on the sites of adult termination, although transient branches and synapses are also formed (Bressoud and Innocenti, 1999). The overproduction and elimination of synapses occur without noticeable changes in the topography of the connections, although presumably it modifies the strength of the connections.

1.15.6 Testing the Role of Exuberance in the Evolution of Cerebral Cortex

The hypothesis that exuberant development of connections could provide a permissive mechanism favoring cortical evolution can be tested against a number of potentially invalidating conditions. Exuberant development of connections should be found across species and systems. The fate of the juvenile connections, whether maintenance or elimination, should be modulated by factors that could have operated during the evolution. Finally, one might expect a greater developmental plasticity of cortical connections in areas that underwent the most massive evolution.

1.15.6.1 Exuberant Development Is Found across Phylogenetically Distant Species

Transient, exuberant projections/connections occur across all the mammalian species that have been studied (Table 1). Most of the studies have involved rodents, carnivores, and primates. However, exuberant corticocortical and/or corticofugal projections have also been demonstrated in rabbit and opossum. These findings can be mapped on to the evolutionary trees of the mammalian radiation (Figure 1). The fact that exuberant projections in development span widely across the mammalian radiation suggests that they were indeed present in the ancestors of most or all the extant mammals, as required by the hypothesis that they played a role in evolution.

1.15.6.2 Exuberant Development Is Found across Systems of Cortical Connections

Exuberant development occurs in several different types of cortical connections, including interhemispheric, intrahemispheric, and local connections, as well as the corticofugal connections. Sensory, motor, and association areas are involved. This is not to say that the magnitude of exuberance/elimination is the same for the different species, systems, and types of connection (Barone *et al.*, 1996), although cross-species comparisons must be made prudently, given the above-mentioned difficulties in the quantification of the transient projections. Furthermore, cross-species comparisons can be complicated by different speeds of axonal development. Interestingly, electron microscopic counts of callosal axons have provided similar estimates of elimination in cat and monkey (reviewed in Innocenti, 1991). However, this similarity might be fortuitous since both studies lack an estimate of the life span of individual axons. Obviously, if the life span of the transient axons differed in the two species, the quantitative estimates of axonal exuberance/elimination would have to be corrected.

The occurrence of developmental exuberance in the different kinds of cortical connections suggests that the newly emerging areas are able to establish an adequate complement of connections in evolution. However, different cortical territories might differ with respect to the type and/or amount of transient, exuberant projections they send and/or receive in development. These hypothetical differences might have favored the emergence of new cortical areas at some specific locations. Thus, precise estimates of the exuberant connectivity of the different cortical territories in a given species might hint at their evolutionary potential.

1.15.6.3 Multiple Factors Regulate the Maintenance/Elimination of Exuberant Connections

Transient connections can be maintained or eliminated by experimental manipulations of the developing cortex.

Although these conditions do not necessarily mimic the evolutionary history of the cerebral cortex, they highlight the mechanisms whose alteration in evolution could have affected the development of corticocortical connections. Particularly important is the evidence linking maintenance/elimination of the juvenile connections to information coming from the sensory periphery, since in evolution changes in body and brain

Table 1 Transient (exuberant) axonal projections in development cerebral cortex (1976–2004)

Species	*Thalamocortical*		*Callosal*		*Intrahemispheric/area*		*Corticofugals*	
	Macro	*Micro*	*Macro*	*Micro*	*Macro*	*Micro*	*Macro*	*Micro*
Rodents (rat, hamster, mouse)	38, 48, 51	61	30, 31, 38, 49, 52		38		1, 5, 14, 15, 29, 50, 54, 62	32, 47
Carnivores (cat, ferret)	7	33, 34, 35, 39	2, 6, 22, 24, 25, 26, 27, 28	2, 3, 6	4, 17, 19, 25, 56, 53	10, 11, 41, 42, 58	8, 40, 46, 63, 64	
Primates (rhesus)	16, 45	59	12, 18, 37		36, 65	60	23	
Others, (rabbit, opossum)			13				9, 20, 21	

Macroexuberance refers to situations where the projection is probably due to long axons.
Microexuberance refers to situations where the projection is due to local branches or synapses. In some cases, however (e.g., in the case of electron microscopic synaptic counts or anterograde transport data), the two conditions cannot be easily differentiated.
Dehay *et al.* (1988a) reported exuberant callosal projections from area 18 of the rhesus monkey but not from area 17.
References: (1) Adams *et al.* (1983); (2) Aggoun-Zouaoui and Innocenti (1994); (3) Aggoun-Zouaoui *et al.* (1996); (4) Assal and Innocenti (1993); (5) Bates and Killackey (1984); (6) Bressoud and Innocenti (1999); (7) Bruce and Stein (1988); (8) Bruce (1993); (9) Cabana and Martin (1984); (10) Callaway and Katz (1990); (11) Callaway (1998); (12) Chalupa and Killackey (1989); (13) Chow *et al.* (1981); (14) Curfs *et al.* (1994); (15) D'Amato and Hicks (1978); (16) Darian-Smith and Darian-Smith (1993); (17) Dehay *et al.* (1984); (18) Dehay *et al.* (1988a); (19) Dehay *et al.* (1988b); (20) Del Caño *et al.* (1997); (21) Distel and Holländer (1980); (22) Feng and Brugge (1983); (23) Galea and Darian-Smith (1995); (24) Innocenti and Caminiti (1980); (25) Innocenti and Clarke (1984); (26) Innocenti and Clarke (1984); (27) Innocenti *et al.* (1977); (28) Innocenti (1981); (29) Iriki *et al.* (1988); (30) Ivy *et al.* (1979); (31) Ivy and Killackey (1981); (32) Joosten and Van Eden (1989); (33) Kato *et al.* (1983); (34) Kato *et al.* (1984); (35) Kato *et al.* (1986); (36) Kennedy *et al.* (1989); (37) Killackey and Chalupa (1986); (38) Kolb *et al.* (1994); (39) LeVay *et al.* (1978); (40) Leonard and Goldberger (1987); (41) Luhmann *et al.* (1986); (42) Luhmann *et al.* (1990); (43) Manger *et al.* (2002a); (44) Manger *et al.* (2002b); (45) Meissirel *et al.* (1990); (46) Meissirel *et al.* (1993); (47) Mihailoff *et al.* (1984); (48) Minciacchi and Granato (1989); (49) Mooney *et al.* (1984); (50) Murakami *et al.* (1993); (51) Nicolelis *et al.* (1991); (52) Olavarria and Van Sluyters (1985); (53) Olavarria (2001); (54) O'Leary and Stanfield (1986); (55) Payne and Siwek (1991); (56) Price (1986); (57) Price and Blakemore (1985); (58) Price and Zumbroich (1989); (59) Rakic (1976); (60) Rakic *et al.* (1986); (61) Rios and Villalobos (2004); (62) Stanfield *et al.* (1982); (63) Tolbert and Panneton (1983); (64) Tolbert *et al.* (1984); (65) Webster *et al.* (1991a); (66) Webster *et al.* (1991b).

had to be coordinated. Strong evidence has accumulated that maintenance and elimination of callosal connections may be under the control of thalamocortical input, conveying information originating more peripherally, in the retina, in the case of the visual system. Studies in the cat and in the rat have shown that callosal as well as corticocortical connections require retinal input for their maintenance (reviewed in Innocenti, 1991; Zufferey *et al.*, 1999). The first evidence of the role of peripheral input in shaping callosal connections came from the work of Shatz (1977), demonstrating abnormal callosal connections in the Siamese cat, as a consequence of the abnormal crossing of retinal axons in this species. The finding that visual callosal axons are either lost or altered in animals binocularly deprived of vision by eyelid suture or eye enucleation (reviewed in Innocenti, 1991) stressed the role of the periphery, mediated in part by activity, in selection of the juvenile axons. The fact that the axons surviving the binocular deprivation are stunted brought further support to the notion that the periphery controls the development of the connections (Zufferey *et al.*, 1999).

Perhaps the strongest argument indicating that evolution did indeed operate through a periphery-driven selection of exuberant connections in development is provided by the analysis of callosal connections at the border between visual areas 17 and 18 in different species. As reviewed by Olavarria (2001), the width of the callosally connected region near the 17/18 border varies across mammals. The callosally connected region near the 17/18 border represents portions of the visual field close to the representation of the visual field midline. It follows that in different species, different fractions of these areas, and consequently different extents of the visual field representations, ought to be callosally connected. Indeed, at comparable elevations, the portion of the visual field represented in the callosally connected portion of the visual areas appears to be wider in the ferret, where it includes azimuths well beyond 25° (Manger *et al.*, 2002a) than in the cat, where it seems not to exceed 15° (Payne and Siwek, 1991). Since in all mammals (including the ferret; Innocenti, unpublished observations) the visual callosal connections develop by exuberance, one can safely infer that some of the projections that are normally transient in one species are maintained in another.

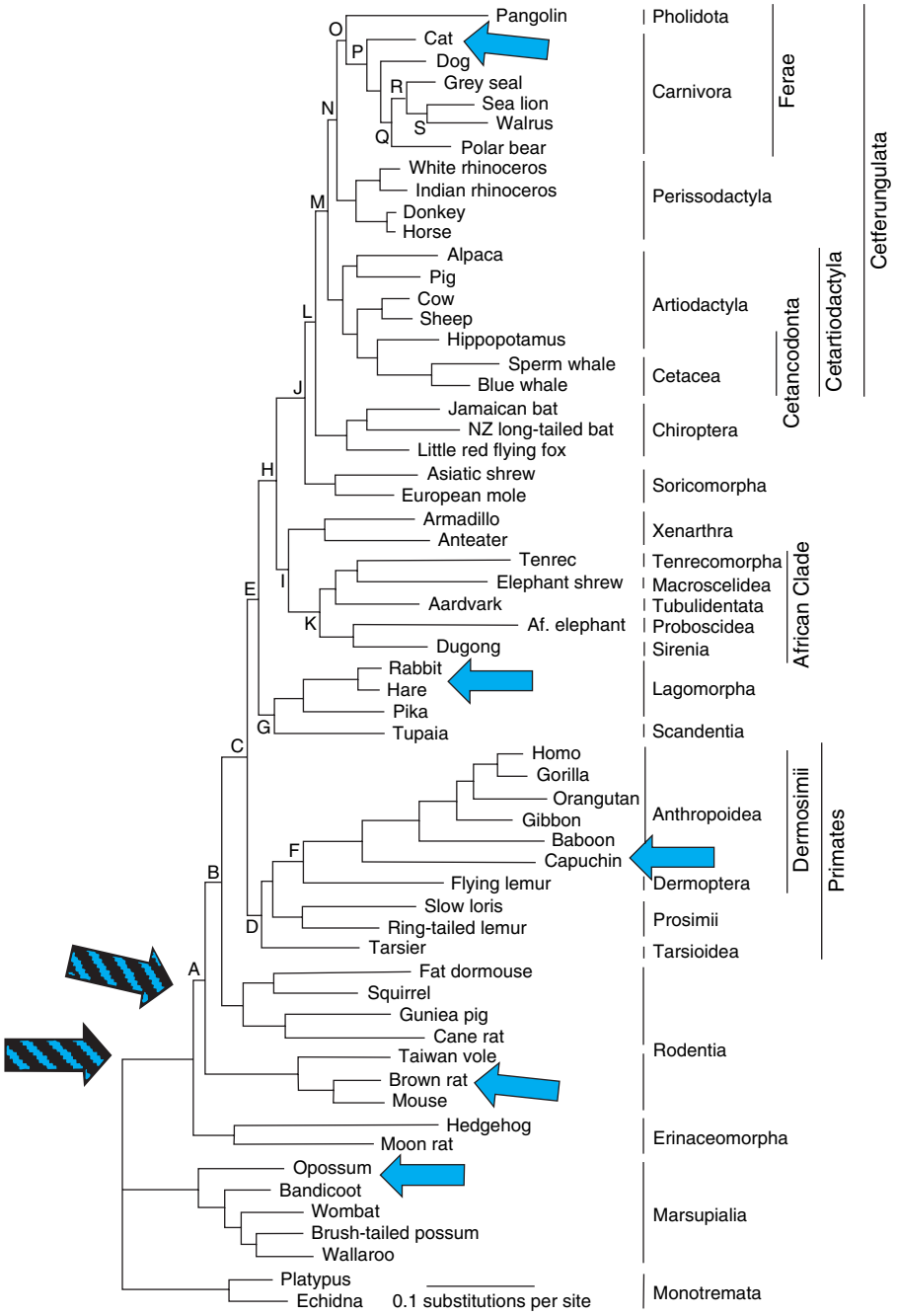

Figure 1 Exuberant connections in development were reported in a number of species (small arrows). This suggests that this mode of development appeared in the earliest ancestors of mammalian radiation (large hatched arrows) and was preserved in evolution. Adapted from Arnason, U., Adegoke, J. A., Bodin, K., *et al.* 2002. Mammalian mitogenomic relationships and the root of the eutherian tree. *Proc. Natl. Acad. Sci. USA* 99, 8151–8156. Copyright (2002) National Academy of Sciences, USA, with permission.

1.15.6.4 More Developmental Plasticity in Cortical Areas Which Evolved More?

Any hint of developmental processes capable of channeling evolution might reveal a source of directedness in the otherwise haphazard emergence of the phenotype by trial and error, which is the legacy of classical Darwinism. Some hypotheses on directedness in the evolution of cortical areas can be derived from what has been discussed above. First, the emergence of cortical areas might have been favored at certain specific locations within the cortical surface. These locations should be those with the richest complement of afferent and efferent exuberant projections in

development. Second, areas at these locations might have evolved to a large extent under the pressure of information coming from the body periphery. Third, these newly emerged areas might also be endowed with the highest degree of developmental plasticity.

Large differences exist in the number of cortical areas across species, but they are probably greatest in the parietal, temporal, and prefrontal cortex. It is unknown whether developmental exuberance is largest in these areas, as would be required by the first of the hypotheses discussed above, although this would only be an approximation of the developmental differences in the ancestors. Consistently with the second hypothesis proposed above, only two areas seem to exist in the posterior parietal cortex of the ferret (Manger *et al.*, 2002b). This is far from the complexity found in the monkey, even taking into account the possibility that some areas might have been missed in the ferret. These differences can be tentatively ascribed to the much more complex repertoire of hand and eye movements, and eye–hand coordination in the monkey compared to the ferret.

Consistently with the third hypothesis mentioned above, experiments with early lesions have revealed an important reorganization of connections in the parietal cortex of the ferret (Restrepo *et al.*, 2003). Similarly, lesions in the temporal cortex of the newborn monkey led to the stabilization of otherwise transient connections (Webster *et al.*, 1991a, 1991b).

1.15.7 Conclusions

Language-related areas are among the most recent acquisitions of the mammalian brain. The integration of these areas into the cortical network, and more generally the functional and structural lateralization of the human brain, would not have been possible without a massive reorganization of corticocortical connectivity, in particular of callosal connections. In this article, I have developed some arguments in favor of the view that developmental exuberance, an interesting blend of directedness and groping around of axons in the formation of cortical connections, appeared early in evolution and was maintained through phyologenesis. Indeed, the exuberant juvenile axonal projections/connections provided the substrate from which new connections could be selected. At the same time, the rules underlying the selection, including information coming from the periphery, channeled evolution. Perhaps the maintenance of rule-driven but flexible developmental syntaxes might be the best key to apprehending how evolution, unlike human-directed mutagenesis, generated the multitude of viable brain architectures we know.

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1.16 Neural Wiring Optimization

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Glossary

adjacency rule	"If components are connected, then they are adjacent." A wire-saving heuristic for laying out a system; also a simple wire-cost measure for such layouts.
component placement optimization	The positioning of a system of interconnected components to minimize total connection cost.
network optimization theory	The characterization of minimized use of limited connection resources (e.g., wire length) in a system.
NP-hard	A set of problems, each conjectured to require computation time typically on the order of a brute-force search of all possible solutions, and often therefore intractable.
size law	For some optimized systems, the smaller a subset, the poorer its optimization.
Steiner tree	A minimum-cost arbor connecting a set of terminal loci, which may include branch junctions not at terminals.

Long-range connections in the brain are a critically constrained resource, hence there may be strong selective pressure to finely optimize their deployment. The formalism of scarcity of interconnections is network optimization theory, which characterizes the efficient use of limited connection resources. The field matured in the 1970s for microcircuit design, typically to minimize the total length of wire needed to make a given set of connections among components. When this simple 'Save wire' idea is treated as a generative principle for nervous system organization, it turns out to have applicability: to an extent, 'instant brain structure – just add wire minimization'. The most salient caveat is that, in general, network optimization problems are easy to state, but enormously computationally costly to solve exactly; those reviewed here are NP-hard. We focus on the Steiner tree concept and on component placement optimization, with emphasis on the latter.

1.16.1 Neuron Arbor Optimization

The basic concept of an optimal tree is as follows: given a set of loci in 3-space, find the minimum-cost tree that interconnects them, e.g., the set of interconnections of least total volume. If branches are permitted to join at sites other than the given terminal loci (the leaves and root), the minimum tree is of the cheapest type, a Steiner tree. If the synapse sites and origin of a dendrite or axon are treated in this way, the optimization of the dendrite or axon can be evaluated. Approximately planar arbors in 2-space are easier to study. The most important feature of naturally occurring arbors – neuronal, vascular, plant, water drainage networks, etc. – is that, unlike much manufactured circuitry, for each internodal junction, trunk costs (e.g., diameter) are higher than the two branch costs. When such Y junctions are examined in isolation, positioning of the junction sites shows minimization of total volume cost to within approximately 5% of optimal (Cherniak, 1992). Furthermore, the relation of branch diameters to trunk diameter fits a simple fluid-dynamical model for minimization of wall drag of internal laminar flow: neuron arbors act like flowing water.

This Y-tree cost minimization constitutes local optimization. Only one interconnection pattern or topology is involved. Such small-scale optimization does not entail larger-scale optimization, where local trade-offs are often required. When more complex portions of a total arbor are analyzed, the optimization problem becomes a global problem, with an exponentially exploding number of alternative possible interconnection topologies. For example, a nine-terminal tree already has 135 135

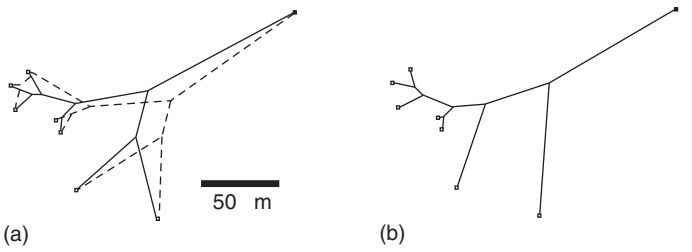

Figure 1 Actual vs. optimal neuron arbors, mouse thalamus extrinsic axon, ascending reticular formation (from data of Scheibel and Scheibel, 1966). The arbor best fits a minimized-volume model. a, Wire-frame representation of an eight-terminal subtree of an observed arbor. Actual tree, with actual topology in its actual embedding, appears as dashed lines. Optimal embedding with respect to volume minimization of the actual topology is superimposed as solid lines. The cost in volume of the actual arbor exceeds that of the optimized embedding of its topology by 2.20%. b, "Best of all possible topologies" connecting the given terminal loci: the optimal topology with respect to volume, optimally embedded. The volume cost of the actual arbor exceeds that of the optimal topology by 2.47%. Only 10 of the 10 395 possible alternative topologies here (approximately 0.14%) have lower total volume costs, when optimally embedded, than the actual topology (Cherniak *et al.*, 1999).

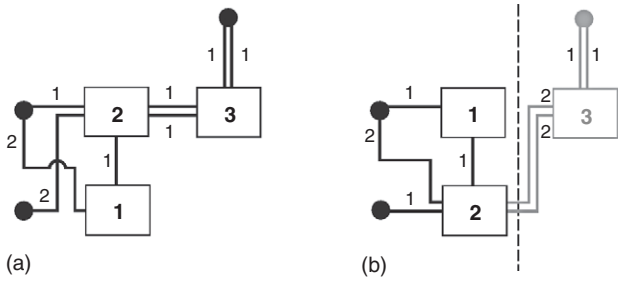

Figure 2 Simple illustration of component placement optimization: minimization of total length of connections. The complete system here consists of a 2-D array of movable components (1, 2, and 3) with given interconnections. All connections are of equal cost per unit length. Component 1 connects to a fixed edge terminal and also to 2; component 2 connects to two fixed edge terminals, and to 1, and also twice to 3; component 3 also connects twice to a fixed edge terminal. a, A globally optimal layout of the three components (cost: 10); cost includes a decussation (connection crossing). b, A complete layout that lacks the decussation, but now is suboptimal (cost: 11). Note also that if the system subset is restricted to only components 1 and 2, including connections to edge terminals, then their layout in (b) is cheaper than their layout in (a), with total connection length reduced from 6 to 5. Hence, these layouts also illustrate global optimization (a) at the trade-off expense of a locally suboptimal cost (b); connection minimization of a total system does not entail connection minimization of its subsets. (A similar pattern holds here for the simpler connection cost measure of adjacency-rule violations explained in text (Cherniak *et al.*, 2004).)

alternative topologies, each of which must be generated and costed to verify the best solution (see Figure 1). Neuron arbor samples, each with three internodal Y junctions, minimize their volume to within approximately 5% of optimal (Cherniak *et al.*, 1999). This optimality performance is consistent for dendrites (rabbit retina ganglion and amacrine cells, and cat retina ganglion cells) and also for some types of axons (intrinsic and extrinsic mouse thalamus).

1.16.2 Component Placement Optimization

Another key problem in microcircuit design is component placement optimization (also characterized as a quadratic assignment problem): given a set of interconnected components, find the placement of the components on a two-dimensional (2-D) surface that minimizes the total cost of connections (e.g., wire length). Again, this concept seems to account for aspects of neuroanatomy at multiple hierarchical levels.

"Why the brain is in the head" is a one-component placement problem. That is, given the positions of receptors and muscles, positioning the brain as far forward in the body axis as possible minimizes total nerve connection costs to and from the brain, because more sensory and motor connections go to the anterior than to the posterior of the body. This seems to hold for the vertebrate series (e.g., humans) and also for invertebrates with sufficient cephalization to possess a main nervous system concentration (e.g., nematodes).

Multiple-component problems again generally require exponentially exploding costs for exact solutions; for an n-component system, $n!$ alternative layouts must be searched (see Figure 2). One neural wiring optimization result is for placement of the 11 ganglionic components of the nervous system of the

roundworm *Caenorhabditis elegans*, with ~1000 interconnections. This nervous system is the first to be completely mapped (Wood, 1988), which enables fair approximation of wire lengths of connections. When all 39 916 800 alternative possible ganglion layouts are generated, the actual layout turns out in fact to be the minimum wire-length layout (Cherniak, 1994a). Some optimization mechanisms provide convergent support for this finding: a simple genetic algorithm, with wire cost as fitness measure, will rapidly and robustly converge on the actual optimal layout (Cherniak *et al.*, 2002). Also, a force-directed placement (mesh of springs) algorithm, with each connection approximated as a microspring acting between ganglion components, attains the actual layout as a minimum-energy state, without much trapping in local minima (Cherniak *et al.*, 2002).

There is statistical evidence that this brain as microchip framework also applies in the worm down to the level of clustering of individual neurons into ganglionic groups and to soma positioning within ganglia to reduce connection costs (Cherniak, 1994a).

Finally, the wiring-minimization approach can be applied to placement of functional areas of the mammalian cerebral cortex. Since wire lengths of intrinsic cortical connections are difficult to derive, one strategy is to explore a simpler measure of connection cost, conformance of a layout to an adjacency rule: if components *a* and *b* are connected, then *a* and *b* are adjacent. An exhaustive search of all possible layouts is still required to identify the cheapest one(s). One promising calibration is that the actual layout of the nematode ganglia is among the top layouts with fewest violations of this adjacency rule. For 17 core visual areas of macaque cortex, the actual layout of this subsystem ranks in the top 10^{-7} layouts best fitting this adjacency costing; for 15 visual areas of cat cortex, the actual layout ranks in the top 10^{-6} of all layouts (Cherniak *et al.*, 2004; see Figure 3) (see The Role of Vision in the Origin and Evolution of Primates, Primate Brain Evolution in Phylogenetic Context, Visual Cortex: Evolution of Maps and Mapping, Captured in the Net of Space and Time: Understanding Cortical Field Evolution, The Evolution of Visual Cortex and Visual Systems).

In general, a Size Law seems to apply to cases with such local–global trade-offs: the larger proportion of a total system the evaluated subsystem is, the better its optimization (see Figure 4). Similar findings have also been reported for rat olfactory cortex and for rat amygdala (Rodriguez-Esteban and Cherniak, 2005). For the largest systems studied (visual, auditory, and somatosensory areas of cat cortex), there is evidence of optimization approaching limits of current detectability by brute-force sampling techniques. A similar Size Law pattern also appears to hold for Steiner tree optimization of neuron arbor topologies (see Figure 1). The

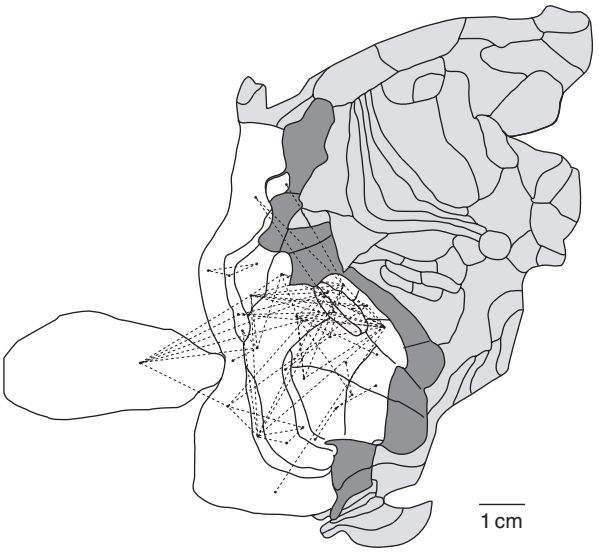

Figure 3 Parcellation of functional areas of macaque cerebral cortex (after Felleman and Van Essen, 1991). Component placement optimization analysis of a layout of 17 core areas (white) of visual cortex, along with immediately contiguous edge areas (dark gray). Reported interconnections among core areas are indicated by dotted lines. Rostral is to the right. In a connection cost analysis, this actual layout of the core visual system ranks in the top one-millionth of all alternative layouts (Cherniak *et al.*, 2004).

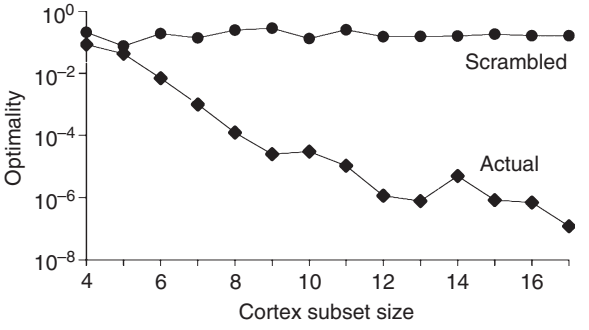

Figure 4 Size Law for macaque visual cortex areas. The system of components here consists of 17 contiguous visual areas of macaque cortex, as in Figure 3. A layout is scored in terms of its violations of the adjacency rule. A series of nested compact subsets of the set of visual areas was generated; each subset was compared with all possible alternative layouts of that subset for adjacency-rule optimality. As subset size increases, optimality ranking of the actual layout consistently improves (with two exceptions, $p < 0.02$). For comparison, the corresponding analysis for a layout of the 17 visual areas with their adjacencies randomly shuffled shows no trend toward improving optimality. Note that this analysis includes only 17 of the total 73 areas of macaque cortex.

picture then is of limited connections deployed very well, a predictive success story. The significance of ultrafine neural optimization remains an open question. Levels of connection optimization in the nervous system seem unlike levels of optimization elsewhere in organisms.

1.16.3 Optimization: Mechanisms and Functional Roles

Mechanisms of neural optimization are best understood against the background that the key problems of network optimization theory are NP-complete, hence exact solutions in general are computationally intractable. For example, blind trial and error exhaustive search for the minimum-wiring layout of a 50-component system (such as all areas of a mammalian cerebral cortex), even at a physically unrealistic rate of one layout per picosecond, would still require more than the age of the Universe (Cherniak, 1994b). Instead, even evolution must exploit quick and dirty approximation/probabilistic heuristics.

One such possible strategy discernible above is optimization for free, directly from physics. That is, as some structures develop, physical principles cause them automatically to be optimized. We reviewed above some evidence for arbor optimization via fluid dynamics, and for roundworm ganglion layout optimization via mesh of springs force-directed placement simulation. Although neuron arbors appear to optimize on an embryological timescale, component placement optimization appears to proceed much more slowly, on an evolutionary timescale. For component placement optimization, there is the chicken–egg question of whether components begin in particular loci and make connections, or instead start with their interconnections and then adjust their positions, or some mix of both causal directions. It is worth noting that both a force-directed placement algorithm for ganglion layout and genetic algorithms for layout of ganglia and of cortex areas suggest that simple 'connections → placement' optimization processes can suffice.

Wiring optimization is, of course, subject to many basic constraints and so cannot be ubiquitous in the nervous system; the question is where it does in fact occur and how good it is. Trade-offs of local optimality for better cost minimization of a total system (as Figure 2 illustrates) are one way in which global optimization can be obscured.

If the brain had unbounded connection resources, there would be no need or pressure to refine employment of wiring. Thus, to begin with, the very fact of neural resource limitations appears to drive 'Save wire' fine-grained minimization of connections. Another part of the functional role of such optimization may be the picture here of 'physics → optimization → neuroanatomy'. Perhaps such an economical means of self-organizing complex structure generation eases transmissibility through the information bottleneck of the genome. This constitutes a thesis of nongenomic nativism that some innate complex biological structure is not encoded in DNA, but instead derives from basic physical principles (Cherniak, 2005).

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1.17 Principles of Brain Scaling

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Glossary

allometry A relationship between the sizes of two biological structures or other quantities that follows a power law. For example, suppose that L is the average length of an animal's life (a biological quantity) for a particular species, and W is the average weight for adults of that species (a measure of total animal size). Further suppose that, across species in some taxonomic group, the length of life is related to body weight by the equation $L = aW^b$ for some quantities a and b that are independent of both L and W. This relationship would constitute an allometry, and lifetime would be said to have an allometric relation to animal size. The constant a is called the scale factor and b is known as the allometric constant for the two quantities L and W.

grade shift If an allometric relationship holds for two different taxa with the same allometric constant but different scale factors, it is said that a grade shift has occurred. For example, there is an allometric relationship between brain size and body weight for both monkeys and teleost fish, but monkeys have larger brains; that is, a monkey always has a larger brain than a fish of the same weight, although a tiny monkey might have a smaller brain than a giant fish.

isometry An isometry is a special case of an allometry for which the allometric constant is unity. That is, two biological quantities are said to be isometric when they are related by a simple proportionality.

map Brain areas, like the thalamus or visual cortex, are said to have a map when the neurons are arranged in a way that preserves the neighbor relationships present in some reference structure. For example, the visual world is projected onto the retina, and the neighbor relations in the visual world are preserved in the projection of the retina to the visual thalamus, and from the visual thalamus to the visual cortex. Thus, both the visual thalamus and the visual cortex have a map of the visual world (the reference structure). Although a map must preserve neighbor relations, in general, other features may not be preserved: ordinary two-dimensional maps of the world keep things on the globe next to each other, but they change the size and shape of the continents. It is generally believed that all brain structures have maps but – as for language cortex or the olfactory cortex, for example – we do not know the reference structure for the map.

scalable architecture A term from computer science that refers to designs for computing circuits that can be made more powerful – that is, can carry out the computation for which they were intended more quickly or accurately – by simply increasing the size of the circuit (by, for example, increasing the number of computing elements) while keeping the same design. Familiar digital computers do not have a scalable architecture, which means that they must be redesigned each time their power is increased.

self-similar function A function that depends on parameters which change its size on a graph without changing its shape. A Gaussian is an example of a self-similar function because a plot of it still has the familiar bell-shape even when its parameters (its mean and standard deviation) are changed.

1.17.1 Introduction

The extent to which the brains of all mammals share a common design is striking: even though the brains of mammalian species vary in size by more than three orders of magnitude, the essential features of their design are unchanging (Butler and Hodos, 1996; Striedter, 2005; see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates, Primate Brain Evolution in Phylogenetic Context, The Evolution of Parallel Visual Pathways in the Brains of Primates, Brain Size in Primates as a Function of Behavioral Innovation, Constraints on Brain Size: The Radiator Hypothesis). This observation means, for example, that rodent, feline, and nonhuman primate brains can serve as model systems for studying principles of structure and function that apply to the human nervous system. From a computational perspective, then, one of the most remarkable features of the mammalian brain is that it has a scalable architecture (Comer, 2005). That is, the computational power of a brain can be increased continuously and gracefully by adding more components while adhering to a single basic design.

Understanding the scalable architecture of the mammalian brain is clearly important for determining how neuronal circuits are designed and how they compute (see The Evolution of Encephalization). And to elucidate scalability, one must learn the rules followed when brain size and computing power are increased. An important quantitative tool for investigating scalability is allometry (Huxley, 1932; Schmidt-Nielsen, 1984), the study of how the size of one part of the brain scales up with the size of another part as the entire brain is enlarged. The rules that describe the relationship between the sizes of two different structures in brains of different sizes are called allometries, or allometric relations, or scaling relations. Two examples of allometric (or, equivalently, scaling) relations are illustrated in Figure 1, where the size of primary visual cortex (V1) – the first cortical processing center for visual information – in various primates is compared with the size of the lateral geniculate nucleus (LGN), the immediate source of the cortex's visual information. Two different measures of size are used here. In Figure 1a, the volume of V1 for a range of primate species is plotted, on double logarithmic axes, as a function of the volume of the LGN (Stephan *et al.*, 1981; Frahm *et al.*, 1984). The relationship is linear on this double logarithmic plot with a slope of 1.125. The sizes of the same two structures are compared again (for the same primate species) in Figure 1b, this time with size being measured by the number of neurons present in each structure rather than by volume; that is, Figure 1b presents the logarithm of the number of cortical neurons processing visual information (the number of neurons in V1) as a function of the logarithm of the number of neurons providing that information (the number of neurons in the LGN) (Stevens, 2001). As before, the data points fall along a straight line, but this time the slope of the line is $3/2 = 1.5$, not 1.125. Here, then, are two scaling laws – allometric relationships – that describe aspects of visual system scalability, and any theory of how the visual system processes information must account for these scaling relationships.

1.17.2 The Interpretation of Scaling Laws

The relationships in Figure 1 have a particular functional form; they are power laws

$$S = as^b,$$

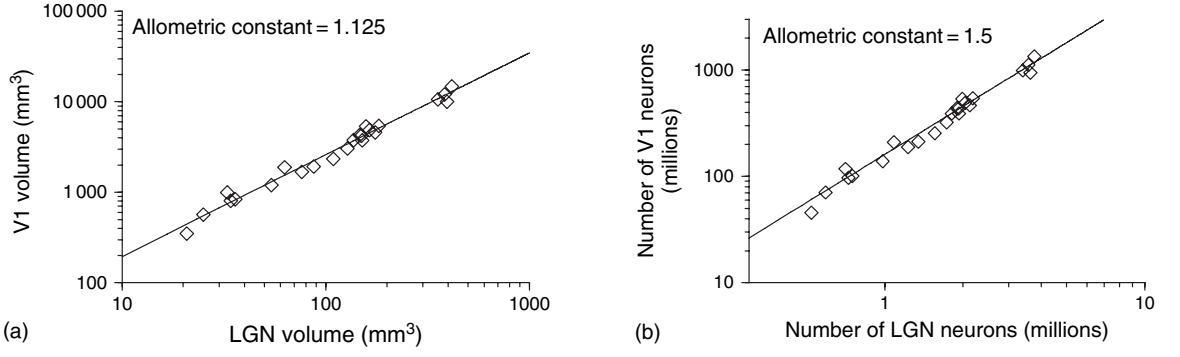

Figure 1 Relationship between the size of primary visual cortex (V1) and the lateral geniculate nucleus (LGN) for 23 primates (haplorhines). a, Size of the two structures is measured as volume (mm^3). The allometric constant is 1.125. b, Size of the two structures is measured in numbers of neurons in each. The allometric constant is 1.5.

with S being the size of the cortex, s the size of the LGN, and a and b constants ($b = 1.125$ in Figure 1a and 1.5 in Figure 1b). Taking the logarithm of this equation, one finds that

$$\log S = b \log s + \log a,$$

an equation with the form

$$y = bx + c$$

if one defines $y = \log S$, $x = \log s$, and $c = \log a$. This means that, on a double logarithmic plot like the ones in Figure 1, a power law results in a straight line with slope b and intercept of $c = \log a$. The constant a is called the scale factor, and the constant b is known as the exponent or the allometric constant. Note that if the exponent $b = 1$, the allometric relationship becomes a simple proportionality

$$S = as.$$

Clearly, although power laws result with either measure of structure size (structure volume in Figure 1a as opposed to number of neurons in Figure 1b), the values of the allometric constants differ (1.125 vs. 1.5 in Figure 1) according to how size is measured.

The relationship displayed in Figure 1 is a power law, but the functional form might, in general, be some other type of function. For example, one could imagine that there might be some pair of variables R and r, representing the sizes of two hypothetical structures, for which the relationship is

$$R = a(1 - e^{-br}),$$

with constants a and b; this is an exponential rather than a power law. Although this exponential equation does relate the sizes of two structures, it would not count as an allometric relationship because the term allometry is reserved for just those pairs of structures whose sizes are related by a power law. Power laws arise in many situations, and their study has been particularly important in various areas of physics (Barenblatt, 1996).

Empirically, many pairs of brain structures have been found to follow power laws with various different allometric constants (Huxley, 1932; Schmidt-Nielsen, 1984; Striedter, 2005). In some of these cases, the actual functional form of the relationship is indeed a power law and, in other cases, the functional form is not really a power law but can be approximated as one over some restricted range of sizes. In general, just as almost any equation can be approximated over a limited range by a linear equation, so can almost any relationship describing the sizes of a pair of structures be approximated, on a double logarithmic plot, as a straight line (which is equivalent to a power law). I am concerned here with those situations that produce actual power laws – that is, true allometric relationships – and I will not consider (except for one case) why functional forms other than power law might occur.

In the following, I give three different ways that true allometric relations can arise. The first is the original interpretation, due to Julian Huxley, of differential growth; Huxley's view was that allometries arose when one structure grew faster than another (Huxley, 1932). If, on the other hand, the growth rate of two structures were always exactly the same, then the relative size of the structures would be constant and they would be said to have an isometric (as opposed to allometric) relationship to one another; this is a special case of the allometric relationship with the allometric constant $b = 1$.

A second way in which allometric relations can be generated is through the preservation of the form of structures. Often, one has the idea that structure and function are intimately related so that form must be evolutionarily preserved in order to preserve function. For example, the hand is a structure beautifully designed for fine manipulations of elements in the environment, and a single species keeps the same form of the hand for small and large individuals by changing hand size and also by altering some dimensions more than others (long, thin fingers as opposed to thick, stubby fingers). Allometric relations can be generated when the basic form of structures is maintained but is stretched in some directions more than in others as the organism is made larger. The production of allometric relationships in this way differs from Huxley's because it does not consider growth of structures but just properties of the final products. This idea is made more precise in an example below by considering what are called self-similar functions (Barenblatt, 1996).

A third possibility for generating allometric relations depends on the unfamiliar notion of changing dimensions in going from one structure to another (Stevens, 2001). For an example of how dimensionality can be changed by an operation, imagine illuminating a three-dimensional object from one direction so that it casts a shadow on a screen. This object (a three-dimensional first structure) casts a two-dimensional shadow on the screen (the second structure) and so one can say that, in some sense, a shadow forms a two-dimensional representation on the screen of a three-dimensional object in space as this object is made smaller and larger. The size of the shadow is related to the size of the object casting the shadow: for example, the area of the shadow will scale as the 2/3 power of the volume

of the object casting the shadow; the size of the two-dimensional shadow, then, will bear an allometric relationship to the size of the original three-dimensional object. As will be described below, certain types of computation can change the dimensions of the space used to represent the input and output of a computation, and this change in dimension can lead to allometric relations between the numbers of neurons in one structure and another.

1.17.3 Huxley's Allometry

How are the scaling laws in Figure 1 to be interpreted? As noted above, the linear relationships of the double logarithmic plots are equivalent to power laws. That is, if S is the size of V1 and s is the size of the LGN, these quantities are related by the power law equation

$$S = as^b,$$

where a and b are constants.

Huxley (1932) discovered a number of scaling relations – it was Huxley who coined the name allometric relation – and attributed them to the differential growth of the two structures being compared. To see how a power law can result from differential growth rates, suppose that one structure (for example, V1), whose size is $S(t)$, grows exponentially with a rate m for a growth duration t. At the end of the growth period, the cortical size would be

$$S = S_0 e^{mt},$$

where S_0 is the size of the structure at the start of the exponential period of growth. The actual final size of S would, of course, depend on the length of the growth period t. Suppose further that another structure (LGN, for example) with size $s(t)$ follows the same exponential growth law for the same growth duration t except this structure has a growth rate n and an initial size s_0; the size s of this second structure, then, is

$$s = s_0 e^{nt}$$

and it also depends on the growth duration t. If one takes the natural logarithm of these two equations and combines them by eliminating the growth duration t (which is assumed to have the same value in both equations), the result is

$$\log S = (m/n)\log s + [\log S_0 - (m/n)\log s_0].$$

That is, S is related to s by a power law whose exponent (or allometric constant) is $b = (m/n)$ and for which the scale factor a above is given by

$$\log a = [\log S_0 - (m/n)\log s_0].$$

For each growth duration, then, different sizes S and s will result, and these sizes are related by a power law. Thus, differential growth rates can lead naturally to scaling relations that are power laws.

1.17.3.1 Detail

The example just given assumed that the growth rate of the structures is constant throughout development and that structure size therefore increases exponentially. A power law results, however, from less restrictive assumptions. Specifically, the growth rate can vary with time during development for two structures and a power law will still result if the two growth rates vary the same way so one is always proportional to the other one.

Let $S_1(t)$ be the size of structure 1 at time t during development, and $S_2(t)$ be the size of a second structure at that time. These structures grow according to the equations

$$dS_1/dt = g(t)S_1(t) \quad \text{or} \quad dS_1/S_1 = g(t)\,dt$$

and

$$dS_2/dt = h(t)S_2(t) \quad \text{or} \quad dS_2/S_2 = h(t)\,dt,$$

where $g(t)$ and $h(t)$ are the growth rates at time t during development; note that the growth rate can vary over the course of development. Eliminate dt between these equations to give a growth equation for the pair of structures:

$$dS_1/S_1 = [g(t)/h(t)]\,dS_2/S_2.$$

If we suppose that the ratio $g(t)/h(t) = b$, where b is a constant – that is, if we suppose that $g(t)$ is proportional to $h(t)$ at all times with the proportionality constant b, even if the growth rates vary over time during development – then the growth equation becomes

$$dS_1/S_1 = b\,dS_2/S_2$$

and integrating this equation one finds that

$$\log(S_1) = b\log(S_2) + c$$

where c is a constant of integration. Thus, even if the growth rates change during development and the growth is not exponential (because growth rate is not constant), as long as the growth rates remain proportional to one another through the time of development, a power law relates the sizes of the two structures (S_1 and S_2) generated by different growth periods.

The problem with this differential-growth explanation for scaling is that one does not know why the growth rates should be different or by how much. A more complete explanation for the scaling relation,

then, would require either some notion of the mechanisms that lead to differential growth rates for two structures or for a reason why having differential growth rates suits the structures to their function. Two alternative explanations for allometric relations are considered below.

1.17.4 When Form Follows Function

Often in biology, the function of a structure and its form are intimately related. If the function is to be preserved as the size of the structure is changed, then the form must also be preserved in order to maintain the form/function connection. This sort of situation can give rise to allometric relations.

To illustrate how preserving form can yield scaling laws, picture a hypothetical axon, much like a retinal axon in the tectum, whose terminal branches form a flat, round disk-like arbor. For this illustration I imagine that all axons in a particular species have arbors of the same size. Suppose we wish to describe how this type of axon distributes synapses in the target structure (the tectum, for example) in a range of comparable species with different brain sizes. The job of this arbor is to distribute information over a map in the target brain area, and the way synapses are distributed should be preserved as the size of the arbor is increased. For example, the computations carried out by the circuit might require that an arbor produce an approximately Gaussian density of synapses over space, and so the form must always be one that will give a Gaussian distribution of synapses for any arbor size (Figure 2). I will denote the size of the target for a particular species by the variable s; this might be, for example, the average lateral length of the tectum, or its average surface area for that species, and I pick some particular species as a reference, for which I choose $s = 1$.

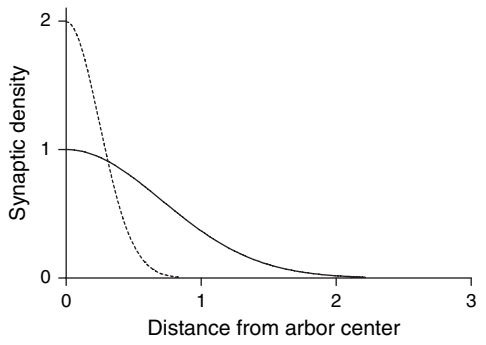

Figure 2 Hypothetical density of synapses formed by an axonal arbor as a function of the distance from the center of the arbor. Two different-sized arbors are depicted, a larger one (solid line) and a smaller, more compact one (dotted line). Both functions are Gaussians.

The size of target structure, then, is measured relative to the size in our reference species, so that, if $s = 3$, the target structure in that species would be three times the size in the reference species.

The average spatial distribution of synapses provided by the arbor will be given by the function $f(r,s)$, where r is the radial distance from the center of the arbor, s is the target size, and the value of the function f gives the average density of synapses in a circular annulus at a distance r from the arbor center. For example, $f(r,s)$ might be a Gaussian whose variance depends on s, as shown by the pair of functions in Figure 2. What does it mean to preserve the form of this arbor? The usual idea for maintaining form is to have the function f be self-similar, which means that its shape is unchanged when s is varied (Barenblatt, 1996); that is, the function might be stretched or compressed in the vertical (y) and horizontal (r) directions, and the amount of the stretching or compression would depend on s. But even when a Gaussian function is stretched in this way, it still is a Gaussian. For example, the function (dotted line) in Figure 2 results when the function presented in a solid line in Figure 2 is stretched vertically by 2 and horizontally by 1/4.

Now suppose we wish to compare the distribution of synapses in the target structure (tectum, for example) across a range of different species. If we take $f(r,1)$ to describe the density of synapses for an axon arbor in the target structure in our reference species (see Figure 2, for example) and if we want to preserve the shape of the function f, then the spatial distribution of synapses for the general target of size s is given by the relation

$$f(r,s) = u(s)f(r/v(s),1).$$

What this means is that, when the target size is s, you can find the spatial distribution of synapses by vertically stretching the distribution of synapses for the reference target by the amount $u(s)$ ($u(s) = 2$ in going from the solid to dotted function in Figure 2), and stretching horizontally by the amount $v(s)$ ($v(s) = 1/4$ in going from the solid to dotted function in Figure 2).

It is easy to see that the vertical stretch is specified by $u(s)$, but perhaps a little harder to understand how $v(s)$ determines the horizontal stretch (in the r-direction). The key is to observe that the value of a function (f in our example) depends on whatever is inside the parentheses $f(\ )$. When r is divided by $v(s)$, the function f decides its value based on the ratio $r/v(s)$ (not just on r), and if $v(s) > 1$, r has to be larger to get the same value of f as when $v(s) = 1$; this stretches the function out along the r-axis.

A remarkable feature of self-similar functions, which has been shown by mathematicians (Aczel, 1969), is that both the vertical and horizontal stretches must have a power law dependence on the size s. Specifically, $u(s)$ and $v(s)$ must both have the form

$$u(s) = as^b$$

and

$$v(s) = As^B$$

for some constants a, b, A, and B if the shape of the function $f(r,s)$ does not change with brain size and if $u(s)$ and $v(s)$ depend smoothly on the size s. Now go back to the synaptic distribution $f(r,s)$, make use of the power law form of $u(s)$ and $v(s)$, and add up the number of synapses in the arbor (integrate $f(r,s)$ over all values of r) to give the total number of synapses $n(s)$ made by each arbor in a target structure of size s. The result can be shown to be the scaling law for synapses per arbor as a function of target size given by

$$n(s) = n(1)s^k$$

for some constant k; here, $n(s)$ is the number of synapses made by an arbor in a brain with target size s and $n(1)$ is the number of synapses made by the reference arbor (whose target size is taken as 1). Thus, preserving the shape of an arbor's distribution of synapses as the target structure is increased or decreased in size results in an allometric relation; often, it is possible to interpret allometric relations in this way. Here I supposed for simplicity that there is only a single variable (r above) and a single parameter (s above for the size of the target structure), but the same sort of argument can be used when more variables are involved.

1.17.5 Changing the Number of Dimensions from One Map to the Next

In addition to differential growth and preserving the form of structures to maintain form/function relations, power laws can also result from certain types of computations and mappings that change the dimension of what is being represented. For example, a two-dimensional map can be transformed into a three-dimensional map, and this can lead to a 3/2 power-scaling law, as will be described below; this happens in the mammalian visual system between the LGN (with a two-dimensional map) and the V1 (which contains a three-dimensional map) (Stevens, 2001).

How can the dimension change in going from one map to another? Describing a curve in a plane – like a circle drawn on a piece of paper – requires two dimensions with an x- and y-coordinate for each point on the curve; Figure 3 (dashed line) illustrates, in perspective, a circle in the x–y plane. This two-dimensional circle can be mapped into three dimensions in a natural way by appending a third number to the (x, y) pair for each point on the circle. For example, each (x, y) pair can be made into an (x, y, z) triplet by setting z equal to the slope of a tangent to the curve at point (x, y). This is seen in Figure 3 (solid line), in which the circle has been 'lifted' into a three-dimensional curve by plotting the orientation of the tangent to the circle at each (x, y) point. Thus, the two-dimensional circle on a plane (dotted line in Figure 3) is transformed into a curve (solid line in Figure 3) in three-dimensional space. The three-dimensional curve can be changed back into the two-dimensional circle by projecting the three-dimensional curve on a plane (for example, by casting its shadow with illumination from above). The three-dimensional space depicted in Figure 3 is called the tangent space of the x–y plane because it not only specifies the position of the curve in the plane (from the (x, y) in each (x, y, z) triplet), but it also give the slope of the tangent to each point along the curve (the z in the (x, y, z) triplet).

When it comes to the brain, it may be slightly difficult to understand what one means by the dimension of maps – say a map of the visual world – represented in some brain region. For example, the visual cortex is essentially a two-dimensional sheet, so how can it contain a representation of the world that is other than two-dimensional? To explain this, I need to consider the essential idea behind the dimensionality of something. The critical notion is: the dimensionality of a space is determined by how many numbers are necessary to specify a point in

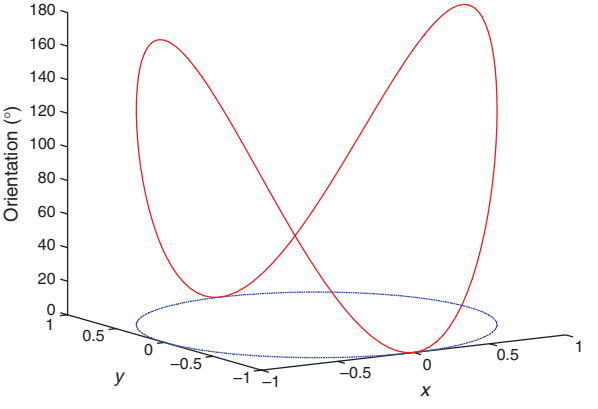

Figure 3 Illustration of how a two-dimensional figure, the circle (dotted blue line) in the x–y plane, might be extended into a three-dimensional volume (solid red line). The vertical axis is the orientation of a tangent to each point on the circle.

that space. For example, because a pair of numbers (x, y) is required to determine the position of any point in a plane, a plane is two-dimensional. Similarly, three numbers (x, y, z) are needed to determine the location of any point in a three-dimensional volume. In the same way, one can imagine (although not picture) a four-dimensional space in which four numbers are required to characterize a point. In physics, it is common to talk about a four-dimensional space, called spacetime, in which the position of a person, for example, is specified by four numbers, three (x, y, z) to define the person's location in space and a fourth number (t) to specify the time at which the person occupied that location. Of course, abstract spaces of any dimension can be constructed.

To understand how a three-dimensional space is represented in the brain, it is convenient to consider a specific example provided by V1. Neurons in V1 respond preferentially to lines or edges, but the response of the neurons depends not only on the location of the line or edge in space but also on its orientation (Hubel and Wiesel, 1959). Thus, if an animal looks at a house at the center of a picture, a particular V1 cell would respond only to a line that is in some specific place in that picture (for example, the line that defines the edge of a tree to the right of the house). But this cell would not respond to just any line there, but only to a line that is, say, vertically rather than horizontally oriented. For each location on the retina, then, there is a population of neurons in V1 that respond only to lines whose images cross that retinal location and, among cells in this population, there are distinct V1 neurons that respond best to each possible direction of the line. This means that each neuron in V1 requires three numbers to characterize its behavior: two numbers to specify the (x, y) position on the retina to which the V1 neuron is assigned, and a third number to give the orientation (from 0° to 180°) of a line that the neuron prefers. Because three numbers (x, y, and orientation) are needed to determine if a particular neuron in V1 is responding, the V1 can be said to have a three-dimensional representation of the visual scene. And because only a pair of numbers are needed to characterize the response of retinal cells, the dimensionality of the retinal representation of the visual scene is two. The visual cortex, then, carries out computations to map a two-dimensional image on the retina into a three-dimensional space in V1. In a similar way, the dimensionality of the representation in any brain area can be described: the dimensionality is determined by how many numbers are needed to characterize the response properties of neurons in that region. As an aside, I should note that the representation of maps in cortical areas is actually greater than three because more than three parameters are needed to characterize completely the response properties of cortical neurons.

How would a scaling law result from such a two-into three-dimensional transformation? To answer this question, one has to recognize that neural representations are grainy because only a relatively small number of neurons are available. Just as the pixel size – and, therefore, the resolution – of a digital camera is determined by the number of pixels available, so is the resolution of a neural map, like the retina or V1, determined by the number of neurons available. If the number of neurons in a hypothetical retina were increased from $1000 \times 1000 = 1$ million to $2000 \times 2000 = 4$ million (a 1-megapixel retina upgraded to a 4-megapixel one), the linear resolution of the retinal image would be doubled because the number of neurons (pixels) would be doubled in each direction. Going from the retina to the LGN (where the image is also represented as two-dimensional), the number of neurons in each direction would also have to be doubled for the LGN to keep up with the resolution available from the retina; there would be no point in making a larger eye, and a larger retina with more neurons, if the increased resolution were just thrown away at the next stage. Going from the LGN to V1, the number of neurons in each direction would also have to be doubled if the resolution in the cortical representation is to keep pace with that available from the eye. The cortex, however, not only has x- and y-directions, each of which must have the number of neurons doubled, but also a z-direction (line orientation) which would also have to double its neurons to make use of the improved resolution available in the image from the larger eye.

To see what happens in general, suppose that the linear resolution in the retina is increased a-fold (doubled in each direction in the example above) so that the total number of retinal neurons is increased by $a \times a = a^2$. For the cortex resolution to keep pace with the resolution available from the retina, the number of cortical neurons would have to be increased a-fold in the x-, y-, and z-directions, so the number of cortical neurons would become $a \times a \times a = a^3$ larger. If n_0 is the initial number of retinal neurons, and n is the number after the increase in size, n would be $n = n_0 a^2$. And if N_0 is the initial number of cortical neurons, and N is the number after the increase in size, N would be $N = N_0 a^3$. Now eliminate a between these two equations to give the result

$$N = (N_0 / n_0^{3/2}) n^{3/2}.$$

Thus, the number of cortical neurons (N) is related to the number of retinal ganglion cells (n) by a power law whose exponent is the ratio of the number of dimensions in the visual cortical representation (3) to the number of dimensions in the retinal representation (2). Such a power law will result whenever the number of dimensions changes in going from one area to another and the resolution in each dimension is increased in parallel.

Certain kinds of computation do not change dimensions whereas others do (Stevens, 2004). For example, if an image is simply filtered – by blurring or sharpening edges, for example – the number of dimensions in the original representation of the image and its filtered versions is the same. This is the sort of operation performed in going, for example, from retina to LGN. On the other hand, some operations do change the number of dimensions. For example, a sonogram of a birdsong takes a one-dimensional function (the sound pressure produced by the bird as a function of time; time is the single independent variable) and displays it as sound intensity of pitches produced as a function of time, a two-dimensional representation of the bird song (the two independent variables are pitch and time). The common image compression schemes, like the modern jpeg format used by digital cameras, also increase the number of dimensions but save space by limiting the resolution.

A widely used class of computations, known as wavelet transforms, always change the dimension of a map. For example, a two-dimensional image becomes four-dimensional after it is wavelet-transformed and it has been argued that V1 carries out such a transform with the firing rate of each neuron representing the magnitude of one coefficient of the transformed image (Stevens, 2004).

In summary, then, allometric relations can arise in different ways and can potentially give information about various underlying mechanisms. According to the original Huxley interpretation, the allometric constant tells you the relative growth rates of two structures. The interpretation of the allometric constant when the allometry arises from self-similarity of structures is more complex, and depends on the details of the situation. Finally, when an allometric relation arises because a computation alters the dimension of the neural representation of a map, the allometric constant specifies the ratio of the number of dimensions in the original representation to the number of dimensions needed for the result. For example, going from a two- to three-dimensional map, the allometric constant would be 3/2.

1.17.6 Some Limitations

Although allometric relations are very important as a tool for describing how brain structures change in size as the brain is made smaller or larger, drawing meaningful conclusions from them requires care. Here I indicate three potential problem areas.

1.17.6.1 Selecting the Appropriate Measures of Size

Figure 1 presents allometric relations between the size of the LGN and V1 with two different measures of size that give two different allometric constants (1.125 and 1.5). Which allometric constant is correct? Both may be correct but not necessarily equally easy to interpret. To decide on the appropriate measure for a structure's size, one must decide, based on the function of the structure, what measure is most natural. For example, number of neurons is an appropriate size measure if one believes that neurons are the computational units of the brain, and volume would be a less natural measure because the essential function does not depend on the volume of the structure (unlike, for example, the liver, where volume is presumably the relevant variable) but rather on the number of computational units.

The volume of a brain structure is related to the number of neurons it contains by the neuronal density (number of neurons per cubic millimeter), and neuronal density is known to decrease as the volume of the structure increases. For the LGN and V1, for example, the neuronal density is related to the structure's volume by power laws (Stevens, 2001), and so the power law for cell numbers, together with the power laws for neuronal densities, dictates that a power law should also result when structure sizes are measured by volumes. To account for the Figure 1a allometric relation, then, one would have to combine the Figure 1b allometric relation and the allometries that relate structure volumes to neuronal densities. The determination of what size measures are relevant for allometric relations depends, then, on one's ideas about how the structures function.

1.17.6.2 Internal Consistency

Figure 4 (open circles) shows an allometric relation between the neocortical volume and the subcortical gray-matter volume for 24 primates (Stephan *et al.*, 1981; Frahm *et al.*, 1982); the allometric constant is 0.73. The subcortical gray matter comprises a number of nuclei, like the thalamus, hypothalamus, and striatum, and each of these structures also obeys a scaling law. For example, Figure 4 (open squares) plots, on a double logarithmic scale, the volume of the striatum as a function of the neocortical volume

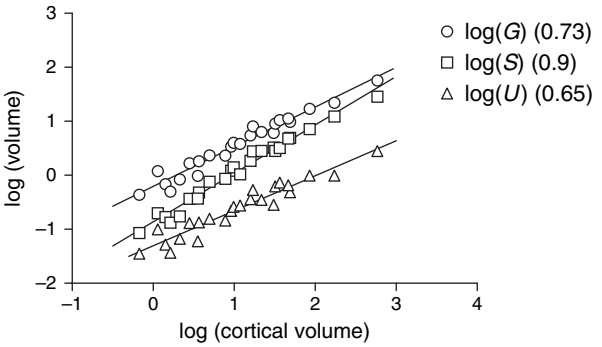

Figure 4 Double logarithmic plots of the volume (mm^3) of subcortical gray-matter structures as a function of the volume of the cortical gray-matter volume for 24 primates. Open circles represent the total volume of the subcortical gray matter, open squares the volume of the striatum, and open triangles the volume of the total subcortical gray matter except for the striatum. Note that the open triangles have been shifted vertically down 10-fold (1 log unit) for clarity.

for the same primate species, and a power law with the allometric constant = 0.9 appears to hold for this pair of structures. The remainder of the subcortical gray-matter volume – including everything except the striatum – is plotted as a function of neocortical volume in Figure 4 as open triangles and the least-squares fitted power function gives an allometric constant of 0.65. Thus, two components whose volume adds together to give the entire subcortical gray-matter volume (striatum and all subcortical structures other than the striatum) each are related to the neocortical volume by power laws with different allometric constants (0.65 and 0.9), and the entire subcortical gray-matter volume is itself related to the neocortical volume by a third power law with an allometric constant of 0.73.

The problem with these allometric relations is that they are internally inconsistent and cannot all be right. The reason is that the sum of power laws with different exponents is not again a power law; for example, there is no value of the constant a that makes $x^2 + x^3$ equal to x^a. Thus, if the striatial volume and the nonstriatal subcortical gray matter are both proportional to different powers of the cortical volume, the total subcortical gray-matter volume (= striatal + nonstriatal) cannot also be proportional to a power of the neocortical volume, as it appears to be from the graph. The sum of power laws with different exponents often can, over some range of values, be approximated by a power law (as illustrated by the data in Figure 4), but the allometric constant for this approximate allometry is not simply related to the allometric constants of the two constituent allometries. This means that the allometric constant for the combined case is difficult or impossible to interpret.

In summary, then, what appears on a double logarithmic plot to be an allometric relation may not, in fact, be one but only an approximation. If it is an approximation that consists of the sum of power laws with different exponents, the allometric constants are not easily interpreted, and may not be useful except as descriptive parameters.

1.17.6.3 Grade Shifts

Allometric relations usually do not hold across all vertebrates or all mammals but rather are restricted to specific taxa. An example is shown in Figure 5a for hominoids (family Hominindae, the great apes and humans) and for monkeys (families Platyrrhini and Ceercopithecoidea), where the volume of the cerebellar hemispheres is plotted, double logarithmically, as a function of the volume of the cerebellar vermis (MacLeod *et al.*, 2003); the allometric constants (the slopes of the lines on the plots) for these hominoids and monkeys are the same (1.4), but the scale factors (determined from the intercepts) are about threefold different (the hominoid curve is displaced vertically from the monkey curve). Allometric relations that differ by their scale factors for different taxa (here, hominoids versus monkeys) are known as grade shifts, and they are very common (Pagel and Harvey, 1989).

The points plotted in Figure 5a are derived from measurements on several specimens from each species, but frequently one uses data that are averaged across all specimens from a given species (for example, the values for all human cerebella are averaged together). The family Hominindae contains only five species, and one generally wishes to seek allometric relations across a larger sample than this. In Figure 5b, data points that appear in Figure 5a have been averaged across species, and the hominoids and monkeys have been plotted together (one point for each species; i.e., one human point, one chimp point, etc.). In Figure 5b, the cerebellar hemisphere volume appears to be related to the vermis volume by a power law with an allometric constant of 1.9 but, from Figure 5a, we know this value for the allometric constant is an artifact that arises from combining allometric data with different scale factors. Ignoring grade shifts in determining allometric constants can therefore lead to incorrect values, and consequently to misinterpretations of the meaning of scaling laws.

In summary, allometric relations reveal orderly rules used by brains when their sizes are increased. These scaling laws can be interpreted in different ways – three have been given here – but these

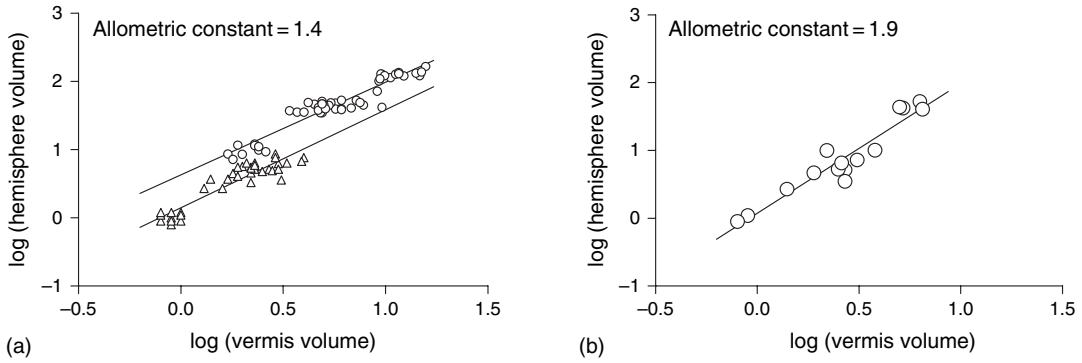

Figure 5 Double logarithmic plot of volume (mm^3) of the cerebellar hemispheres as a function of the volume of the vermis of the cerebellum for five hominoids and 10 species of monkey. a, Each data point represents an individual specimen. b, Each data point is the average of all of the specimens for a particular species.

interpretations are the start, not the end, of the job of understanding the scalable architecture of the brain. For each allometric relation, one must determine the mechanisms that generated it and understand why evolution has chosen a particular value for an allometric constant.

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1.18 Relevance of Understanding Brain Evolution

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Glossary

afferent Arriving input; said of nerve fibers carrying impulses into the brain or any center.

EEG Electroencephalogram; used to embrace the electrical activity seen by electrodes on the scalp or on the brain or in the brain including the compound field potentials of many cells from unknown but presumably multiple sources and generartors. Normally understood to have been filtered by amplifiers that pass only activity above about 0.2 Hz and up to several hundred hertz.

efferent Output leaving the brain or any reference center; said of nerve fibers carrying impulses away.

EP Evoked potential; electrical activity responding to some stimulus; used for the compound local field potentials recorded with EEG electrodes as passed by specified filters, generally used with 'adequate' = normal stimuli delivered at a known moment.

ERP Event-related potential; electrical activity, usually a slow wave attributable to an event which in humans would be called mental and is well defined in time of onset.

glia Neuroglia; non-neuronal cells of the central nervous system, other than cells of the vascular system; normally used to include astrocytes, oligodendroglia, and microglia.

PTP Post-tetanic potentiation; a term referring to a physiological phenomenon where, following the end of a period of tetanus (a series of imposed stimuli such as electrical shocks at a frequency and strength causing sustained contraction of muscle), there is a period of enhanced effectiveness of single test shocks.

rebound A physiological phenomenon where excitability changes for a period in the opposite direction from whatever effect imposed stimuli cause; for example, rebound excitation would follow the end of a period of imposed inhibition.

rhythmicity The degree to which a time series of more or less wide band nature (in terms of the Fourier or frequency/power spectrum) consists of rhythms defined as periodicities within some limit of irregularity of period, sustained for more than some arbitrary number of cycles without major phase shift.

taxon A scientifically defined category of animal populations at a defined level of species, genus, family, order, class or phylumor other groupings, relatedness such as intermediates.

trait A character or feature of structure or function, whether behavioral, anatomical, or physiological.

A case can be made for the proposition that the most neglected aspect of biology is the evolution of complexity and for the assertion that the evolution of complex nervous systems – in short, of the brain – is an outstanding fact, manifesting a span of difference in grade of complexity from the simplest exemplars to the most advanced, far greater than any other systems known except systems made up of many brains. The aim of this article is not to make this case, which I take to be self-evident, but to point out how little studied are the specifics that manifest and define the grades of complexity, in other words, the consequences of neural evolution. A major step in opening this field of study would be to recognize some way of measuring complexity, relevant to animal biology.

It seems feasible, in principle, to attempt to measure neurological complexity by the number of kinds of (1) elementary cellular units, neurons and neuroglia cells and (2) anatomical and physiological interrelationships among groupings of units at each integrative level from single, whole cells and pairs of pre- and postsynaptic cells to organized assemblies, both smaller and larger, plus (3) their resulting repertoire of alternative discriminations and behaviors. Obviously, real numbers would be unavailable in practice and educated guesses as to relative numbers must suffice. Even with relatively

[†] Deceased.

simple animals, it is clear that there are many variables underlying the recognition of repertoire of responses, self-initiated acts, and distinguishable sensory stimuli.

Instead of merely multiplying numbers of like neurons, nature has differentiated them at least with respect to their receptive fields and effector fields (input and output connectivity) with decisive consequences for the animal so that the number of kinds of cells becomes the number of essentially distinct connectivities, afferent and efferent, allowing for overlap. Behind this anatomically basic differentiation is a rich domain of dynamic differentiation in functional properties. We know a great deal about these at the neuronal and subcellular levels but much less at levels of assemblies and functional groupings. At the neuronal level, more than 48 traits or variable properties have been listed (Bullock *et al*., 1977), within each of which there can be a spread from high to low degree of expression; e.g., the threshold of a given neuron can be relatively high or low or intermediate.

Evolution has operated mainly at the higher levels of the brain. Some examples are familiar, particularly in anatomical and connectivity features. Among invertebrates, it is commonly observed that otherwise more advanced taxa have parts of the brain composed of many small, chromatin-rich, tightly packed cells called globuli cells, principally connected intrinsically, among themselves. More advanced or higher brains are also frequently laminated in their more rostral regions and may have a cortex of cells overlying a subcortex of fiber tracts instead of the more primitive condition in which a fiber- and terminal-rich core is surrounded by a rind of somata (cell bodies). The subcortex or fiber core tends to segregate into regions of pure fibers and regions with many terminals – axonal, dendritic, or both – so that more advanced taxa show more differentiated textures of neural tissue and neuropil, with many terminal ramifications and synapses, confined to certain areas. Such areas are likely, in advanced taxa, to be defined or delimited by texture boundaries into nuclei, centers, or laminae, which are more numerous, more distinct, and more subdivided in the most evolved animals. Structures such as commissures and the highly specialized corpus callosum exemplify what I am advocating. We should discover and characterize, even list, the features that distinguish grades of complexity with the estimated degree of differentiation and the names of the taxa displaying them. Conspicuous examples such as the corpus callosum and the cerebral cortex are relatively well known but I assert that others are not yet familiar or even known, especially semiquantitatively in estimated degree of differentiation. Comparative hodological study is likely to uncover differences in the prevalence of reciprocal connections, in marked asymmetries of afferent and efferent connections or in the mapping of either, or in the proportion of cells that respond bimodally or multimodally to two or more kinds of stimulation.

These are only some of the possible innovations that might have evolved in some taxa, probably at the level of order or class. As long as we do not know where such characters appear, we will not appreciate what evolution has brought about or what intermediate stages the brain has had between the simplest and the most evolved brains. A real challenge for new research is to identify specific traits that distinguish grades of brains and behavior.

Even more relevant than anatomical traits are dynamic physiological processes – an area that is much less known and studied. Basic neural mechanisms are still poorly known, e.g., the relative roles of spikes and slow and infraslow potentials, of oscillations, both intracellular and circuit-dependent, of degrees of synchrony, and of correlation dimensions greater than 3 or 4, among other dynamic features. Our understanding even of the occurrence and distribution of slow waves, of synchrony, and of correlated activity among organized assemblies of cells is based on too little comparative data from animal groups that are widely different in complexity.

What we know of such signs of complexity (Bullock and Horridge, 1965) strongly suggests that they are commonly more prominent in the taxa that evolved later and that this is true between and within invertebrate phyla, classes, and orders and between and within vertebrate classes and orders commonly though far from inevitably – long before there were primates or mammals or vertebrates – so that it is not an anthropomorphic bias.

To this writer it is not at all obvious what the selective advantage or survival value was for the stepwise advances in grade of complexity. Vague references to improved discrimination and adaptability are not convincing and plausibility by itself is a treacherous guide. Lacking a satisfactory selective value, it is all the more important to investigate just what evolution has brought to pass, in specific detail, structurally, biochemically, and functionally as well as in susceptibility to viral attacks, immunological disorders, and degenerative diseases. Survival value should support the less complex animals that have passed the test of survival convincingly again and again.

I have elsewhere argued (Bullock, 1992) that most neural evolution has been horizontal adaptation by specialization within the same general grade of

complexity and that vertical differences in grade of complexity are relatively rare, being clear only when one compares taxa sufficiently far apart, rarely at the level of families or orders. This use of grade is therefore quite distinct from the usual use in phylogenetic literature.

In some quarters, there is a lingering prejudice against the terms higher and lower and equivalent words. I take it as *prima facie* that the nervous system of corals is simpler than that of polyclad flatworms and that these are simpler than those of many polychaete annelids, which in turn are not as complex as those of many insects. Higher simply means more complex. Nothing is implied about phylogenetic relationship, moral value, or engineering efficiency.

I will consider cephalopods more complex than gastropod mollusks and polychaete annelids more complex than oligochaetes, at least when comparing the more advanced families in each class. With this proviso, I find it difficult to arrange Chelicerata, Insecta, and Crustacea in order of complexity of their respective most advanced members. Among the vertebrates, the best teleosts are more complex than elasmobranchs, which are in turn more complex than agnathans, although the brain of hagfish is quite advanced histologically. Advanced reptiles are higher than the best amphibians but not much more so than the most advanced teleosts, according to my impression (see Evolution of the Nervous System in Fishes, Evolution of the Amphibian Nervous System, Evolution of the Nervous System in Reptiles). Gallinaceous birds are probably less complex than psittacines and corvines, which are approximately equal to some mammalian orders (see The Evolution of Neuron Classes in the Neocortex of Mammals). Some orders of Mammalia seem more complex than others and this, like all the foregoing cases, was true long before the advent of higher primates. Zoologically and giving due weight to behavior, considered as a broad congeries more than a specialized modality, I place the human species a large step in complexity beyond the next nearest taxon. No doubt some of these assertions are arguable and all suffer from an extreme lack of even semiquantitative data for our definition of complexity. The interest and importance of the question, however, justify our best guessing, as I have attempted to do, beginning with an estimate of the number of distinct (only partially overlapping) kinds of neurons and ending with the above summary, based mainly on the predicted length of the complete ethogram with all the discriminable stimuli and responses, especially the subtler social stimuli and responses. Whatever the arrangement or sequencing, I am willing to assume that the separable grades are different in more than a few traits of brain anatomy or physiology. It is these upon which I am focusing in this article; we should be searching for them at each grade level, particularly for the dynamic physiological properties.

I must stretch to name examples; this is the domain I am advocating for new research. We know little about the comparative physiology of kindling, a well-studied phenomenon in laboratory mammals, in which brain stimulation subthreshold for eliciting seizure discharges reveals a sensitization such that weak stimuli delivered 10 or more hours later trigger full seizures, but not if delivered too soon. Frogs are said to be triggered after mere minutes (Morrell and Tsuru, 1975; Ono *et al*., 1980). It seems likely that the presence or absence of a six-layered cortex would make still further differences in such physiological properties as post-tetanic potentiation or inhibition or in rebound excitation or inhibition (Bullock, 1986b). We have tentatively reported that ongoing local field potentials such as the electroencephalogram (EEG) can show more or less coherence, on average, between electrodes a short distance apart (Bullock, 1991). Coherence is the fraction of the energy at each frequency that maintains the same phase in two electrode loci for the chosen duration of sampling. The regression with distance is a measure of volume synchrony (Bullock and McClune, 1989) of the slow fluctuations (usually *c*. 1–25 Hz, at better than 1 Hz resolution). We believe, from a small number of tectum opticum samples in teleost (catfish and electric fish) and elasmobranch fish, that there is less coherence in these than in mammals and that geckos and turtles may have an intermediate level of coherence. Synchronization of subthreshold slow waves may have evolved from more stochastic activity in lower vertebrates. Invertebrates may have still less, based on a single study of ongoing spontaneous background activity in ganglia of the gastropod, *Aplysia*, with microelectrodes less than a millimeter apart in loci not dominated by large unit spikes (Bullock and Basar, 1986).

Bicoherence is a variable that measures the quadratic phase coupling between any two frequencies in the same or different sites and is a nonlinear higher moment of an ongoing wideband time series (Bullock *et al*., 1996, 1998). It can be low or high for different pairs of frequencies if there is enough nonrandom structure mixed with a stochastic component. We do not have enough data as yet to make comparisons but this property might show evolution between major taxa.

Among other variables that might show evolution, such as mutual information or correlation dimensions, a new variable has been pointed out: an estimate of the rhythmicity. Among the wide band of the EEG, some bands show more than a coincidental degree of repetitive pattern in some samples some of the time, whereas many individuals show no significant rhythmicity much of the time (Bullock, 2003). When a good rhythm appears, it is usually unaccompanied by others, but exceptionally a second rhythm at another frequency is seen. Again, we have insufficient experience to assert any comparative statements at present.

In the very early stage of understanding we are in, it seems that we should not worry about finding principles or ranking emergent traits in importance or defending proposals for selective survival value. We should act as naturalists and attempt simply to list differences and estimate their degree of expression in taxa well separated in overall advancement. In how many ways are the brains of the best (most complex) reptiles different from those of the best amphibians? (see Evolution of the Amphibian Nervous System, Evolution of the Nervous System in Reptiles). Once we have reasonably representative lists, interpretive propositions will be in order and, it may be hoped, insightful. It is to be expected that some comparisons will be ambiguous with respect to which taxon is more complex. Special adaptations, such as echolocation and electrosensory processing, add complexity but in a particular modality. Traits often evolve independently and perhaps the taxa I am calling more complex are in fact those with many more independent traits, especially behavioral traits.

One category of dynamic property is reflected in the compound field potentials of ongoing spontaneous activity frequently called the EEG (which I will use not only for the human scalp-recording, but also for that derived from microelectrodes on or in the brain, also called the local field potential). The EEG is not well understood in origin or causation but manifests spontaneity and volume conduction of algebraically summed activity of neurons and, probably, neuroglia and other sources. Usually described in terms of the Fast Fourier Transform as so much energy at each of the frequency components between chosen limits at a chosen resolution (commonly from <1 to >50 Hz at 1 Hz or finer resolution), we are puzzled by the finding that all classes of vertebrates are much alike (Bullock and McClune, 1989), whether they possess a cerebral cortex or not! The only difference between the EEGs of lower vertebrates and mammals is in amplitude, which is markedly higher in the latter class practically independent of species and brain size (Bullock, 2004).

Another part of the puzzle, in addition to the great similarity of the EEG power spectra in elasmobranchs, teleosts, amphibians, reptiles, and mammals, is the great difference between these and the EEGs of most invertebrates. Despite large differences in anatomy and ethology, the ongoing, spontaneous electrical recordings from the brains of insects, crustaceans, snails and other gastropods, and earthworms and other annelids are all much alike but strikingly different from those of all the vertebrates (Basar and Bullock, 1986; Bullock, 1986a). The invertebrate activity is very spikey with much energy above 100 Hz and very little below 25 Hz, whereas the activity of any vertebrate is mainly below 25 Hz with very little above 100 Hz. Spikes can be found by searching with suitable electrodes but they contribute little energy. The exception is the cephalopod EEG, which is vertebrate-like but, inexplicably, can be switched on or off (Bullock, 1984)!

Beyond the realm of spontaneous, ongoing background activity, such as the EEG, many features and traits are available in the evoked potential (EP) and the event-related potential (ERP), time-locked to the instant of stimulation or of an event that we would consider a mental event in humans (such as "There's one!" or "What's that?"). I prefer to maintain the distinction of earlier literature, between these two categories, one more exogenous, dependent on the stimulus parameters, such as intensity, linearity, and rate of onset, and the other more endogenous and cognitive – dependent on brain state and recent history. We (my associates and I) have much experience with EPs in different taxa and modalities (sharks, rays, bony fish, amphibians, lizards, snakes, turtles, doves, penguins, rats, rabbits, cats, sloths, seals, sea lions, dolphins, manatees, and bats) and invertebrates (earthworms, polychaetes, insects, crayfish, crabs, mantis, shrimp, lobsters, *Limulus*, snails, slugs, *Aplysia*, octopus, squid, and cuttlefish) and some experience with ERPs in a few species of vertebrates.

The main finding relevant to the present topic is that EPs have evolved very little (Basar and Bullock, 1986; Bullock and Basar, 1986). ERPs have been represented by the omitted stimulus potential (OSP), a wave emitted by the eye or brain at a characteristic delay after the due time of a missing stimulus from a regular train at *c.* 2–15 s^{-1}. It is quite dependent on brain state, is barely sensitive to the intensity, placement, or physics of the stimulus, and can be considered a sign of expectation unmet. The defining feature is that, for different

interstimulus intervals in the conditioning train, a fixed and consistent latency is found in each individual, measured from the due time of the omitted stimulus (Bullock *et al*., 1990). Humans have two kinds of OSP: (1) a short-latency, fast one that does not depend on attention and (2) a long-latency, slow one that requires the subject's focused attention (Karamürsel and Bullock, 2000). The other species studied thus far have the fast kind but we have not yet controlled the animal's attention to look for the slow kind except by eliminating it with sleep during which the crayfish show no OSP. The surprise is that a very similar OSP occurs in humans, cats, turtles, rays, and crayfish; i.e., no evolution has thus far been detected. In some taxa, it has been shown that this wave arises in the retina, even after the optic nerve is cut to prevent efferent activity from the brain. This suggests that the OSP can be a relatively low-level phenomenon, cognitively.

I expect the ongoing EEG in nonmammalian species to be less complex than that of mammals (Bullock, 2002a), in correlation with the anatomy of the cerebrum and the evolution of a laminated cortex but thus far have not identified the differences in complexity of electrical activity (Bullock, 2002b, 2003). Also correlated with the anatomy, I expect the ethogram, including discriminable social situations, degrees of danger or attraction, and range of emotional responses, to be longer, more detailed, and hence more complex but thus far cannot cite convincing authors or quotations to support this assumption. The vast difference in complexity between clearly simpler and more advanced phyla, classes, and orders in brain anatomy and behavior remains little studied and a pregnant reservoir of explanatory traits.

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1.19 Origin and Evolution of the First Nervous System

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Glossary

anterior class Hox *genes* — Group of *Hox* genes that are involved in the specification of the anterior-most part of the anteroposterior body axis of bilaterians. The bilaterian Hox cluster genes are believed to be descended from an ancestral ProtoHox cluster which included four genes, the ancestor of the present-day Hox classes (anterior, group-3, central, and posterior).

basal Metazoa — Here used to refer to Porifera, Cnidaria, Ctenophora, and Placozoa. Other authors include the Platyhelminthes (flatworms).

Bilateria — A monophyletic group of metazoan animals that is characterized by bilateral symmetry. Traditionally, this group includes deuterostomes (e.g., chordates, echinoderms, and hemichordates), and protostomes (e.g., arthropods, nematodes, annelids, and mollusks).

Coelenterata — Cnidaria and Ctenophora were traditionally joined together as Coelenterata based on the presence of a single gastrovascular system serving both nutrient supply and gas exchange.

deuterostome — A bilaterian animal whose mouth forms embryonically as a secondary opening, separate from the blastopore. Deuterostomes include chordates, hemichordates, and echinoderms.

effector cell/organ — Single cells or group of specialized cells transducing external stimulation or neuronal signals into a specific response like contraction, secretion, bioluminescence, or electricity.

Eumetazoa — A monophyletic group of animals including all metazoans except the phylum Porifera.

excitable epithelia — Epithelia which can conduct electrical signals over wide areas without decrement.

expressed sequence tag (EST) — A nucleic acid sequence that is derived from cDNA as part of sequencing projects.

four-domain Na^+ channel — A single protein ion channel composed of four linked domains, each of which consists of six transmembrane segments. The whole protein folds up into a channel forming a pore that is selective for Na^+ ions. The four-domain Na^+ channels are believed to have evolved from structurally similar Ca^{2+} channels.

gap junctions — Membrane protein complexes (connexons) that join the plasma membranes of two neighboring cells creating a communication between the cytoplasm of the two cells. This allows the exchange of molecules and the direct propagation of electrical signals.

higher Metazoa — We use these terms as a synonym of Bilateria.

homologue — A gene related to a second gene by descent from a common ancestral DNA sequence. The term, homologue, may apply to the relationship between genes separated by the event of speciation (see orthologue) or to the relationship between genes separated by the event of genetic duplication (see paralogue).

hypostome — The terminal region of a polyp, on which the mouth is situated.

low resistance pathway — A tract of multiple cells which are cytoplasmically connected through specialized pores in the cell membranes allowing the fast conduction of electrical signals.

medusa — Mobile form (jellyfish) of life history in the cnidarian classes Hydrozoa, Scyphozoa, and Cubozoa (Medusozoa).

Medusozoa — Comprises three of the four cnidarian classes (Hydrozoa, Scyphozoa, and Cubozoa), which produce a sexually reproducing medusa (jellyfish) as part of the life cycle.

mesenteries — Longitudinal sheets of tissues that extend radially from the body wall of polyps into the body cavity.

mesogloea (also known as mesoglea) — The body layer between ectoderm and endoderm in cnidarians, ctenophores and acoelomates, which is traditionally distinguished from mesoderm on the basis of the former being acellular and the latter cellular.

myoepithelium — A single-layered tissue of contractile cells.

orthologue — Orthologues are genes in different species that evolved from a common ancestral gene by speciation. Orthologues often retain the same function in the course of evolution.

pacemaker — Single cell or group of cells (neuronal or muscular) that spontaneously drive rhythmic activity in neighboring cells.

paralogue — Paralogues are genes related by duplication within a genome. Paralogues may evolve new functions.

planula — The free-swimming, ciliated larva of a cnidarian.

polyp — The sessile form of life history in cnidarians; for example, the freshwater *Hydra*.

posterior class Hox *gene* — Group of *Hox* genes that is involved in the specification of the posterior part of the anteroposterior body axis of bilaterians. The bilaterian Hox cluster genes are believed to be descended from an ancestral ProtoHox cluster which included four genes, the ancestor of the present day Hox classes (anterior, group-3, central, and posterior).

protomyocyte — An evolutionary antecedent of muscle cells.

protoneuron — Term coined by Parker (1919) for the type of nervous cell from which modern ganglionic neurons evolved.

protostome — A bilaterian animal whose mouth and anus develop embryonically from the same invagination (the blastopore) during embryogenesis.

Radiata — Animals that are traditionally considered to have radial symmetry. This group includes the Ctenophora and the Cnidaria.

Siphonophora — Cnidarian order of marine colonial hydrozoans.

statocyst — The statocyst is a balance organ and consists of a pouch lined with sensory hairs, within which sits a heavy granule called the statolith. The sensory hair cells are connected by nerve fibers to the animal's nervous system. The sensed motion of the statolith in response to gravity allows the animal to orientate itself.

1.19.1 Introduction

1.19.1.1 Tracing Back the First Nervous System

By definition, the first nervous system evolved after the evolutionary shift from unicellular to multicellular life forms. Complex, coordinated behavior controlled by a primitive nervous system in early metazoan animals must have conferred strong selective advantages and thus contributed significantly to the evolutionary success of nervous systems within metazoan animals. Ultimately, more advanced nervous systems, including our own, evolved into the most complex structures found in living matter. In order to learn more about the origins of complex nervous systems in highly evolved animal species, research on the more simple nervous systems that characterize basal metazoan phyla was initiated more than two centuries ago. Then, as today, understanding the origin and early evolution of these simple nervous systems may lead to more profound insight into fundamental principles of development, organization, and function of modern nervous systems.

It is highly likely that the emergence of the first nervous system predated the evolutionary divergence of Bilateria and Radiata 600–630 Mya (Peterson *et al.*, 2004) given the fact that neurons and nervous systems are present in both animal groups. However, the independent evolution of the Bilateria and Radiata during this long period of time implies that most extant animals cannot be regarded as primitive in terms of the organization of their nervous systems. Moreover, for the Radiata, which are generally considered to be basal eumetazoan groups, the fossil record is poor and does not allow reconstruction of fossil nervous systems (Chen *et al.*, 2002). Thus, in the quest to understand the origin of the first nervous systems, it seems best to pursue a comparative approach, in which the structure, function, and development of nervous systems in several basal metazoan phyla are considered and compared in terms of key molecular, cellular, and morphological aspects.

In this review, we will begin by defining what neurons and nervous systems are and then present a current version of the phylogenetic relationships that characterize the systematic groups that are relevant for subsequent considerations. Following this, we will give a brief historical overview of the ideas concerning the origin and evolution of the first nervous system. The main part of the review will then present a detailed comparative analysis of nervous systems in the basal metazoan phyla which may have participated in the origin of the nervous system. Here the main emphasis will be on Cnidaria, but Porifera, Ctenophora, and Placozoa will also be presented, and electrical conduction outside of the animal kingdom will be considered. Finally, we will discuss the implications of recent molecular genetic findings on neurogenesis and axial patterning in cnidarians and bilaterians for our current understanding of the origin of the first nervous system.

1.19.1.2 Definition of the Nervous System

All living cells respond to stimuli and engage in signal processing. Thus, even in the absence of a nervous system, reactions to external stimuli do occur. In most metazoans however, a discrete subset of specialized somatic cells form an interconnected network, called the nervous system, in which multiple sensory stimuli can be processed and conducted to specific effector organs, achieving coordination of complex behaviors. A useful general definition of nervous systems has been given by Bullock and Horridge (1965): "A nervous system is an organized constellation of cells (neurons) specialized for the repeated conduction of an excited state from receptor sites or from other neurons to effectors or to other neurons." An additional aspect was put forward by Passano (1963), who pointed out that the ability to generate activity endogenously is as much a part of the definition of a nervous system as is the ability to respond to stimulation. It follows, from these considerations, that connectivity, specialization for propagating an excited state, and spontaneous generation of activity are important anatomical and physiological criteria for a true nervous system.

The functional units of nervous systems are nerve cells or neurons, which are specialized for the reception of stimuli, conduction of excitation, and signal transmission to other cells. Neurons appear in the most simple animals as specialized conducting, secreting, and spontaneously active cells within epithelia which themselves may show sensory, conducting, and pacemaker features. Given their role in conduction, a key point about neurons is that they are elongated, which enables them to transmit beyond their immediate neighbors without exciting all the interspersed cells (Horridge, 1968).

Some extant animals have a diffuse nerve net representing either an ancestral organization or a secondary loss of centralized structures as often observed in parasitic or sedentary life forms. A nerve net has been defined by Bullock and Horridge (1965) as "a system of functionally connected nerve cells and fibers anatomically

dispersed through some considerable portion of an animal and so arranged as to permit diffuse conduction of nervous excitation, that is, in relatively direct paths between many points. The paths, as opposed to indirect routing through a distant ganglion or central structure, are multiple and confer a tolerance of incomplete cuts."

1.19.1.3 Basal Metazoan Phylogeny

A comparative approach to nervous system structure, function, and origin requires an understanding of the phylogeny that underlies the animal groups considered. It is now commonly agreed that all metazoan phyla including Porifera have a monophyletic origin (reviewed in Müller, 2001; Müller *et al.*, 2004). In this section the phylogenetic relationships of major extant taxonomic groups at the stem of bilaterian animals will be presented (Figure 1).

Choanoflagellata, which show a striking structural resemblance to the choanocytes found in sponges, have been hypothesized to be the closest relative to multicellular animals, and Porifera have been proposed to derive from a colonial form of choanoflagellates (James-Clark, 1867). Recent molecular phylogenetic data provide further support for this hypothesis, indicating that choanoflagellates are indeed more closely related to animals than are fungi and, thus, form a monophyletic sister group of metazoans (Medina *et al.*, 2001; Brooke and Holland, 2003).

Porifera represent the earliest known metazoan phylum and consist of three major taxa: Hexactinellida, Demospongiae, and Calcarea. The molecular sequence analysis of key proteins from these three poriferan classes, suggest that Hexactinellida are the phylogenetically oldest taxon, while Calcarea represent the class most closely related to higher metazoan phyla (Medina *et al.*, 2001; Müller *et al.*, 2004).

The relative positions of the potential sister groups to the bilaterians, namely Cnidaria, Ctenophora, and Placozoa are controversial. Classically the Cnidaria and Ctenophora have been grouped together as the sister group to bilaterians. Together, they are also referred to as the Radiata based on their radially symmetrical appearance (this term may be inappropriate given that biradial and even bilateral symmetry are also common among these animals). On morphological and embryological grounds, such as the presence of mesoderm as a third germ layer, multiciliated cells or a simplified through gut, Ctenophora have been suggested to be the closest relative to Bilateria (Nielsen, 1997; Martindale and Henry, 1999). However, recent molecular phylogenetic analyses support the notion that Cnidaria are more closely related to Bilateria than are Ctenophora, and Cnidaria are therefore often considered as the true sister group of Bilateria (Collins, 1998; Kim *et al.*, 1999; Medina *et al.*, 2001; Martindale *et al.*, 2002). Within Cnidaria recent molecular data based on ribosomal

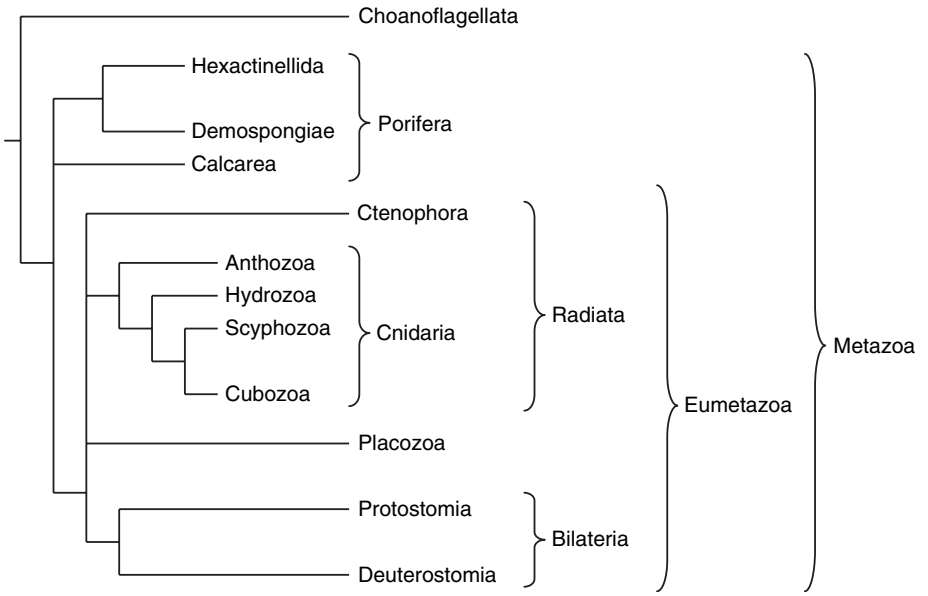

Figure 1 Phylogeny of metazoan animals at the stem of Bilateria. Choanoflagellata have been included as the closest unicellular relatives to the metazoans. The phylogeny is based on widely accepted molecular data and the currently uncertain relationships between the different sponge classes as well as among the potential bilaterian sister groups (Ctenophora, Cnidaria, and Placozoa) have been left open. Terms used in the text for higher classification of animal phyla are indicated on the right-hand side.

DNA sequence analysis and mitochondrial genome organization are in agreement with the view that the Anthozoa, which have only a polyp stage, are basal to the other three classes, Hydrozoa, Scyphozoa, and Cubozoa, which are characterized by an additional medusa stage in their life cycle (Medusozoa; Petersen, 1979).

The Placozoa, represented by a single known species, *Trichoplax adhaerens*, were long believed to be cnidarians with a simple organization as the result of secondary reduction (Bridge *et al.*, 1995). Analysis of molecular data, however, has shown that Placozoa are not derived cnidarians (Ender and Schierwater, 2003). Furthermore, Bilateria and Placozoa may have a more recent common ancestor than either does to Cnidaria (Collins, 2002).

The rapidly increasing amount of molecular data from basal metazoans such as sponges, ctenophorans, cnidarians, placozoans are expected to further clarify the phylogenic relationships among these groups in the coming years (see Metazoan Phylogeny). A robust phylogeny based on different sets of molecular data and, importantly, including a large number of representing species for each taxonomic group will be essential to understand early metazoan evolution and, thus, gain more insight into the origin of the first nervous system.

1.19.2 Historical Concepts and Theories about the Evolutionary Origin of Nervous Systems

1.19.2.1 The Elementary Nervous System

The cornerstone for studies of the evolution of nervous systems at the cellular level was the application of the cell theory (Schleiden, 1838; Schwann, 1839) to the anatomical units of the nervous system in the neuron doctrine which was put forward by Cajal, Kölliker, Waldeyer, and others at the end of the nineteenth century (reviewed in Shepherd, 1991). Subsequently, with improved anatomical staining methods, it became possible to specifically label nervous structures in basal metazoan organisms. With experimental access to the neurons and nervous systems of basal metazoans, it became conceivable to address the question of which cell lineages originally gave rise to nerve cells and how the first nervous system was organized at the cellular level. Hypothetical considerations were initially based on the conceptual model of an elementary nervous system, defined as "a group of nerve cells with the minimal number of specializations required to perform the basic functions of nervous tissue" (Lentz, 1968). However, Lentz pointed out that this simplified conceptual approach does not necessarily determine the actual characteristics of an evolutionarily early, simple system.

Nerve cells are likely to have arisen in multicellular organisms from epithelial cells that turned out to become able to transduce external information (pressure, light, and chemicals) into chemical and electric signals, and then transmit these signals to neighboring cells (Mackie, 1970; Anderson, 1989). Assuming an epithelial layer of equivalent cells, all having the potential of receiving stimuli and producing some form of effector response, different evolutionary theories on the origin of specialized sensory cells, nerve cells, and muscle cells have been proposed. In the following, a brief historical overview of the most influential theories about the evolution of the first nervous system will be given.

1.19.2.2 Proposals for the Evolution of the First Nervous System

One of the earliest theories on the origin of the nervous system was that of Kleinenberg (1872), which he based on the discovery of 'neuromuscular cells' in the freshwater hydrozoan *Hydra*. He viewed this cell type as a combination of receptor, conductor, and effector cell. The apical ends of the described cells were exposed on the surface of the epithelium and were believed to act as nervous receptors. Their basal ends were drawn out into muscular extensions and supposedly served as effectors which received signals from the cell bodies. Kleinenberg postulated that comparable 'neuromuscular cells' gave rise to nerve and muscle cells in the course of evolution. In 1878 the Hertwig brothers described sensory cells, ganglionic cells, and muscular cells in Cnidaria, and postulated that each element was differentiated from a separate epithelial cell but still in a physiologically interdependent way (Hertwig and Hertwig, 1878). In contrast to this notion, Claus (1878) and Chun (1880) suggested that nerve and muscle cells arose independently and became associated only secondarily.

The theory of the Hertwigs in which nerve and muscle were thought to have evolved simultaneously was generally accepted until Parker's publication of *The Elementary Nervous System* in 1919. In this influential publication, Parker proposed a succession of three major evolutionary stages in the organization of the neuromuscular system (Figure 2; Parker, 1919). In sponges, which Parker considered as extant representatives of the first evolutionary stage, muscle is present at the absence of nerve cells. This stage is characterized by the appearance of 'independent effectors' such

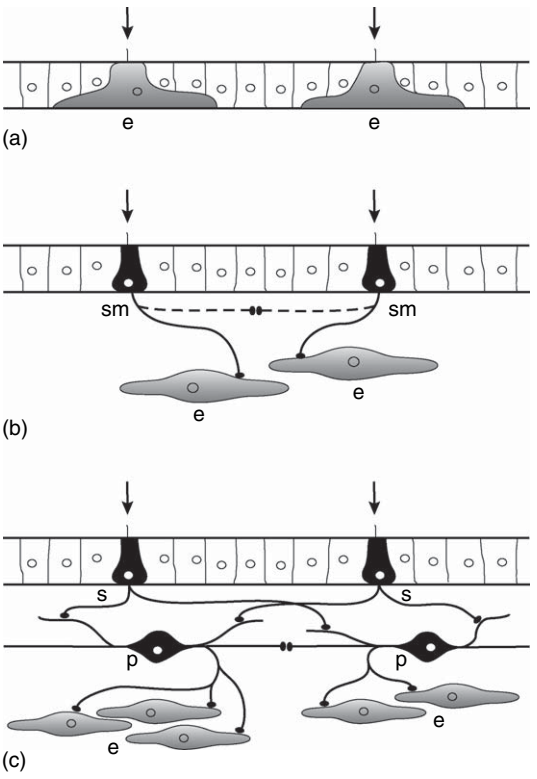

Figure 2 Succession of three evolutionary stages of neuromuscular organization according to Parker (1919). a, Independent effectors. Single contractile effector cells surrounded by epithelial cells are directly stimulated, which leads to a response in the cell. b, Receptor–effector system. Sensory motor neurons directly conduct external stimuli to the underlying muscle cells. In a more complex form, sensory motor neurons can be interconnected among each other (dashed lines). c, Nerve net. A second type of neuronal cell termed "protoneuron" by Parker intercalates between the sensory cells and the muscle cells and forms a highly interconnected neuronal network. Parker proposed that nerve cells of higher animals had their origin in protoneurons. e, effector/muscle cell; p, protoneuron; s, sensory cell; sm, sensory-motor neuron. Arrows indicate the site of stimulation.

as the contractile cells of the oscula sphincters in sponges, which respond directly to environmental stimuli. Although sponges lack nerves, Parker pointed out that they do have a slow type of conduction due to elementary protoplasmic transmission, and he suggested that this 'neuroid transmission' might be considered the forerunner of nervous activity. The second stage of evolution was postulated to be a receptor–effector system such as that believed to exist "in the tentacles of many cnidarians" (Parker, 1919). Receptors were thought to arise from epithelial cells that were in close proximity to the already differentiated muscle cells and, in its simplest form, directly connected to the subjacent muscle cells. However, the separate existence of this type of receptor–effector system has never been directly observed and even Parker admitted that this organizational level might frequently be complicated by the fact that receptor cells not only innervate muscle cells but are also interconnected among each other. In the final stage of early nervous system evolution, a third type of cell, termed "protoneuron" by Parker, was intercalated between the sensory and effector cells forming a true nerve net. This stage was thought to be represented by the nerve nets of extant Cnidaria, and Parker suggested that nerve cells of higher animals were derived from this third type of protoneuronal cell. In a nutshell, Parker proposed that the first nervous system evolved as a consequence of the selective advantage obtained by coordinating independent effectors.

In the second half of the twentieth century, a number of alternative theories for the evolutionary origin of the nervous system were put forward. Based on morphological and physiological studies on sea anemone nerve nets, Pantin (1956) proposed that nervous systems functioned from the beginning to coordinate the behavior of the whole animal. He argued that the nervous system did not evolve on the basis of single cells, but rather originated as whole networks innervating multicellular motor units. Only later would specific conducting tracts have become associated with specific reflexes in the nerve net and given rise to the reflex arc, which according to this view, is not primitive. Pantin's major objection to Parker's theory was the lack of evidence for the independent existence of a receptor–effector system. Based on studies of *Hydra* and scyphomedusae, Passano (1963) postulated that the nervous system evolved from specialized pacemaker cells whose function was to generate contractions within groups of protomyocytes from which they derived. In this view, nerve cells would have derived from pacemaker cells, retaining rhythm generation as their primary function, and only later becoming specialized for conduction over long distances and as sensory receptors. Grundfest (1959, 1965) postulated that the ancestral neuron was derived from a secretory cell that developed a conducting segment between its receptive and secretory poles. Accordingly, true neurons were originally formed when the secretory activity became confined to the terminations of the cells' processes. Thus, this theory is based on the notion that secretion is a primitive feature of the nervous system (Figure 3). A few years earlier, Haldane (1954) proposed that signaling by means of neurotransmitters and hormones had its origin in chemical signaling in protists exemplified by the chemical signals involved in the control of conjugation among different mating types in ciliates. Lentz (1968) noted that protists

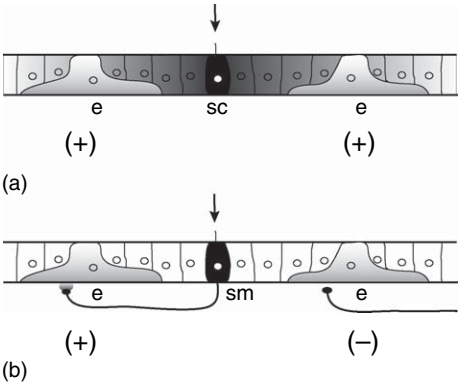

Figure 3 Cell signaling by diffuse secretion preceded synaptic innervation according to Grundfest (1959, 1965). a, Ancestral state. Single cells secrete biologically active substances upon stimulation, which diffuse throughout the epithelium and activate all surrounding effector cells. b, Emergence of neurons. Upon stimulation, sensory neurons specifically activate their target cells by local synaptic release of neurotransmitters. e, effector cell; sc, secretory cell; sm, sensory-motor neuron. Arrows indicate the site of stimulation, (+) stands for an active state and (–) for an inactive state of the effector cell.

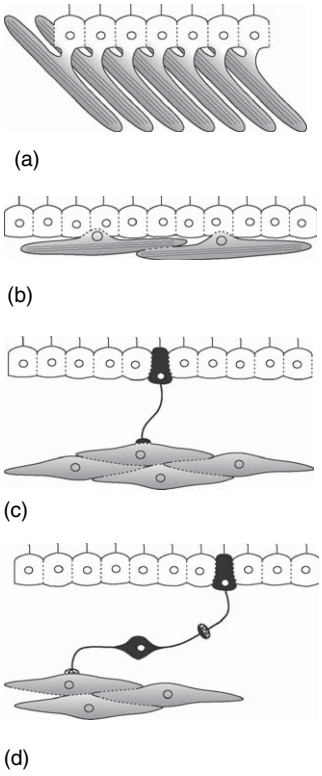

Figure 4 The evolution of nerve and muscle cells from electrically coupled myoepithelial cells according to Mackie (1970). a, Primordial myoepithelium. b, Protomyocytes start to leave the epithelium and move into the interior. c, Protoneurons evolve, conveying excitation to the myocytes from the exterior. All cells are still shown as electrically coupled. d, Neurosensory cells and neurons evolve. They are connected to one another and to the myocytes by chemically transmitting, polarized synapses. Electrical coupling persists in many epithelia and muscles. However, conduction of impulses becomes increasingly a property of the nervous system. Dashed lines at junctions between cells indicate low resistance pathways through which electrical currents can flow. Modified from Mackie, G. O. 1970. Neuroid conduction and the evolution of conducting tissues. *Q. Rev. Biol.* 45, 319–332, Copyright 1970, The University of Chicago Press.

as well as many non-nervous cells have excitable and conductile properties and, furthermore, that 'neurohumors' occur in protists, indicating that these substances could have evolved before the appearance of neurons. He therefore suggested "that the nerve cell arose by the coupling of electrical activity with secretion of biologically active substances so that a chain of events in response to stimuli resulted in alteration of effector activity." In contrast to Grundfest's proposal that the ancestral neuron was a secretory cell which developed specialized receptive surfaces and a conductile intermediate component, Lentz proposed that both neuronal functions evolved simultaneously.

Horridge (1968) and Mackie (1970) described excitable epithelia in hydromedusae and siphonophores, which conduct action potentials and serve as pathways mediating certain types of behavior. Based on this discovery, they proposed that nerves evolved from tissue whose cells were already interconnected by pathways for metabolic exchange and electrical current flow, thus making cell-to-cell propagation of action potentials possible. According to Horridge, the primary function of neurons was neurosecretory or growth regulatory and only later did their elongated axons become effective in impulse propagation. Nerve cells, with their elongated form and functional isolation from surrounding tissues, would have arisen in response to a need for a more selective type of excitation within conductile epithelia in which effector subgroups could be controlled independently (Horridge, 1968). Mackie proposed that the starting point for a metazoan nervous conducting system resembled a myoepithelial tissue sheet in coelenterates. The cells in the tissue capable of reception, transmission, and contraction were connected by cytoplasmic pathways, which also served metabolic exchange among the cells (Figure 4). Specialized muscle cells arose by segregation from the primordial epithelium, whereas cells that lost their contractile component but retained their conducting ability gave rise to nerve cells (Mackie, 1970). Westfall propagated the idea that receptive, electrogenic, and neurosecretory functions co-evolved in primitive protoneurons. This proposal was based on his demonstration with

electron microscopic resolution that nerve cells in *Hydra* not only have receptor poles with a sensory cilium and basal neurites making synaptic contact with effectors but also contain neurosecretory material (Westfall, 1973; Westfall and Kinnamon, 1978). He further proposed that specialized neurons found in modern higher animals derived from multifunctional neuronal ancestors comparable to those found in *Hydra* (e.g., Grimmelikhuijzen, 1996).

In more recent studies, Seipel *et al.* (2004), working on the development of the hydrozoan *Podocoryne carnea*, have found molecular evidence supporting the hypothesis that muscle and nerve cells derive from a common myoepithelial precursor. In bilaterian animals, neuronal determination and differentiation is controlled by genes encoding basic-helix-loop-helix (bHLH) transcription factors and among these are the genes of the Atonal gene family (reviewed in Lee, 1997; Dambly-Chaudiere and Vervoort, 1998). In *Podocoryne*, the cnidarian Atonal-like 1 (Atl1) gene is expressed in a subset of nerve cell precursors of the medusa and additionally in developing striated muscle cells. Simlarly, the neuronal marker gene coding for the cnidarian RFamide neuropeptide is expressed not only in mature nerve cells but also transiently in the developing muscle of *Podocoryne* (Seipel *et al.*, 2004). Based on these developmental genetic similarities, the authors propose that nerve and muscle cells are likely to have been linked closely in evolution and share a common ancestor. In contrast, Miljkovic-Licina *et al.* (2004) studying regulatory genes involved in differentiation of neuronal cell lineages in *Hydra* have proposed a scenario in which mechanoreceptor cells would have preceded neuronal cell types in evolution. Their work shows that the nematocyte and neuronal cell differentiation pathways share regulatory genes that exhibit a high level of conservation during metazoan evolution (Miljkovic-Licina *et al.*, 2004). Nematocytes can sense chemical and mechanical stimuli, transduce these signals, and react to them through nematocyst discharge. The authors propose that this type of fast and cell-autonomous response was a hallmark of very primitive nerve cells and that nematocytes were a derived cnidarian byproduct of these ancestral 'neuro-epithelial' cells. In subsequent evolutionary steps, the 'neuro-epithelial' cells could have differentiated into neuronal cells with elongated processes that began to establish connections with myoepithelial cells and involve them in the response to the stimulus. During later stages, neuronal cells would have become progressively more interconnected with each other in a nervous system allowing coordinated behavior.

In summary, a variety of alternative theories implying different origins of the nervous system have been suggested in the last 150 years. Most of these theories are based on extrapolations of observations made on extant protists, sponges, and cnidarians. The origin of neurons is generally attributed to epithelial cells; however, the characteristics of these ancestral cells are variously considered to have been contractile, neurosecretory, conductile, chemoreceptive, or mechanoreceptive, and each theory emphasizes one or several of these features as being driving force for the evolution of the nervous system. While many of these proposals appear plausible and inspiring for further discussion, it seems impossible to rate one of the theories as more relevant than the others. However, all of the proposed scenarios for the evolution of the nervous system do focus attention on the cell biology of excitable cells in the basal animal groups, and this focus will be explored in more depth in the following pages.

1.19.3 Origin of the First Nervous System: A Comparative Phylogenetic Approach

1.19.3.1 Introduction

Although the nervous system must have arisen in a multicellular organism, unicellular organisms such as protists show a variety of behavioral programs in response to their environment. In protists, behavioral responses to external stimuli are achieved at a subcellular level by organelles specialized for signal reception, signal conduction, and effector response (Deitmer, 1989; Febvre-Chevalier *et al.*, 1989; Hennessey, 1989). Thus, molecular machineries capable of reception of chemical, mechanical, or light stimuli, secretion of biologically active substances, propagation of electrical potentials along membranes, and conversion of stimuli into effector responses were probably already present in the ancestor of metazoans. Assuming colonial protists with equivalent cells as an intermediate form between unicellular protists and early metazoans, an increasing specialization of subgroups of cells must have occurred during evolution. Porifera represent the most basal extant metazoan phylum and are thought to have derived from a colonial form of choanoflagellates. Although a variety of different cell types can be found in sponges, no nerve cells could be identified so far (Jones, 1962; Pavans de Ceccatty, 1974; Mackie, 1979). Nevertheless, contractile cells encircling the oscular openings in sponges are able to react upon mechanical stimulation. In cnidarians and

ctenophores, the closest metazoan relatives of sponges, nerve cells are present and can form sophisticated nervous systems capable of solving complex behavioral tasks. This evolutionary step from poriferan to cnidarian or ctenophoran organization may harbor the emergence of nerve cells and nervous systems.

1.19.3.2 Non-Nervous Conduction Outside of the Animal Kingdom

Many key characteristics of nerve cells can be found in non-nervous cells of metazoans, plants, fungi as well as in unicellular organisms like protists and even prokaryotic bacteria. These characteristics include reception and transmission of signals to other cells, intercellular communication by secretion of biologically active substances, and the propagation of electrical potentials. Nevertheless, the combined appearance of these features in morphologically and functionally specialized nerve cells is unique to the nervous systems of metazoan animals.

Ion channels, which can be gated by ligands, voltage, or mechanical forces and are permeable to specific ions, such as K^+, Ca^{2+}, Na^+, and Cl^-, play a major role in the generation of neuronal excitability in higher animals. Moreover, ionic fluxes across cellular membranes mediate a great variety of biological processes that are essential for viability of most life forms. A large number of genes presumably coding for ion channels have been identified in prokaryotes, but although structural or electrophysiological information has been obtained for some of these proteins, their biological roles are mostly unknown. Presumably, prokaryote channels are involved in metabolic function, osmoregulation, and motility (Ranganathan, 1994; Kung and Blount, 2004). In the bacterium *Escherichia coli*, genome sequencing suggests the presence of six putative mechanosensitive channels, one putative voltage-gated K^+ channel, and two Cl^- channel-like structures. Three of the mechanically gated channels are involved in osmoregulation and release solutes upon osmotic down-shock, whereas Cl^- channels apparently function in short-term acid tolerance. Although, the function of the K^+ channel is still unknown, its protein shares extensive topological and structural similarity with eukaryotic K^+ channels suggesting a common ancestral origin from which K^+ and later probably Ca^{2+} and Na^+ channels evolved (Milkman, 1994; Ranganathan, 1994; Kung and Blount, 2004). Voltage-dependent and stretch-activated ion channels have been found in the plasma membrane of yeast (Gustin *et al.*, 1986, 1988; Zhou *et al.*, 1995). In addition, the yeast genes involved in the pheromone response show high similarity to signal transduction genes of higher animals. For example, the mating factor receptor STE2 of *Saccharomyces cerevisiae* belongs to the rhodopsin/beta-adrenergic receptor gene family (Marsh and Herskowitz, 1988), and the alpha-type mating factor shows amino acid sequence similarities with the vertebrate reproductive gonadotropin-releasing hormone (Loumaye *et al.*, 1982).

In addition to the transmission of information through substrate flux, plants have electrical and hormonal signaling systems. Action potentials in plants were described for the first time in 1873 by Burdon-Sanderson. He recorded electrical signals from a specimen of the Venus's flytrap, *Dionaea muscipula*, which he received from Charles Darwin (Burdon-Sanderson, 1873; Sibaoka, 1966). The leaves of *Dionaea* are divided into two lobes, each of which carries three tactile sense hairs functioning as trigger for an all-or-nothing electrical signal that is followed by the fast closing of the lobes entrapping the prey. In plants like the Venus's flytrap, action potentials are part of a signaling system that responds to mechanical stimulation by changing cell turgor, which leads to relatively rapid movements. Propagation of action potentials from the site of stimulation to the effector cells has been studied in the seismonastic movements of the leaves of *Mimosa pudica* (Sibaoka, 1966; Simons, 1992). Non-nervous electrical conduction in plants involves low-resistance pathways (plasmodesmata) between the phloem cells, comparable with gap junctions that electrically couple cells in excitable epithelia and muscles in animals. Action potentials in plants have been studied in detail in the giant internodial cells of the freshwater algae *Chara* and *Nitella*. In these large cells, a motility system based on actin and myosin drives cytoplasmic streaming, which serves to equally distribute organelles and nutrients around the central vacuole. Upon mechanical or electrical stimulation, an action potential is generated, which spreads in both directions along the shoot and immediately stops the cytoplasmic streaming probably to avoid leakage of the cell in case of injury. In contrast to the action potentials of higher animals where the influx of Na^+ and Ca^{2+} supports the depolarizing phase, in *Chara* and *Nitella* Ca^{2+} and Cl^- are the key components of depolarization, a situation which is typical for plant action potentials. A fast initial influx of Ca^{2+} ions is followed by the efflux of Cl^- through Ca^{2+} activated Cl^- channels across the vacuolar and plasma membranes. The falling phase of the action

potential is due to an increase in K^+ permeability, similarly to what occurs in nervous cells of higher animals (Sibaoka, 1966; Simons, 1992; Wayne, 1994; Kikuyama, 2001). Although molecules that act as neurotransmitters in higher animals such as glycine, GABA, glutamate, and acetylcholine have been isolated from plants, no chemical transmission of electrical signals between cells of plants has been observed. Rather, these substances are involved in a variety of functions related to metabolism, circadian rhythm, or light response of plants (Simons, 1992; Mackie, 1990; Hille, 1984).

A number of neuroactive substances including adrenalin, noradrenalin, 5-HT, DOPA, dopamine, and beta-endorphin as well as receptors for acetylcholine, catecholamines, and opiates have been reported in protists (Zipser *et al.*, 1988; Carr *et al.*, 1989; Görtz *et al.*, 1999). Furthermore, receptor tyrosine kinase genes, known to be involved in cell–cell signaling in metazoans, have been recently isolated from choanoflagellates, suggesting that this family of signal receptor molecules evolved before the origin of multicellular animals (King and Carroll, 2001; Brooke and Holland, 2003; King *et al.*, 2003). Some protists can respond to mechanical stimulation with depolarizing or hyperpolarizing membrane potentials. Their membranes are equipped with ion channels gated mechanically, or by ligand or voltage, and in some cases, action potentials are elicited when the cell membrane is depolarized up to a threshold level by receptor potentials. In most protists, Ca^{2+} ions are responsible for carrying ionic currents and coupling membrane excitation to motile response or contractile activity (Febvre-Chevalier *et al.*, 1989). In some ciliates, ion channels are not distributed uniformly over the cell membrane; this is reminiscent of neuronal cell membranes that have distinct channel populations in dendrites, soma, axon, and presynaptic terminals. For example, in *Paramecium* and *Stylonychia*, different ion channels can be found at the front and back poles of the cell generating different ion currents, which lead to opposed escape behaviors away from the source of mechanical stimulation (Kung, 1989; Deitmer, 1989; Kung and Blount, 2004). Behavioral responses in protists elicited by action potentials often involve changes of cell shape or alterations in the pattern of ciliary or flagellar beating (Febvre-Chevalier *et al.*, 1989; Hennessey, 1989). The complexity of effector responses driven by different types of electrical potentials within a unicellular organism is nicely illustrated by the dinoflagellate *Noctiluca*. Two different kinds of flagellar movements and a bioluminescent light response are controlled through different action potentials involving different ion currents across the cytoplasmic and vacuolar membrane. In this manner, multiple bioelectric activities in *Noctiluca* are able to control altered effector responses within a single cell (Oami, 2004). Thus, in the absence of a nervous system, protists exhibit complex behaviors which incorporate features of sensory receptors and effectors into a single, highly structured eukaryotic cell.

1.19.3.3 Porifera: Specialized Cells and Electrical Conduction

Sponges, the most basal extant metazoans, probably evolved from a colonial choanoflagellate. At this stage of phylogeny a number of specialized cell types including muscle-like contractile cells has made its appearance, however, nerve cells are lacking (Jones, 1962; Pavans de Ceccatty, 1974; Mackie, 1979). Some of the actin-containing contractile cells (myocytes) are concentrated as sphincters around the osculum and pore canals of sponges. To contract, the sphincters have to be directly stimulated and they thus represent "independent effectors" as proposed by Parker (1919). Slow contractile responses that spread over short distances have been described in several sponge species, but the responsible cells do not seem to be electrically excitable, and there is no evidence of associated changes in membrane potentials (Mackie, 1979). Thus, some form of mechanical interaction between neighboring cells seems likely. The sponge epithelial cells that build the external and internal boundary of the mesenchyme are not joined together with occluding junctions and, therefore, the internal milieu may not be very well isolated from the external. Nevertheless, the mesenchyme provides an environment in which electrical and chemical gradients could be generated and nutrients and hormones diffuse without excessive leakage through the body wall (Mackie, 1990). Acetylcholinesterase, catecholamines, and serotonin have been shown, by histochemical techniques, to be present in sponges (Lentz, 1968). Further, some neuroactive substances have been demonstrated to influence the water circulation in the sponge *Cliona celata* (Emson, 1966), but so far there is no clear evidence that they are involved in intercellular signaling processes. Interestingly, a recent finding has shown that cells isolated from the marine sponge *Geodia cydonium* (Demospongiae) react to the excitatory amino acid glutamate with an increase in intracellular calcium concentration (Perovic *et al.*, 1999). Extracellular agonists as well as antagonists known from metabotropic glutamate/GABA-like

receptors in mammalian nerve cells were found to elicit similar effects in these sponge cells. In addition, a cDNA coding for a seven-transmembrane receptor was isolated from *Geodia*, which has high sequence similarity to metabotropic glutamate/GABA-like receptors in mammals. Although these findings suggest that Porifera possess a sophisticated intercellular communication and signaling system, so far there is no evidence for the type of specialized intercellular signal transmission in sponges that might foreshadow the evolutionary origin of nervous systems.

The tissue of glass sponges (Hexactinellida) is syncytial, allowing the rapid propagation of electrical events, which is a fundamental difference between this class and the other two cellular sponge classes, Demospongiae and Calcarea (Müller, 2001). All-or-nothing electrical impulses were recorded from the glass sponge *Rhabdocalyptus dawsoni*. Tactile and electrical stimuli evoke impulses, which lead to the abrupt arrest of water flow through the body wall, presumably due to the coordinated cessation of beating of the flagella in the flagellated chambers. From the superficial pinacoderm, impulses are conducted through the trabecular reticulum, a multinucleate syncytial tissue draped around the spicules of the sponge skeleton, to the flagellated chambers. Impulses are propagated diffusely at 0.27 ± 0.1 cm s^{-1}, a value that falls within the lower range of action potential conduction velocities in non-nervous tissues. It is assumed that signal propagation through the syncytium depends on Ca^{2+} influx and that Ca^{2+} channels may also mediate the flagellar arrest (Leys *et al.*, 1999). The trabecular syncytium seems to be a derived feature specific to the most ancient sponge class Hexactinellida. Since calcareous sponges and demosponges lack comparable syncytial tissue, they would require low-resistance pathways equivalent to eumetazoan gap junctions to conduct electrical signals from cell to cell, but no similar structures have been found so far (Leys *et al.*, 1999; Müller, 2001).

Larvae of many sponge species exhibit rapid responses to external stimuli including light, gravity, and current (reviewed in Wapstra and van Soest, 1987). Demosponge larvae have a spheroid body shape and consist of an outer epithelial layer of monociliated cells and a solid center of amoeboid cells in an extracellular matrix of collagen. The spheroid-shaped body is polarized anteroposteriorly with respect to the swimming movement of the larvae, and a ring of pigmented cells that gives rise to long cilia is located at the posterior end. In the demosponge *Reneira*, directional swimming is mediated by the long cilia of the posterior pigmented cells and incorporates an asymmetric response of these cells to different light intensities (Figure 5). Increased light intensity causes a bending of the cilia such that they shield the pigment vesicles, whereas decreased light intensity reverses this process. This results in steering the larva away from bright light (Leys and Degnan, 2001). Interestingly, re-analysis of the action spectrum of the ciliary response to light reveals that the photoreceptive pigment in the sponge larva has the characteristics of rhodopsin, similar to the situation in other metazoans that have a rhodopsin-like protein as their primary photoreceptive pigment (Leys *et al.*, 2002; Leys and Meech, 2006). In *Reneira* the light response of the posterior cells has been suggested to depend on the depolarization of the membrane potential and the influx of Ca^{2+} into the cilium. Since sponge larvae lack neurons or gap junctions that would allow coordination of signals among cells with long cilia, each posterior cell appears to respond independently to changes in light intensity. On the other hand, no intercellular coordination seems to be required, given the inherent photokinetic

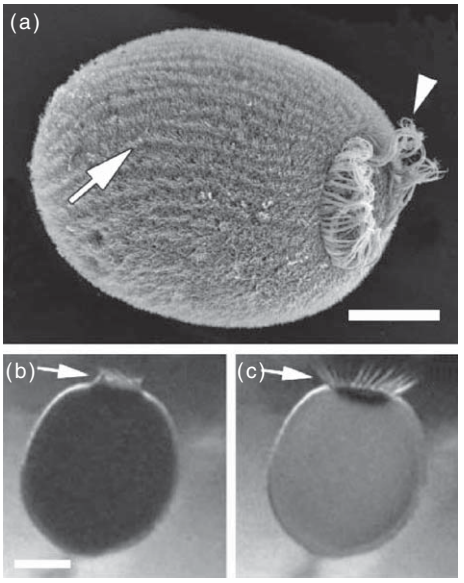

Figure 5 Photosensitive cells and ciliary light response of the sponge *Reneira* larva. a, Scanning electron micrograph showing the structure of the demosponge larva. Monociliated epithelial cells form most of the outer layer (arrow). The posterior pole is circumscribed by a ring of long cilia (arrowhead). Video recording of bending (b) and straightening (c) of the long posterior cilia (arrows) in response to shutting and opening of a shutter in front of the light source. Scale bars: 100 μm. Reproduced from *J. Comp. Physiol. A*, vol. 188, 2002, pp. 199–202, Spectral sensitivity in a sponge larva, Leys, S. P., Cronin, T. W., Degnan, B. M., and Marshall, J. N., figures 1a and 2b (I + II). With kind permission of Springer Science and Business Media.

responses of each ciliated cell depending on its position relative to the light source (Leys and Degnan, 2001). Therefore, in some cases 'independent effectors' in sponges may mediate coordinated behavior. Although sponges emerged at an early level in multicellular animal evolution when nervous systems had not yet evolved, they do represent the oldest extant metazoans with specialized cells responding to different stimuli and performing behavioral tasks.

1.19.3.4 Ctenophora and Cnidaria: The Oldest Extant Nervous Systems

Ctenophora and Cnidaria are the lowest animal phyla that have a nervous system. The two phyla were traditionally joined together in one group, termed Coelenterata, based on the presence of a single gastrovascular system serving both nutrient supply and gas exchange among the body parts. Molecular phylogenetic data, however, suggest an independent origin of the two phyla in the prebilaterian line, and their relative position in early metazoan phylogeny is controversial (Martindale and Henry, 1999; Medina *et al.*, 2001; Podar *et al.*, 2001; Ball *et al.*, 2004). Whereas most molecular data support the more basal position of ctenophores with cnidarians forming the sister group to bilaterians, other evidence, including the presence of true subepithelial muscles and multiciliated cells, supports the view that ctenophores are more closely related to bilaterians than cnidarians (Nielsen, 1997). Thus, it is presently not clear whether Ctenophora or Cnidaria are the closest extant metazoan relatives of Porifera. Nevertheless, it is likely that the first nervous system evolved at the evolutionary step from Porifera to either of the two coelenterate phyla.

Ctenophores are medusoid gelatinous animals, which generally have two tentacles for capturing prey and eight ciliary comb rows on their outer surface for locomotion. The nervous systems of ctenophores are organized into diffuse nerve nets, which show some local tract-like accumulations below the ciliary comb rows and around the mouth and pharynx. At the ultrastructural level, polarized as well as symmetrical chemical synapses have been shown to be present in these nerve nets. Sensory nerve cells are interspersed among the epithelial cells, except at the aboral pole where sensory and nerve cells constitute, together with a statocyst, the apical organ. Locomotory movements of ctenophores involve metachronal beating of eight comb plate rows radiating from the aboral region. The apical organ serves as pacemaker of the comb plate rows and coordinates geotactic responses (Satterlie and Spencer, 1987). Transmission of ciliary activity among comb plate cells is non-nervous by mechanical coupling (Tamm, 1982). In addition, comb cells are electrically coupled through gap junctions, probably allowing the synchronous response of neighboring cells to modulatory synaptic input (Hernandez-Nicaise *et al.*, 1989). In *Pleurobrachia* different inhibitory and excitatory pathways coordinate the electromotor behavior of comb plate cells with tentacle movements during prey capture and ingestion (Moss and Tamm, 1993). In their basic elements the ctenophoran nervous systems already share many features with nervous systems of higher animals, thus allowing well-coordinated behavioral programs in a basal metazoan animal.

1.19.3.5 Cnidarian Nervous Systems: Multiple Levels of Organization

It is often assumed that nervous systems probably evolved first in Cnidaria or a closely related ancestor, and their nervous systems are, thus, often considered to be among the simplest forms and reflect an early stage of evolution. This view prevailed until few decades ago and is still present in many textbooks (Brusca and Brusca, 1990; Ruppert and Barnes, 1994). However, cnidarians have been evolving independently for some 600–630 million years, and have therefore had plenty of time to develop sophisticated solutions for comparable behavioral tasks and under similar conditions as have many higher animals. During this long evolutionary time period, a wide spectrum in nervous system complexity emerged within the cnidarian phylum, ranging from the diffuse nerve nets of sessile polypoid species to the multiple ring-shaped nerve tracts, giant axons, and highly specialized sensory organs in actively swimming medusoid species. Thus, in some cases, the complexity of nervous systems in modern cnidarians may reflect more the behavior tasks of the species considered than any ancestral organization. Many physiological and structural solutions found exclusively in the nervous systems of cnidarians deal with the problem of generating coordinated behavior in a radially symmetrical animal (Mackie, 1990). Ring-shaped nerve nets or diffuse epithelial conduction may, therefore, represent adequate systems for specific behavioral functions rather than remnants of a primitive nervous system. Nevertheless, many basic features of bilaterian nervous systems can be found in cnidarian nervous systems and consequently are likely to have been present in their common ancestors in which the first nervous system probably

evolved. These features, which have been the subject of considerable research, are considered in more detail below.

Different levels of nervous system organization are encountered in the phylum Cnidaria and often even in the same animal. The spectrum of levels ranges from independent effector cells, as already found in sponges, to the first trends of centralization of integrative and coordinative functions in the nerve rings of some medusae (Bullock and Horridge, 1965; Mackie, 2004). In many aspects, the cnidarian nematocytes can be considered as 'independent effectors' (Miljkovic-Licina *et al.*, 2004). Nematocytes are mechanoreceptor cells found in the ectodermal tissue of cnidarian tentacles that discharge the toxic content of a highly specialized capsule named the cnidocyst upon contact with the prey. Although most nematocytes are innervated, they are still able to discharge in the absence of nerve cells (Aerne *et al.*, 1991) and thus respond to direct stimulation. Another example of an 'independent effector' in cnidarians are the photoreceptor cells of the cubozoan *Tripedalia* planula. These unicellular photoreceptors contain the photoreceptor and shielding pigment granules within the same cell, which in addition carries a motor cilium that enables the larva to perform phototactic behavior. Ultrastructural analysis further reveals that there is no nervous system to which these photosensitive cells transmit visual information. These cells are thus self-contained sensory-motor entities that respond directly without a coordinating nervous system (Nordström *et al.*, 2003). The unicellular photoreceptors of the *Tripedalia* larva represents an interesting parallel to the photosensitive ciliated cells of sponge larvae in that each cell has a well-developed motor-cilium, which directly responds to light stimulation (Leys and Degnan, 2001; Leys *et al.*, 2002; Nordström *et al.*, 2003). However, since no similar autonomous photosensory motor cells have been described in more basal cnidarian larvae, the homology of these two structures can be most likely excluded.

Excitable epithelia are another non-nervous element involved in signal conduction that can be found in Cnidaria side by side with highly specialized nervous conduction pathways. Excitable epithelia are present in the endodermal radial canals of hydrozoan medusae where they conduct signals involved in motor control of behavioral responses such as 'crumpling' (protective involution), feeding, or swimming. In the pelagic jellyfish *Aglantha*, this epithelial pathway is preserved despite the presence of a highly complex nervous system consisting of several neuronal conduction systems that include diffuse nerve nets, nerve rings, and giant axons (Mackie, 2004). Thus, relatively slow, non-nervous signal conduction of the type known from sponges and even plants can offer alternative pathways in parallel to highly specific, fast nervous conduction. Epithelial conduction consisting of electrically coupled equivalent cells, from which more specific pathways evolved with the emergence of elongated nerve cells, has been proposed as a characteristic of the hypothetical metazoan ancestor (Horridge, 1968; Mackie, 1970). Whether epithelial conduction is indeed an ancient feature or rather arose several times during evolution is unclear. Nevertheless, this mode of conduction can be found throughout the animal kingdom, from ctenophores to the early tadpole larvae of amphibians (Roberts, 1969; Mackie, 1970).

A diffuse, two-dimensional nerve net formed by bi- or multipolar neurons is considered to be a simple form of nervous system organization. A classical example of this simple type of neural ground plan is found in *Hydra*. This cnidarian has a network of multifunctional nerve cells, which combine sensory and motor tasks and have processes that conduct impulses bidirectionally. Traditionally, the nervous system of *Hydra* has been illustrated with a simple meshwork of equally spaced neurons, as it is still the case in many textbooks (e.g., Brusca and Brusca, 1990). However, detailed neuroanatomical analysis of the *Hydra oligactis* nerve net shows that its neurons are not equally distributed throughout the polyp body wall but rather form a ring-shaped area between tentacles and mouth opening and local concentrations in the peduncle suggesting a level of regional specialization (Figure 6a; Grimmelikhuijzen and Graff, 1985). Furthermore, distinct neuronal subsets can be distinguished morphologically or neurocytochemically based on neuropeptide expression (Grimmelikhuijzen *et al.*, 1996). In *Hydra*, new nerve cells are constantly generated by interstitial cells in a specific zone of the polyp body column and migrate toward the body extremities where old nerve cells are lost. As they migrate, nerve cells can undergo morphological and neurochemical transformations and give rise to the different neuronal subsets (Bode *et al.*, 1988; Grimmelikhuijzen *et al.*, 1996). In addition to their roles in behavior, nerve cells in *Hydra* are directly involved in the regulation of growth and in the production of chemical morphogenetic gradients (Schaller *et al.*, 1996). Thus, the nervous system of *Hydra* is not a simple, diffuse meshwork of interconnected nerve cells and it is unlikely to represent an ancestral situation within the Cnidaria. In the sea pansy, *Renilla koellikeri*, belonging to the

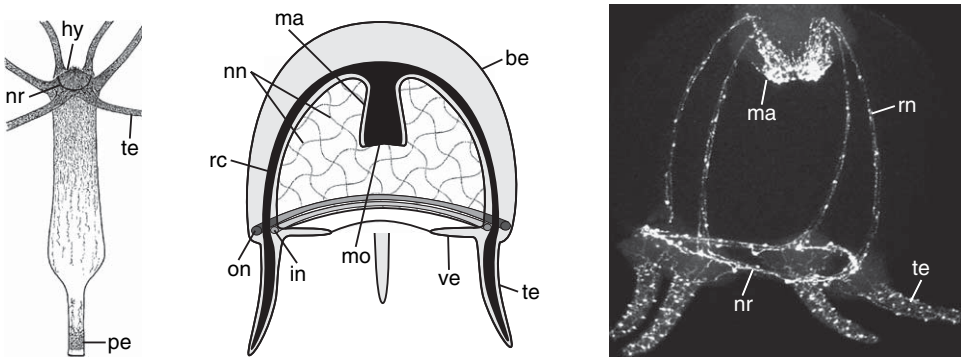

Figure 6 Nervous system organization of hydrozoan polyps and medusae. a, Drawing showing the RFamide-positive nervous system in *Hydra oligactis*. This species has a dense plexus of immunoreactive neurites in the hypostome and a nerve ring between hypostome and tentacle bases. A collar of neurons can be found in the peduncle. b, Nerve net and nerve rings in a hydromedusa. Nerve nets underlying the ectodermal and endodermal tissues span the inner surface of the bell. An inner and an outer nerve ring encircle the bell near the margin. These nerve rings connect with fibers innervating the tentacles, muscles, and sensory organs. c, Fluorescent RFamide staining of the hydromedusa *Podocoryne carnea*. Nerve cells expressing RFamide can be detected in the nerve ring around the margin of the bell and the radial nerves which line the four radial canals. In addition many RFamide positive cells are found around the mouth opening at the tip of the manubrium and scattered over the surface of the tentacles. be, bell; hy, hypostome; in, inner nerve ring; ma, manubrium; mo, mouth; nn, nerve net; nr, nerve ring; on, outer nerve ring; pe, peduncle; rc, radial canal; rn, radial nerve; te, tentacle; ve, velum. a, Reproduced from *Cell Tissue Res.*, vol. 241, 1985, pp. 171–182, Antisera to the sequence Arg-Phe-amide visualize neuronal centralization in hydroid polyps, Grimmelikhuijzen, C. J. P., figure 9b. With kind permission of Springer Science and Business media. c, Courtesy of V. Schmid.

phylogenetically basal cnidarian class of Anthozoa, the nervous system is also found to consist of multiple interconnected nerve nets with local concentrations at specific organs involved in feeding or reproduction (Pernet *et al.*, 2004; Umbriaco *et al.*, 1990). Indeed, it appears that the simplest form of nervous system organization found in extant cnidarians is that of multiple interconnected nerve nets formed by different neuronal subtypes and showing local concentrations.

An important feature of nerve nets is diffuse conduction, characterized by the spreading of an impulse in all directions from the site of stimulation. Symmetric synapses are frequently seen in cnidarian nerve nets, especially in Scyphomedusae, where they can transmit excitation bidirectionally (Anderson and Spencer, 1989). Although, bidirectionality can often account for diffuse conduction, symmetrical synapses are apparently not an absolute requirement for this and diffuse conduction can also be obtained by the distributed arrangement of many unidirectional pathways (Bullock and Horridge, 1965). Asymmetrical as well as symmetrical chemical synapses have been identified in all cnidarian classes, whereas electrical synapses have been demonstrated only in hydrozoans by electrical and dye coupling and by the presence of conventional gap junctions (Anderson and Mackie, 1977; Spencer and Satterlie, 1980; Westfall *et al.*, 1980). In the multiple nerve net system of hydrozoans, neurons belonging to the same nerve net are generally electrically coupled by gap junctions or even represent true syncytia, whereas chemical synapses are restricted to the interfaces between different nerve nets or utilized for excitation of epithelia, including myoepithelia (Satterlie and Spencer, 1987; Mackie, 2004). The restriction of gap junctions within the phylum Cnidaria to Hydrozoa raises the question of whether electrical signaling between neighboring cells via gap junctions could have preceded the evolution of true nervous conduction. If gap junctions evolved before neurons did, the ancestors of Anthozoa and Scyphomedusae must have independently lost their gap junctions secondarily during evolution, which is rather unlikely. Alternatively, gap junctions arose *de novo* in the ancestor of Hydrozoa after nervous cells had already evolved (Mackie, 1990).

Cnidarian nerve rings and nerve tracts have been proposed to correspond to 'compressed nerve nets' (Spencer and Schwab, 1982), although nerves consisting of parallel axon bundles, which are not interconnected by synapses have also been described (Mackie, 2004). A nerve ring, which has been taken as a simple example of neuronal centralization in Cnidaria, is located near the oral pole of the polyp *Hydra oligactis* (Figure 6a; Grimmelikhuijzen and Graff, 1985). Even more obvious is the presence of nerve rings in medusae at the margin of the bell (Figures 6b and 6c). These nerve rings are

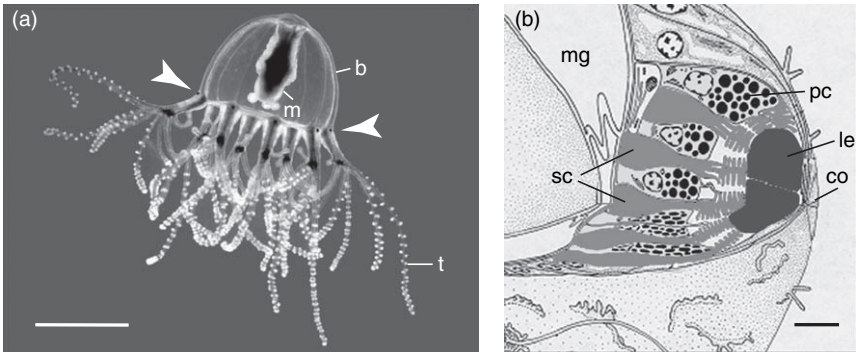

Figure 7 Photosensitive organs in the hydromedusa *Cladonema radiatum*. a, Photograph of an adult medusa with lens eyes located at the base of the tentacles at the margin of the bell (arrowheads). b, Structure of the lens eye in a schematic cross section. The lens eye consists of sensory cells, pigment cells, and a tripartite lens that is covered by a cornea. b, bell; co, cornea; le, lens; m, manubrium; mg, mesogloea; pc, pigment cell; sc, sensory cell; t, tentacle. Scale bar: 700 μm (a); 10 μm (b). Reproduced from Stierwald, M., Yanze, N., Bamert, R. P., Kammermeier, L., and Schmid, V. 2004. The sine oculis/six class family of homeobox genes in jellyfish with and without eyes: Development and eye regeneration. *Dev. Biol.* 274(1), 70–81, with permission from Elsevier.

integrative centers, where different peripheral pathways from sensory organs converge and where activity patterns that result in coordinated behavior are generated. A further striking example is found in the two marginal nerve rings of *Aglantha*, a pelagic hydrozoan medusa. In these interconnected nerve rings, information from 14 conduction systems, including multiple nerve nets, giant axons, and two epithelial pathways, are processed and result in the generation of complex behavioral patterns (Mackie, 2004). A ring-shaped central nervous system (CNS) has been proposed to be appropriate for a radially symmetrical organism, where the term 'central' is not meant morphologically but rather in terms of the functions carried on within it (Spencer and Arkett, 1984; Mackie, 1990). Cnidarian nerve rings may therefore represent the first integrating concentrations of nervous tissue in the animal kingdom (Bullock and Horridge, 1965).

Ganglionic centers, which contain a variety of sensory structures including statocysts, ocelli, or even, lens eyes, can be found spaced around the bell margin at the base of the tentacles of many medusae (Figure 7a). The occurrence of photosensitive structures in Cnidaria includes a wide range of complexity and specializations. The sessile polyps of all cnidarian classes respond to light (Tardent and Frei, 1969) but until now no photoreceptive structures or specialized cells for light detection have been identified in polyps. The free-swimming medusa stage, however, can have differentiated photoreceptor organs, which range from simple ocelli to highly evolved lens eyes (Figure 7b; Land and Fernald, 1992; Stierwald *et al.*, 2004; Piatigorsky and Kozmik, 2004; Gehring, 2005). The diversity of photosensitive structures is illustrated by the cubozoan *Tripedalia cystophora*, where the planula develops unicellular photoreceptors scattered over the posterior epidermis of the larva, whereas the adult jellyfish forms elaborate multicellular lens eyes (Nordström *et al.*, 2003).

The presence of giant axons is another feature of nervous systems that is common to cnidarians and higher invertebrates. Giant axons are distinguishable from normal axons by their large diameter and relatively high speed of signal conduction. Indeed, the first intracellular neuronal recordings in Cnidaria have been carried out from the giant axons in the stem of the siphonophoran *Nanomia*, a colonial hydrozoan (Mackie, 1973). Giant axons may have evolved independently in different cnidarian groups, most probably by axonal fusion within nerve nets or endomitotic polyploidy (Mackie, 1989). In the hydromedusa *Aglantha*, several giant axons have been shown to be involved in rapid escape behavior. Interestingly, motor giant axons of *Aglantha*, which synapse onto swimming muscles, can conduct two types of action potentials. Rapidly conducted Na^{+}-dependent action potentials result in fast swimming associated with escape behavior, whereas slow swimming movements depend on low-amplitude Ca^{2+} action potentials. Thus, two kinds of impulse propagation within the same giant axon subserve different behavioral responses in *Aglantha* (Mackie and Meech, 1985), showing that structural simplicity does not allow inference of functional simplicity in Cnidaria.

1.19.3.6 Cnidarian Nervous Systems: Ion Channels and Neuroactive Substances

Cnidarian nervous systems have electrophysiological properties which are similar to those of higher

animals. Neurons exhibit conventional action potentials with Na^+ inward currents and K^+ outward currents, miniature end-plate potentials, Ca^{2+}-dependent quantal transmitter release, and with spatial and temporal synaptic summation and facilitation (Spencer, 1989). Typical four-domain Na^+ channels are found in Cnidaria, although these channels are not tetrodotoxin sensitive as in higher metazoans (Mackie, 1990). Whereas most protists use Ca^{2+} as the inward charge carrier, purely Na^+-dependent action potentials are common to metazoans, including cnidarians. This prompted Hille (1984) to speculate that Na^+ channels evolved from Ca^{2+} channels in parallel with the evolution of the first nervous system. With the emergence of voltage gated Na^+-selective channels, neurons that generate action potentials at high frequency would have become possible; if Ca^{2+} were the only positive charge carrier, high-frequency discharges would probably cause intracellular Ca^{2+} to accumulate to toxic levels (Anderson and Greenberg, 2001). Hille further suggested that oubain-sesitive Na^+–K^+ ATPase molecules, involved in maintaining the electroosmotic gradient of these two ions, evolved coincidentally with Na^+ channels (Hille, 1984).

Two different classes of neuroactive substances, classical neurotransmitters and neuropeptides, have been detected in cnidarian tissues. The major difference between these two classes is their mode of synthesis. While classical transmitters are synthesized in nerve terminals, neuropeptides are synthesized in neuronal cell bodies, processed within vesicles and then transported along the axons to the nerve terminals. A large percentage of cnidarian neurons show immunoreactivity with antisera against neuropeptides that have either an Arg-Phe-NH2 or Arg-Trp-NH2 carboxyterminus (LWamide, RFamide). Furthermore, from a single anthozoan species, *Anthopleura elegantissima*, 17 different neuropeptides have been isolated so far, some of which are specifically expressed in at least six identified neuronal subpopulations (Grimmelikhuijzen *et al.*, 1996). Cnidarian neuropeptides occur only in neurons and have been shown to have behavioral effects in several species. Interestingly, some of these neuropeptides also play an important role in growth regulation, morphogenesis, and the induction of metamorphosis (Schaller *et al.*, 1996). This dual role is exemplified in the planula of the hydrozoan *Hydractinia echinata*, where LWamide and RFamide neuropeptides form an antagonistic system that influences both planula migratory behavior and initiation of larval metamorphosis in response to environmental cues (Katsukura *et al.*, 2003, 2004; Plickert *et al.*, 2003). Although, the cnidarian nervous system is primarily peptidergic, there is growing evidence for the involvement of classical neurotransmitters in signal transmission. This is supported by the presence of biogenic amines and acetylcholine in the tissues of several cnidarian species and the role of these substances in modulating behavior. Furthermore, serotonin-immunoreactive neurons have been described in the colonial anthozoan *Renilla*, and GABA and glutamate receptors mediate a modulatory function of pacemaker activity and feeding response in *Hydra* (Umbriaco *et al.*, 1990; Concas *et al.*, 1998; Kass-Simon *et al.*, 2003; Pierobon *et al.*, 2004). However, it remains controversial to what extent neuronal signal transmission in Cnidaria is accomplished by the use of classical transmitters since their action at the synaptic level has not yet been demonstrated (Mackie, 1990; Grimmelikhuijzen *et al.*, 1996; Anctil, 1989). Nevertheless, the presence of both aminergic and peptidergic neurotransmitters in cnidarians indicates a parallel evolution of the two transmitter systems (Prosser, 1989).

1.19.3.7 Placozoa versus Cnidaria

The phylogenetic position of Placozoa, which is currently represented by a single-known species, *T. adhaerens*, is controversial. Recent evidence, however, favors the localization of Placozoa between Cnidaria and Bilateria, rather than within medusozoan cnidarians. Placozoa have a low level of tissue organization consisting of only four different somatic cell types arranged in a functional lower and upper side enfolding a number of intermediate cells (Grell and Ruthman, 1991). Although *Trichoplax* apparently lacks nerve cells, some cells react with antibodies raised against the neuropeptide RFamide (Schuchert, 1993). The possible presence of neuropeptides in *Trichoplax* may indicate a secondary loss of a nervous system, in accordance with the notion that placozoans are reduced derivatives of an early metazoan. Alternatively, RFamides could have a primitive pre-nervous role in growth regulation or differentiation. Be that as it may, extant placozoans do not have neurons and do not have nervous systems. Thus, we are left with the Cnidaria.

The analysis of signal conducting systems in cnidarians representing the most basal extant phyla with nervous systems, leads to the conclusion that many basic features characterizing nervous systems of higher animals were already present in the last common ancestor of cnidarians and bilaterians. Cnidarian neurons structurally resemble those of higher animals. Furthermore, the biophysical basis of electrogenesis in

neurons is conventional, and chemical and electrical synapses are similar to those found in all higher metazoans, although the common use of bidirectional synapses in cnidarians is somewhat unusual. Therefore, the 'simplicity' of the cnidarian nervous system does not lie at the level of individual neurons, but rather in the organization of such cells into conducting systems, such as nerve nets. The evolutionary origin of the neuron remains elusive.

1.19.4 Origin of the First Nervous System: A Comparative Developmental Genetic Approach

1.19.4.1 Conserved Genes in Neuronal Development

In 1990, Mackie relaunched Parker's discussion of the elementary nervous system and proposed that the evolutionary origin of the nervous system should be reconsidered in the light of recent results form molecular biology and developmental genetics (Mackie, 1990). Indeed, over 80 years after Parker first put forward his theoretical views, it seems appropriate to consider not only the origin of the cell lineages that initially gave rise to neurons, but also the origin of the genes involved in neurogenesis and neuronal differentiation. Ideally this type of molecular evolutionary developmental approach should allow identification of a basal set of genes that are likely to have been involved in generating the first nervous system. Thus, a novel and promising approach to nervous system evolution is the comparative analysis of the genes that control neuronal proliferation and differentiation in key metazoan phyla. Which key phyla should be subjected to such a molecular genetic analysis? Although, impulse conduction and sensitivity to neuromodulatory substances have been shown in different Porifera, extant sponges lack nerve cells and a nervous system and are therefore not ideal for studies on the molecular genetics of neuronal development. In contrast, true neurons as well as different levels of nervous system organization can be found in the Cnidaria, and, in consequence, a comparative developmental genetic analysis of cnidarian versus bilaterian nervous systems is likely to be useful. In the following, the evolution and origin of the first nervous system will be considered in light of the molecular genetic control elements for neurogenesis, axial patterning, and eye development that are conserved between Cnidaria and Bilateria. A caveat for all of these considerations is, however, the fact that functional analyses of key control genes are still lacking in the Cnidaria.

1.19.4.2 Genetic Control of Neurogenesis in Cnidaria and Bilateria

Key genetic regulators of neurogenesis have been studied in a number of vertebrate (mouse, chick, frog, zebra fish) and invertebrate (*Drosophila, C. elegans*) model organisms. Several transcription factors involved in early neurogenesis events have been identified that are structurally and functionally conserved among protostome and deuterostome phyla (Arendt and Nübler-Jung, 1999; Bertrand *et al.*, 2002; Reichert and Simeone, 2001). This suggests that similar transcription factors might already have been involved in neurogenesis of the common ancestor of all bilaterians. Different classes of regulatory genes involved in neurogenesis have been isolated and their expression patterns studied in the cnidarian model organism *Hydra*, and homologues of regulatory genes expressed during neurogenesis in deuterostomes and protostomes have been found.

Two homeobox genes *prdl-a* and *prdl-b* are expressed in nerve cell precursors and neurons in the body column of the *Hydra* polyp (Gauchat *et al.*, 1998, 2004; Miljkovic-Licina *et al.*, 2004). They are both related to the paired-like *aristaless* family, members of which have been shown to be important for normal forebrain development in vertebrates (Seufert *et al.*, 2005). The *COUP-TF* genes which encode orphan nuclear receptors are implicated both in neurogenesis and in CNS patterning during embryogenesis as well as in the adult nervous system of vertebrates and *Drosophila* (Gauchat *et al.*, 2004). The *Hydra* homologue *hyCOUP-TF*, was found to be expressed in a subset of neurons and in the nematocyte lineage (Miljkovic-Licina *et al.*, 2004). The bHLH transcription factor *CnASH* is related to the *achaete-scute* gene family in *Drosophila*, which has proneural activity (Grens *et al.*, 1995). *CnASH* is expressed in the differentiation of sensory neurons in the tentacles of *Hydra* (Hayakawa *et al.*, 2004). Another bHLH transcription factor *Atonal-like1* (*Atl1*), which belongs to the Atonal gene family, has been isolated in the hydrozoan *Podocoryne*. Atonal homologues are responsible for the determination of neural fate in sense organs as well as in the peripheral system and CNS of bilaterian model organisms (reviewed in Hassan and Bellen, 2000). In the medusa of *Podocoryne, Atl1* is expressed in subsets of presumed nerve cells of the tentacle and the feeding organ (Seipel *et al.*, 2004). These findings suggest that some elements of the genetic network underlying neuronal development may be conserved from cnidarians to vertebrates, implying that the

molecular genetic control of neuronal development evolved only once.

1.19.4.3 Genetic Control of Anteroposterior Patterning in Cnidaria and Bilateria

The bilateral symmetry of bilaterian animals is achieved by the orthogonal intersection of an anteroposterior and a dorsoventral body axis. Different genetic mechanisms are responsible for patterning each axis and the underlying gene networks are widely conserved between Protostomia and Deuterostomia. Thus, *Hox* genes play an evolutionary conserved role in patterning the anteroposterior axis of all bilaterians studied to date (Slack *et al.*, 1993). Interestingly, Hox genes are also responsible for the anteroposterior patterning of bilaterian nervous systems as has been shown in genetic experiments carried out for arthropods and vertebrate model systems. The anteroposterior expression pattern of the *Hox* genes during nervous system development largely reflects their pattern of expression in the embryonic body and corresponds to the spatial arrangement of the *Hox* genes in their chromosomal clusters (spatial colinearity). Similarly, the homeobox transcription factors of the *orthodenticle* (*otd/Otx*) and *empty spiracles* (*ems/Emx*) families have evolutionarily conserved expression domains in the anterior cephalic regions of all bilaterian animals studied to date. Moreover, both gene families are known to play an important role in the development of the most anterior part of the nervous system, the anterior brain, in arthropods and vertebrates. Mutations in these genes lead to severe brain phenotypes such as the absence of large neurogenic regions of the brains of both insects and vertebrates. Thus, bilaterian brains are universally characterized by a rostral region specified by genes of the *otd/Otx* and *ems/Emx* family and a caudal region specified by genes of the *Hox* family (Figure 8a; Shankland and Bruce, 1998; Sharman and Brand, 1998; Arendt and Nübler-Jung, 1999; Hirth and Reichert, 1999; Reichert and Simeone, 2001; Lowe *et al.*, 2003; Lichtneckert and Reichert, 2005).

Homologous genes involved in anteroposterior patterning of the body wall and nervous systems of bilaterians have been isolated from different cnidarian species. *otd/Otx* family genes have been cloned from two hydrozoans, *Hydra* (Smith *et al.*, 1999) and *Podocoryne* (Müller *et al.*, 1999). Whereas *Podocoryne Otx* is only expressed in the striated muscle of the developing medusa, which seems unrelated to *otd/Otx* function in Bilateria, *Hydra Otx* expression can be found in ectodermal epithelial cells throughout the body column. In addition, *Hydra Otx* expression has been detected in nerve cells by cell type Northern; however the *Otx*-positive neural subpopulation has not yet been identified. In gastrozooid polyps of the hydrozoan *Hydractinia symbiolongicarpus*, expression of *Emx* is detected at the oral 'head' end of the oral–aboral axis, specifically in endodermal epithelial cells of the hypostome (Mokady *et al.*, 1998). No *Emx* expression in nervous systems of cnidarians has been described so far.

The question whether true *Hox* genes are present in cnidarians is controversial (reviewed in Galliot, 2000; Ball *et al.*, 2004; Finnerty, 2003). Based on sequence analysis, several authors have argued for the presence of anterior class and posterior class *Hox* genes in cnidarians. The chromosomal linkage of these genes in clusters is still a matter of debate. The expression data from hydrozoans and anthozoans show that different *Hox* genes are expressed in specific regions along the oral–aboral body axis. Five *Hox* genes were recovered from the sea anemone *Nematostella vectensis*; their expression was studied during larval development (Finnerty *et al.*, 2004). Two cnidarian-specific gene duplications appear to have produced two pairs of sister genes *anthox1-anthox1a* which are homologous to bilaterian posterior group *Hox* genes, and *anthox7-anthox8*, which are homologous to the anterior *pb/Hox2* genes in vertebrates and flies (Figure 8b). Whereas expression of *anthox1* is restricted to the ectoderm at the aboral tip of the polyp, a nested expression of *anthox1a, anthox7*, and *anthox8* is found in the endoderm layer all along the body column. The *lab/Hox1* homologue, *anthox6* is expressed in the endodermal body layer of the pharynx, the oral-most part of the polyp. Therefore, during development *Nematosella Hox* gene expression spans nearly the entire oral–aboral axis, which is similar to the situation in the body of bilaterian animals. Whether expression of anthozoan *Hox* genes is present in the nerve cells of *Nematosella* is currently unknown. Cnidarian *Hox* gene expression has also been reported in larval development of the hydrozoan *P. carnea* (Masuda-Nakagawa *et al.*, 2000; Yanze *et al.*, 2001). Three *Hox* genes, *cnox1-Pc, cnox2-Pc*, and *cnox4-Pc* are expressed in restricted domains along the oral–aboral axis in ectodermal and endodermal germ layers of the planula larva. Although, an anteroposteriorly polarized nerve net has been described in the planula larva of *Podocoryne* (Gröger and Schmid, 2001), the presence of the *Hox* genes in the cells of this nerve net has not been investigated yet. Interestingly, comparison of orthologous *Hox* genes between *Nematostella* and *Podocoryne* reveals that their axial expression patterns in the planula are reversed.

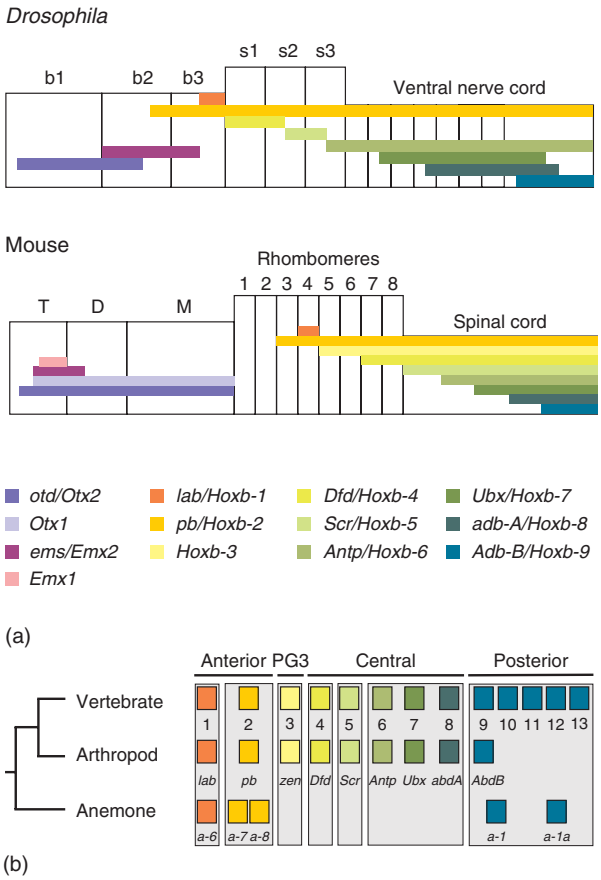

Figure 8 Conserved anteroposterior order of gene expression in embryonic CNS development of bilaterians and occurrence of Hox genes in bilaterians and anthozoans. a, Schematic of *otd/Otx, ems/Emx*, and *Hox* gene expression patterns in the developing CNS of *Drosophila* (stage 14 embryo) and mouse (stage 9.5–12.5 embryo). b, Homology of *Nematostella vectensis Hox* genes to vertebrate and arthropod orthologues based on phylogenetic analysis of homeodomains. Vertebrate *Hox* paralogues are numbered from 1 to 13. Arthropod *Hox* paralogues are named with *Drosophila* terminology (*lab, labial; pb, proboscipedia; zen, zerknüllt; Dfd, Deformed; Scr, Sex combs reduced; Antp, Antennapedia; Ubx, Ultrabithorax; abd-A, abdominal-A; Abd-B, Abdominal-B*). Paralogue groups are classified as anterior, paralogue group 3 (PG3), central, and posterior *Hox* genes. *a-1, anthox1; a-1a, anthox1a; a-6, anthox6; a-7, anthox7; a-8, anthox8;* b1–b3, segments in the *Drosophila* brain (proto-, deuto-, and trito-cerebrum, respectively); s1–s3, mandibular, maxillary, and labial segments, respectively, of the fly subesophageal ganglion; T, telencephalon; D, diencephalon; M, mesencephalon. a, Reproduced from Sharman, A. C. and Brand, M. 1998. Evolution and homology of the nervous system: Cross-phylum rescues of otd/Otx genes. *Trends Genet.* 14(6), 211–214, with permission from Elsevier. b, Reprinted with permission from Finnerty, J. R., Pang, K., Burton, P., Paulson, D., and Martindale, M. Q. 2004. Origins of bilateral symmetry: *Hox* and *dpp* expression in a sea anemone. *Science* 304, 1335–1337. Copyright 2004 AAAS.

For example, the anterior *Hox* gene, *cnox1-Pc* is expressed at the apical end of the planula in *Podocoryne*, while the *Nematostella* homologue, *anthox6* is expressed at the blastoporal end of the planula. This apparent contradiction may be attributed to a developmental reversal of spatial polarity that has been described for *Hox* expression in *Podocoryne* during metamorphosis (Masuda-Nakagawa *et al.*, 2000). Thus, while clear homologues of bilaterian anterior and posterior class *Hox* genes are present in cnidarians, the correlation between cnidarian and bilaterian *Hox* gene expression patterns remains ambiguous. Moreover, the expression and function of cnidarian *Hox* genes in nerve cells has not been explicitly investigated so far, leaving the question of their involvement in nervous system patterning unanswered.

1.19.4.4 Genetic Control of Dorsoventral Specification in Cnidaria and Bilateria

A hallmark of dorsoventral polarity in many bilaterians is the dorsoventral location of the CNS. Whereas in vertebrates the CNS is located dorsally, in arthropods the CNS is located ventrally. This reversal in the relative position of the CNS led Geoffroy Saint-Hilaire to propose that the dorsoventral axes of vertebrates and arthropods are inverted with respect to the position of their mouth openings (Geoffroy

Saint-Hilaire, 1822). This 'dorsoventral inversion' hypothesis has gained strong support in recent years, since homologous, but spatially inverted patterning mechanisms were found to be operating in vertebrates and insects (Holley *et al.*, 1995). The *transforming growth factor-β* (*TGF-β*) superfamily members *decapentaplegic/Bone Morphogenetic Protein 4 (dpp/BMP4)* are required for patterning the dorsal region in arthropods and for promoting ventral fates in vertebrates (Figures 9a and 9b). In both animal groups *dpp/BMP4* have strong anti-neurogenic properties, and therefore, the nerve cord can only develop where *dpp/BMP4* activity is inhibited or absent. In *Drosophila*, the ventral expression of the *dpp* antagonist *short gastrulation* (*sog*) allows the development of the ventral neuroectoderm, whereas in vertebrates, the same effect is achieved dorsally by the *sog*-related *Chordin* gene (Reichert and Simeone, 2001; Lichtneckert and Reichert, 2005).

Although textbooks usually characterize cnidarians as radially symmetrical (Brusca and Brusca, 1990; Campbell *et al.*, 2004; Johnson, 2003), it has long been recognized that many anthozoan cnidarians exhibit bilateral symmetry (Stephenson, 1926; Hyman, 1940). In many sea anemones a secondary body axis, referred to as the directive axis, crosses the pharynx orthogonally to the primary oral–aboral body axis. For example, a cross section through the sea anemone *N. vectensis* reveals that the mesenteries and their associated retractor muscle fibers exhibit a bilateral symmetry in their orientation around the pharynx. Genes involved in specifying the dorsoventral axis in Bilateria have recently been found to be expressed asymmetrically along the directive axis of anthozoans. In the gastrulating embryo of *Acropora millepora*, expression of *bmp2/4-Am* (a *dpp/BMP4* homologue) is not symmetrical about the primary body axis, which runs through the blastopore. Rather *bmp2/4-Am* mRNA is concentrated in one quadrant of the surface ectoderm next to the blastopore (Hayward *et al.*, 2002). This suggests that the *bmp2/4-Am* expression domain defines a second polarized axis, in addition to the one defined by the blastopore. A similar distribution of *dpp/BMP* mRNA has been reported during early embryogenesis of the sea anemone *N. vectensis* (Finnerty *et al.*, 2004); at later developmental stages of *Nematostella, dpp/Bmp4* is expressed in the pharynx and the mesenteries in a bilaterally symmetrical fashion relative to the directive axis (Figure 9c). Within the Cnidaria, bilateral symmetry is a characteristic of anthozoans and thus probably represents an ancestral trait of the phylum that might have been lost

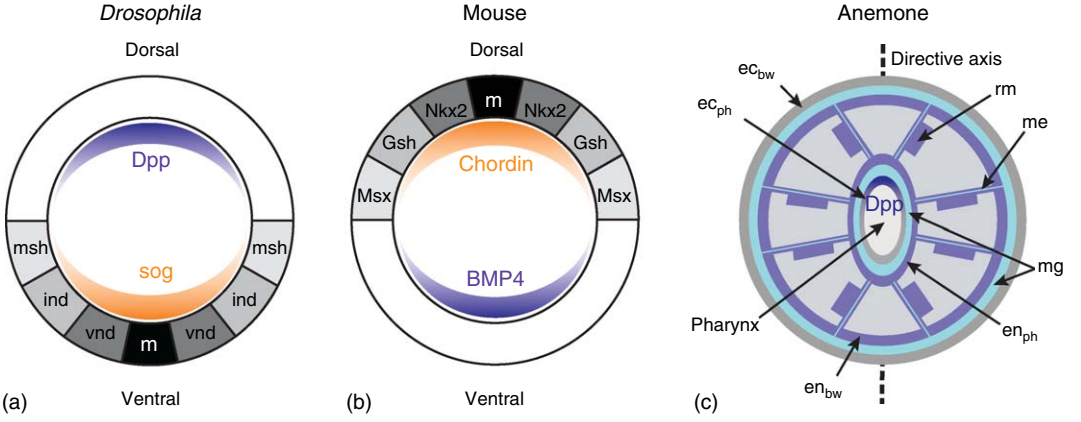

Figure 9 Asymmetric Dpp/BMP4 signaling along the dorsoventral and directive axis of bilaterians and anthozoans. a and b, The secreted products of the homologous genes *dpp/Bmp4* form a dorsoventrally inverted gradient in mouse (Deuterostomia) with respect to *Drosophila* (Protostomia). Sog/Chordin act from opposing dorsoventral poles in both insect and vertebrate embryos antagonizing the antineurogenic effect of Dpp/BMP4. The neuroepithelium is further subdivided by a set of homeobox genes into medial (*vnd/Nkx2*), intermediate (*ind/Gsh*), and lateral (*msh/Msx*) neurogenic domains in *Drosophila* and mouse. c, Cross section through the pharyngeal region of the anemone *Nematostella* reveals bilateral symmetry about the directive axis. The pharynx is attached to the outer body wall via eight endodermal mesenteries. Each mesentery bears a retractor muscle on one face. The only plane of mirror symmetry passes through the directive axis. During development, Dpp is expressed throughout the endoderm. In addition, Dpp expression is transiently found in the pharynx ectoderm in an asymmetric distribution relative to the directive axis. ec_{bw}, body wall ectoderm; ec_{ph}, pharyngeal ectoderm; en_{bw}, body wall endoderm; en_{ph}, pharyngeal endoderm; m, midline; me, mesentery; mg, mesogloea; rm, retractor muscle. a and b, Modified from Reichert, H. and Simeone, A. 2001. Developmental genetic evidence for a monophyletic origin of the bilaterian brain. *Philos. Trans. R. Soc. Lond. B* 356, 1533–1544, Copyright 2001, The Royal Society. c, Reprinted with permission from Finnerty, J. R., Pang, K., Burton, P., Paulson, D., and Martindale, M. Q. 2004. Origins of bilateral symmetry: *Hox* and *dpp* expression in a sea anemone. *Science* 304, 1335–1337. Copyright 2004 AAAS.

secondarily in medusozoans due to the emergence of a clearly radial symmetric medusoid life stage. Although at least part of the dorsoventral patterning system that has antineural function in bilaterians is present in anthozoan polyps, no morphological regionalization of the nervous system along the directive axis of polyps has yet been observed.

In arthropods and vertebrates, initial regionalization of the dorsoventral axis by *dpp/Bmp4* and their antagonists is followed by further patterning of the neuroectoderm along its dorsoventral axis by a group of conserved homeobox genes. In *Drosophila*, *vnd* (*ventral neuroblasts defective*), *ind* (*intermediate neuroblasts defective*), and *msh* (*muscle segment homeobox*) are involved in the dorsoventral specification of a ventral, intermediate, and lateral column of neuroblasts in the developing ventral neuroectoderm (Figures 9a and 9b). During vertebrate neurogenesis, genes closely related to *Drosophila msh* (*Msx*), *ind* (*Gsh*), and *vnd* (*Nkx2*) are expressed in domains corresponding to those in *Drosophila* along the dorsoventral axis of the developing CNS suggesting that this system was conserved throughout evolution (reviewed in Arendt and Nübler-Jung, 1999; Reichert and Simeone, 2001; Lichtneckert and Reichert, 2005).

All three of these dorsoventral patterning genes (*vnd/Nkx2, ind/Gsh, msh/Msx*) are present in cnidarians (Schummer *et al.*, 1992; Grens *et al.*, 1996; Hayward *et al.*, 2001). In the anthozoan *A. millepora, cnox-2Am*, the orthologue of the vertebrate *Gsh* gene, is expressed in scattered ectodermal cells of the larva with a restricted distribution along the oral–aboral body axis. Based on morphology, these cells have been characterized as transectodermal neurons (Hayward *et al.*, 2001). The expression of *cnox-2Am* in a subset of neurons is consistent with the restricted expression of *Gsh* orthologues in bilaterians. The presence of all three dorsoventral patterning homeobox genes in Cnidaria, together with the spatially restricted neuronal expression of *cnox-2Am* along the anteroposterior axis of the planula larva, suggests that the *msh/ind/vnd* system may have had an ancient evolutionary origin that predated the Cnidaria/Bilateria split, and thus might represent an ancient nervous system patterning process. It remains to be shown, however, if the cnidarian orthologues of *vnd/Nkx2* and *msh/Msx* are also expressed in nerve cells and if their expression specifies different neuronal subsets located on a secondary body axis, as in bilaterians.

1.19.4.5 Genes Involved in Eye Development in Cnidaria and Bilateria

A conserved gene regulatory network including members of the *Pax6, six, dachshund*, and *eyesabsent* families has been shown to orchestrate eye development in a wide range of bilaterian animals. *Pax6* mutations in the mouse or fly cause a reduction or absence of eyes. On the other hand, ectopic expression of *Pax6* from various bilaterian species induces ectopic eyes in *Drosophila*, implying that *Pax6* might represent a 'master control' gene for eye development (reviewed in Piatigorsky and Kozmik, 2004; Gehring, 2005). The fundamental, evolutionarily conserved role of the genetic network underlying eye development led to the suggestion of a monophyletic origin of the eye (Gehring and Ikeo, 1999). The *Pax2/5/8* family comprises one single *D-Pax2* gene in *Drosophila* (Fu and Noll, 1997), whereas in mammals three genes, *Pax2, Pax5, and Pax8*, arose by duplications at the onset of the vertebrate lineage (Pfeffer *et al.*, 1998). The *Pax2/5/8* genes play an important role in brain patterning and are also implicated in eye development.

In cnidarians, eyes are found sporadically in some hydrozoan (see Figure 7b) and cubozoan medusae, and it is not known whether other jellyfish have lost their eyes in the course of evolution or whether they never acquired them (Piatigorsky and Kozmik, 2004; Gehring, 2005). Four *Pax* genes (*PaxA, PaxB, PaxC,* and *PaxD*) have been isolated from anthozoans (Miller *et al.*, 2000) and a number of other cnidarian species (Sun *et al.*, 1997, 2001; Gröger *et al.*, 2000; Kozmik *et al.*, 2003), but none of these have a protein domain structure that corresponds of bilaterian *Pax6*. In the cubomedusa *T. cystophora, PaxB* is expressed in the lens and the retina of the complex eyes as well as in the statocyst. Interestingly, it has been shown that *PaxB* is structurally a mosaic between *Pax2* and *Pax6*. This is further supported by functional studies in *Drosophila*, where *PaxB* complements *Pax2* mutants (*sparkling*) and also induces ectopic eyes like *Pax6* (Kozmik *et al.*, 2003). Therefore, *PaxB* of *Tripedalia* might resemble an ancestral gene of the *Pax6* and *Pax2/5/8* subfamilies, which arose by duplication of the ancestral form in the bilaterian line (Kozmik *et al.*, 2003; Piatigorsky and Kozmik, 2004). Thus, the competence to regulate eye development was either inherited from the ancestral *PaxB*-like gene by cnidarian *PaxB* and bilaterian *Pax6*, which would support the monophyletic origin of eyes (Gehring and Ikeo, 1999; Gehring, 2005), or it emerged parallely during the evolution of the two *Pax* genes following the cnidarian bilaterian split (Piatigorsky and Kozmik, 2004). Interestingly, a *PaxB* orthologue has been isolated from sponges (Hoshiyama *et al.*, 1998); however, it is not known whether the expression of this gene is associated with the photoreceptive cells in sponge larva. Additional support for the monophyletic origin

of the eyes was obtained from the hydrozoan *Cladonema*. Orthologues of two *Six* family members, which are known to control eye development in vertebrates and arthropods, are expressed in the lens eyes of the hydromedusa and are involved in eye regeneration (Stierwald *et al.*, 2004). This implies that the common ancestor of Cnidaria and Bilateria may already have possessed some kind of photoreceptive organ. Moreover, it suggests that at least part of the gene regulatory network used for the development of eyes by modern species, was already used by the eumetazoan ancestor. Taken together, the presence of photosensitive cells, probably autonomous receptor–effector cells, in multicellular animals, as exemplified by certain sponge larvae, may have anticipated the emergence of a nervous system. If this were the case, then the sensory input from these photoreceptors might have had a strong influence on the early evolution of the nervous system.

1.19.5 Conclusions and Outlook

The origin and evolution of the first nervous system remains elusive. Over the last 150 years, the evolution of the first nervous system has been a central issue in notions about the emergence of eumetazoan animals, and a variety of theories have been proposed. The main question has been the identification of the primordial cell lineage from which nerve cells might have been derived. During the last decade, however, advances in molecular genetic techniques have focussed our interest on the genes that might have been involved in the generation of the first nervous system. In terms of comparative developmental genetics, it appears that genes involved in patterning of the anteroposterior axis in bilaterians, such as the *Hox* genes, are also expressed in restricted domains along the main body axis during cnidarian larval development as well as in the adult polyp. However, the validity of comparing gene expression patterns along the oral–aboral axis of cnidarians to those found along the anteroposterior axis of bilaterians is questionable. Moreover, in contrast to bilaterians, *Hox* gene expression in cnidarian nerve cells has not yet been unequivocally demonstrated. Similar considerations apply to most of the genes involved in dorsoventral patterning in cnidarians and bilaterians. Thus, although there is morphological and genetic evidence for bilateral symmetry with respect to the directive axis in anthozoans, no regional restriction of neurogenesis in the cnidarian body has been reported to date. Does this mean that the restriction of nervous tissue to one side of the dorsoventral body axis by early genetic pattering mechanism evolved only in bilaterian animals?

One of the most intriguing findings to emerge from preliminary expressed sequence tag (EST) projects on several cnidarian species is that the gene sets of cnidarians and, by implication, the common metazoan ancestor, are surprisingly rich and complex (Kortschak *et al.*, 2003). A long-held assumption is that fewer genes should be required to build a sea anemone than a fly, but this seems not to be true. This paradox is exemplified by the fact that, whereas anthozoan cnidarians have the simplest extant nervous systems, the *A. millepora* genome contains many of the genes known to specify and patterns the much more sophisticated nervous systems of vertebrates and insects. It has been proposed that the first major wave of gene duplications in metazoans predated the Parazoa and Eumetazoa split ~940 Mya resulting in large genomes in basal metazoans (Nikoh *et al.*, 1997; Suga *et al.*, 1999). Gene number seems to be a poor indicator of the sophistication of gene use; it is now widely accepted that alternative splicing and transcriptional regulation are generally more complex in mammals than in insects and that this difference accounts for the execution of more complex molecular programs in complex animals (Ball *et al.*, 2004).

A comparative genetic approach including Cnidaria and Ctenophora as well as different bilaterian groups may help to reconstruct different aspects of the nervous system of the last common ancestor, which might have resembled the first nervous system in evolution. Moreover, the availability of genomic data from Porifera in the near future (Leys *et al.*, 2005), should pave the way for the identification and analysis of further sponge homologues to genes involved in neurogenesis or in sensory organ development in Eumetazoa, thus providing more information about the origin and the evolution of the first nervous system.

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1.20 The Evolution of Arthropod Nervous Systems: Insights from Neural Development in the Onychophora and Myriapoda

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Glossary

anlage(n)	Embryonic primordium or rudiment of an organ.
apomorphy	A character that has been derived from, but differs from, the ancestral condition.
commissure	Axon pathways that connect right and left sides of the central nervous system.
basal	At the base of an evolutionary lineage.
efferent	Axons that exit the central nervous system, i.e., motor axons.
fascicle	A bundle of axons.
invagination	Folding of an epithelial sheet to produce a depression, which may separate from the overlying sheet as a vesicle or tube.
monophyletic	Derivation of a taxonomic group from a single ancestral lineage.
neuroblast	A neural stem cell, whose progeny develop either as neural precursors or neurons.
neurogenesis	Developmental processes underlying the generation of an immature, postmitotic neuron. This excludes processes associated with neuron differentiation, e.g., axon growth, neurotransmitter production.
neuromere	A segmental unit of the nervous system.
paraphyletic	A taxonomic grouping that includes some, but not all, of the descendants of a single common ancestor.
polyphyletic	Derivation of a single taxonomic group from two or more different ancestral lineages.
synapomorphy	A character present in two or more related lineages that is derived from a character present in an ancestor shared by only those lineages.

1.20.1 Introduction

Comparative studies of neural development in living animal groups can provide insights into both the evolutionary origins of those groups and how their nervous systems have been modified in the course of evolution. The arthropods are a natural choice for such an analysis, as we know more about cellular and molecular mechanisms of neural development for one arthropod, the fruitfly *Drosophila melanogaster*, than for any other animal. Other representatives of the Hexapoda and the remaining arthropod taxa – Chelicerata, Crustacea, and Myriapoda – have, in comparison, received much less attention. However, insights into developmental mechanisms provided by studies in *Drosophila* have generated a renewed interest in these groups. Each has been the subject of several recent studies, which have employed a range of modern molecular and cellular tools supplementing the classical histological methods used in

descriptions of arthropod embryology dating back to the late nineteenth century.

This article reviews the literature concerning neural development in Myriapoda and a group generally held to be closely related to the Arthropoda, the Onychophora. These taxa have long held a special interest for those seeking to understand the evolution of the Arthropoda and their relationships to other metazoans (Heymons, 1901; Sedgwick, 1888). For example, similarities in the functional morphology (Manton, 1973) and early embryology (Anderson, 1973) of these groups were central to the development of a long-standing model for arthropod evolution, the Uniramian hypothesis (see Origin and Evolution of the First Nervous System, Adult Neurogenesis and Neuronal Regeneration in the Teleost Fish Brain: Implications for the Evolution of a Primitive Vertebrate Trait).

1.20.2 Evolutionary Relationships between Onychophora, Myriapoda, and Other Arthropods

To understand how nervous systems have been modified during the course of evolution, it is necessary to have some knowledge of the phylogenetic relationships between the animal groups in question. I will therefore briefly review our current understanding of the position of the Onychophora and Myriapoda with respect to other arthropods as a prelude to reviewing neural development in these groups. This issue has been a long-standing one in biology and remains a lively topic of debate.

1.20.2.1 Onychophora

Onychophora were originally considered to be a transitional group between the Annelida and the Arthropoda (Sedgwick, 1884; Snodgrass, 1938). Annelidan-like features of their body plan include a soft body and metameric segmentation, while arthropod affinities include presence of a cuticle, dorsal blood vessel, hemocoel, and appendages with extrinsic and intrinsic muscles.

Recent phylogenetic analyses using a range of gene sequences place the Onychophora close to the Arthropoda: either as one of a number of taxa, including Tardigrada, closely related to the Euarthropoda – a taxon comprising the Hexapoda, Myriapoda, Crustacea, and Chelicerata (Figure 1a; Boore *et al.*, 1995; Aguinaldo *et al.*, 1997; Mallatt *et al.*, 2004); as the sister group to the Euarthropoda (Figure 1b; Wheeler *et al.*, 1993); or as a sister group to chelicerates and crustaceans plus hexapods (Figure 1c; Ballard *et al.*, 1992). A parsimony analysis employing a suite of 100 brain morphological features supports a sister group relationship between Onychophora and Arthropoda (Strausfeld, 1998).

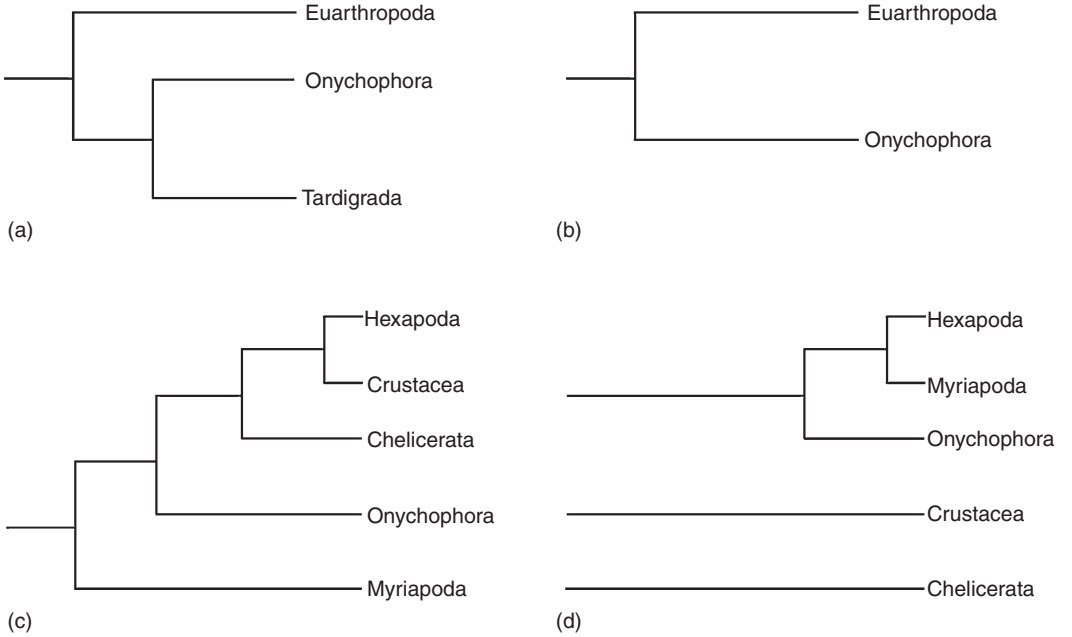


Figure 1 Four alternative views of the phylogenetic relationships between the Onychophora and Euarthropoda (Hexapoda + Crustacea + Chelicerata + Myriapoda). Part a has the Onychophora as one of a number of taxa, including Tardigrada, closely related to the Euarthropoda. Part b has the Onychophora as a sister group to the Euarthropoda. Part c has the Onychophora as a sister group to the chelicerates and crustaceans plus hexapods. Part d depicts the Uniramian hypothesis, which postulates a polyphyletic origin of the arthropods, with Onychophora being most closely allied to the Myriapoda and Hexapoda.

The Uniramian hypothesis (Tiegs and Manton, 1958; Anderson, 1973) placed the Onychophora with the Myriapoda and the Hexapoda in a separate phylum to the other arthropods – the Crustacea and Chelicerata (Figure 1d). The methodology used in the construction of the Uniramian hypothesis has been criticized by several authors and it is no longer widely accepted.

1.20.2.2 Myriapoda

The Myriapoda consists of four classes of terrestrial arthropods (Chilopoda, Diplopoda, Symphyla, and Pauropoda) that share a number of morphological features. However, the status of the Myriapoda as a monophyletic group is controversial. The traditional view of monophyly, based on morphology (Ax, 1987; Boudreaux, 1987), is supported by several recent molecular analyses (Friedrich and Tautz, 1995; Regier and Schultz, 1997; Mallatt *et al.*, 2004). In contrast, Dohle (1997), Kraus (1997), and Shear (1997) maintain that there are no positive morphological characters shared by all of the myriapod classes that can be regarded as genuine synapomorphies – only the absence of characters such as median eyes, scolopidia, and typical ommatidia. Cladistic analyses led them to the conclusion that Myriapoda is a paraphyletic group, with Chilopoda being more closely related to the Hexapoda than to the other three classes of myriapods, which they group together in a taxon called the Progoneata.

Leaving aside the question of monophyly of the Myriapoda, what is the likely sister group of these arthropods? Until recently, the favored view (Figure 2a) was to group the Myriapoda and the Hexapoda in a taxon known as the Antennata (Kristensen, 1991). Morphological characters supporting this division include absence of second antennas and presence of Malpighian tubules, organs of Tömösvary and tracheae in Myriapoda and Hexapoda. A plausible evolutionary scenario for the evolution of the insects from a myriapod-like ancestor involves tagmatization of trunk segments, with loss of limbs on posterior segments to form the abdomen and gain of wings on the two thoracic segments adjacent to the abdomen. This idea seems particularly attractive given the existence of homeotic mutations in *Drosophila* and other insects that result in a transformation from abdominal to thoracic or metathoracic to mesothoracic segmental identity.

In recent years the concept of an Antennata taxon has come under attack and an increasing amount of morphological, developmental, and molecular evidence now points to the malacostracan crustaceans, rather than the Myriapoda, as being the sister group to the Hexapoda (Figures 2b and 2c). Several of the characters supporting this case involve the structure and development of the nervous system. Some of this evidence is detailed below and has been reviewed elsewhere (Strausfeld, 1998; Dohle, 2001; Whitington, 2004; Harzsch *et al.*, 2005). In brief, several aspects of the organization of the compound eyes, the brain, and the ventral nerve cord (VNC) in adult insects show much closer affinities to malacostracan crustaceans than to myriapods. The mode of generation of neurons in the central nervous system (CNS) and the pattern of early axonogenesis in insect embryos are more closely related to malacostracans than to myriapods.

In addition, analyses of several rRNA gene sequences have led to the conclusion that Crustacea, not Myriapoda, is the sister group to Hexapoda. These molecular studies have either grouped the Myriapoda with the Chelicerata (Figure 2b; Friedrich and Tautz, 1995; Hwang *et al.*, 2001; Mallatt *et al.*, 2004; Pisani *et al.*, 2004) or have placed them basal to the rest of the Arthropoda (Figure 2c; Ballard *et al.*, 1992). Similarities between Myriapoda and Arachnida in the pattern of neurogenesis (see Section 1.20.4.1.2) provide support for the former view. In any event, the weight of evidence now speaks against the

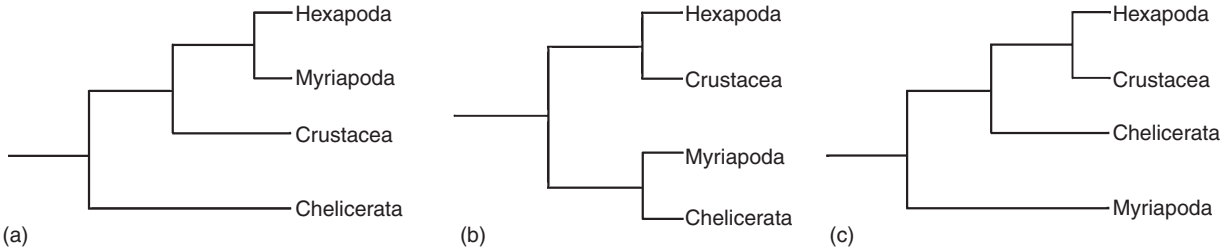


Figure 2 Three alternative views of phylogenetic relationships between the arthropods. Part a has the Myriapoda as the sister group to the Hexapoda, while (b) and (c) depict the more widely held recent view that Crustacea is the sister group to the Hexapoda. Part b has Chelicerata and Myriapoda as sister groups, while (c) has the Myriapoda basal to the other arthropods.

traditional view (Figure 2a) of a sister group relationship between the myriapods and insects.

1.20.3 Neural Development in the Onychophora

Studies of neural development in Onychophora are almost exclusively restricted to morphological descriptions using classical histological techniques (Balfour, 1883; Sedgwick, 1885, 1887; Evans, 1902; Pflugfelder, 1948; Manton and Harding, 1949). A recent study (Eriksson *et al.*, 2003) has supplemented these methods with an immunohistochemical staining procedure that reveals developing axon tracts. These studies, on species from a wide geographical range and including both ovoviviparous and viviparous groups, provide a consistent view of neural development, which is likely to reflect an ancestral mode.

1.20.3.1 Neurogenesis

Neurogenesis in Onychophora commences at an early embryonic stage, when the first segmental borders are beginning to appear in the head and 7–9 pairs of somites are evident (Figure 3; stage 1 according to the staging system of Eriksson *et al.*, 2003).

The region of the ventral ectoderm from which the CNS arises – the neuroectoderm – consists of a simple stratified layer of epithelial cells, whose nuclei are elongated in the apical–basal axis. Scattered mitotic divisions are seen in enlarged cells in this layer. Further internally, a layer of smaller, more rounded cells with heterochromatic nuclei is present – presumably the products of these divisions (Figures 3 and 4). Sedgwick (1887) and later Eriksson *et al.* (2003) suggest that these darker cells are neuron precursors. However, the manner in which they arise from the outer cells remains unclear, as is their distribution over the neuroectodermal cell sheet.

At stage 1 the left and right sides of the neuroectoderm are separated, in all but the most anterior and posterior regions of the embryo, by a medial layer of thin, extraembryonic ectoderm (Figure 3).

Slightly older embryos, in which antennas are just beginning to form (stage 2), show an additional layer internal to the two layers described above, which consists of small cells with strongly heterochromatic nuclei (Figures 4 and 5). Some of these

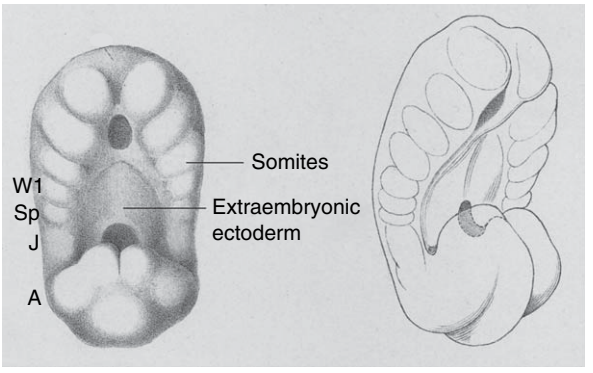


Figure 3 *Peripatus capensis* embryo at stage 1 of Eriksson *et al.* (2003). A, antennal segment; J, jaw; Sp, slime papilla; W1, walking leg 1. Reproduced from Sedgwick, A. 1885. The development of *Peripatus capensis*. *Q. J. Microsc. Sci.* 25, 449–468, with permission from The Company of Biologists.

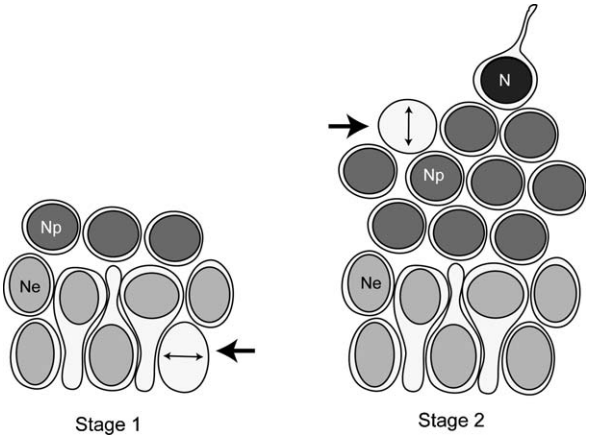


Figure 4 Schematic representations of the neuroectoderm and ganglion anlagen of stage 1 and 2 onychophoran embryos. Ne, neuroectodermal cells; Np, neural precursor cells; N, neurons. Arrows indicate regions of mitotic activity. Lines with double arrowheads indicate orientation of long axis of mitotic spindle within the dividing cells. Neural precursor cells are presumed to arise from divisions of cells in the outer neuroectodermal layer. Mitotic divisions of these neural precursor cells are thought to generate neurons in the ganglion anlage.

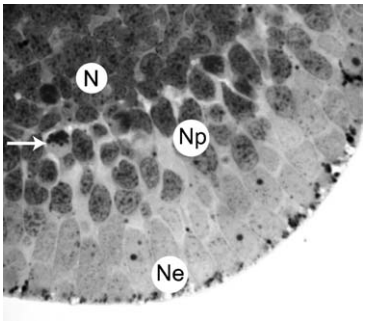


Figure 5 Cross section of a stage 4 *Euperipatoides kanangrensis* embryo showing neuroectoderm and ganglion anlage. Ne, neuroectodermal cells; Np, neural precursor cells; N, neurons. Arrow indicates a dividing cell at the border between the neural precursor and neuron layer. Reproduced from 'Head development in the onychophoran *Euperipatoides kanagrensis* with particular reference to the central nervous system', *J. Morphol.*; Eriksson, B. J., Tait, N. N., and Budd, G. E.; Copyright © 2003, Wiley-Liss. Reprinted with permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.

cells are apparently early-differentiating neurons as they possess small axon-like protrusions (Eriksson *et al.*, 2003). These cells are even more rounded than the cells in the neural precursor layer. There is a rapid increase in the number of these cells and the thickness of the innermost layer over the following developmental period, during which time the outer two layers remain relatively constant in thickness. How the innermost cells are generated from the presumed neural precursor cells in the second layer is unclear. While mitotic activity is evident in the second layer (Figure 5), there is no indication that these cells undergo the asymmetrical, stem cell divisions typical of neuroblasts of insects and malacostracan crustaceans. Nor is there any sign of the internal nuclear migration characteristic of neurogenesis in the Myriapoda (see Section 1.20.4.1.2). Pflugfelder (1948) describes asymmetrical divisions in the neuroectodermal layer that produce neurons, but this is not apparent in his figures. He also describes an internal movement of these immature neurons through a thick basement membrane that separates the neuroectoderm from the ganglion anlagen. Mayer (2005) presents a series of cross sections of the onychophoran neuroectoderm at different developmental stages which provide additional support for the sequence of cellular events described above.

It is unclear whether there is a segregation of neural precursor and epidermal precursor cell fates within the neuroectoderm of the onychophoran embryo. It seems likely that cells remaining in the outer neuroectodermal layer after neurogenesis has slowed down develop into epidermal cells, although this has not been definitively shown. There is no indication from classical histological studies that the epidermis arises by a migration of ectodermal cells from lateral or medial positions, as occurs in myriapod (see Section 1.20.4.1.3), crustacean, and chelicerate embryos.

A stereotypic pattern of early-differentiating, individually identified neurons, such as is seen in insect and crustacean embryos, cannot be readily discerned in the VNC of onychophoran embryos. Large numbers of neurons are rapidly produced soon after neurogenesis commences. Neuromeres in the VNC of adult onychophorans contain much larger numbers of neurons (Schürmann, 1995, estimates 20000–40000 neurons per neuromere) than in insect and crustacean VNC ganglia (approximately 700 neurons per neuromere). The majority of these neurons are small cells (nuclei of diameter 5–9 μm), which cannot be individually identified. While small cells of this type are found in certain regions of the brain in insects and crustaceans (Strausfeld, 1976, 1998; Strausfeld *et al.*, 1995), they are not a feature of ventral ganglia in these groups.

1.20.3.2 Development of Central Axon Tracts

The pattern of axon growth in the embryonic onychophoran CNS is summarized diagrammatically in Figure 6. This schema is based largely on data presented in Eriksson *et al.* (2003), where antiacetylated tubulin immunohistochemistry was used to reveal axons in the developing CNS of *E. kanangrensis*.

1.20.3.2.1 Brain Axon growth within the developing CNS commences when the ganglion anlage already contains many neurons (stage 5). The first axon tract to appear is the antennal commissure, which links left and right sides of the developing antennal hemispheres. Efferent axons linking the first neuromere to the base of the antenna – the

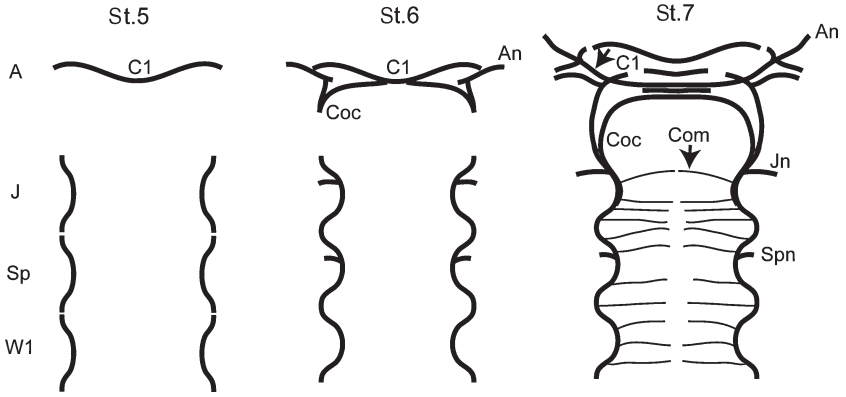


Figure 6 Schematic representation of formation of major axon tracts in the brain and VNC of the onychophoran embryo from stages 5–7. A, antennal neuromere; J, jaw neuromere; Sp, slime papilla neuromere; W1, first walking leg neuromere; Coc, circumesophageal connectives; Com, commissural axons; C1, first (antennal) commissure indicated by arrow; An, antennal nerve; Jn, jaw nerve; Spn, slime papilla nerve.

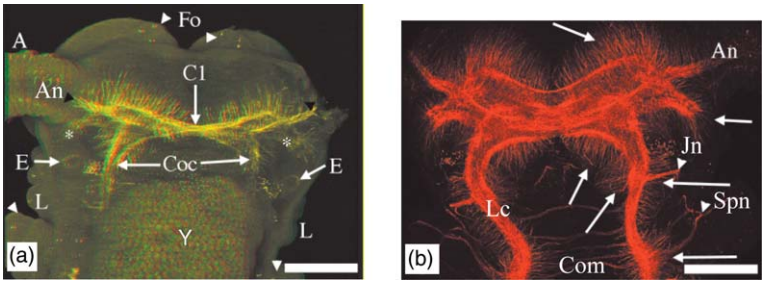


Figure 7 a, Whole-mount stage 6 *E. kanangrensis* embryo immuno-stained with antiacetylated tubulin antibody. C1, first (antennal) commissure; E, eyes; Coc, circumesophageal connectives (longitudinal axons projecting posteriorly); Fo, frontal processes; L, lips; An, antennal nerve (black arrowheads); Y, yolk; asterisks, antennal glomeruli; white arrowheads, slime papillae. Projection of a confocal microscope image stack. Scale bar: 200 μm. b, Whole-mount stage 7 *E. kanangrensis* embryo. An, antennal nerve; Jn, jaw nerve; Spn, slime papilla nerve; Com, commissural axons; Lc, longitudinal connectives. Arrows indicate position of cell bodies of neurons contributing axons to the brain neuropile. Scale bar: 200 μm. Reproduced from 'Head development in the onychophoran *Euperipatoides kanagrensis* with particular reference to the central nervous system', *J. Morphol.*; Eriksson, B. J., Tait, N. N., and Budd, G. E.; Copyright © 2003, Wiley-Liss. Reprinted with permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.

antennal nerve – appear shortly thereafter, as do posteriorly projecting axons (Figure 7a). The brain becomes much more complex from stage 7 onwards, as many more neurons contribute axons to the neuropile, antennal glomeruli develop, and additional commissural tracts appear (Figure 7b). During this period, the antennal nerve becomes redirected, projecting in an anterior rather than a lateral direction.

1.20.3.2.2 Ventral nerve cord Axons begin to appear in the neuromeres that innervate the jaws, the slime papillae, and the most anterior legs at the same time as the antennal commissure begins to form. Most of these axons project longitudinally, meeting axons from adjacent neuromeres during stage 5 (Figure 6). Axonal connection between the brain and the VNC is delayed as a thin ectodermal sheet initially separates the antennal and jaw segments. By stage 7, when the connection between the brain and the VNC is established, a broad, continuous bundle of axons runs longitudinally down each side of the VNC (Figures 6 and 7b). These longitudinal connectives, which are widely separated and lie lateroventrally, are joined by commissural axon bundles.

Commissure formation in trunk segments is retarded with respect to the antennal neuromere, presumably because of the split in the germ band in the trunk. Pioneering axons do not begin projecting towards the ventral midline in trunk segments until late stage 6 to early stage 7 (Figures 6 and 7b). By this stage the left and right sides of the germ band have begun to move towards the ventral midline and are separated by only a narrow strip of thin, extraembryonic ectoderm. In contrast, the antennal commissure is strongly developed at this stage. Growth of efferent axons from trunk neuromeres is well under way by stage 6–7.

The pattern of commissural growth in trunk segments of Onychophora, as revealed by antiacetylated tubulin staining of *E. kanangrensis* (Eriksson *et al.*, 2003) and antihorseradish peroxidase (anti-HRP) immunostaining of *Acanthokara kaputensis* embryos (P. M. Whitington and D. Leach, unpublished) is quite irregular, contrasting with the stereotypic mode of commissure formation in crustaceans and insects (Klämbt *et al.*, 1991; Whitington *et al.*, 1993, 1996). A relatively large and variable number of separate axon fascicles (between five and nine, compared to two in insects and crustaceans) make up the commissural pathways in an individual trunk neuromere (Figure 8a). These fascicles are unevenly spaced in the anteroposterior axis and the course taken by single fascicles, at least during early stages of commissure formation, can be quite irregular. Fascicles often do not grow directly across the midline, but may wander in the anteroposterior axis, sometimes fusing with other fascicles (Figure 8b). Some of the irregularity in the pattern of commissural axon tracts is maintained into late embryonic stages.

Unlike the situation in most arthropod embryos, the developing VNC comprises a continuous mass of neural tissue – at no stage is a morphological separation of individual neuromeres into distinct ganglia linked by connectives evident. The leg nerves are the only gross structures in the CNS that show a clear metameric organization.

The above description of the final structure of the embryonic onychophoran nervous system closely matches the organization of the adult onychophoran nerve cord, as described in several early studies (reviewed in Schürmann, 1995). One difference is

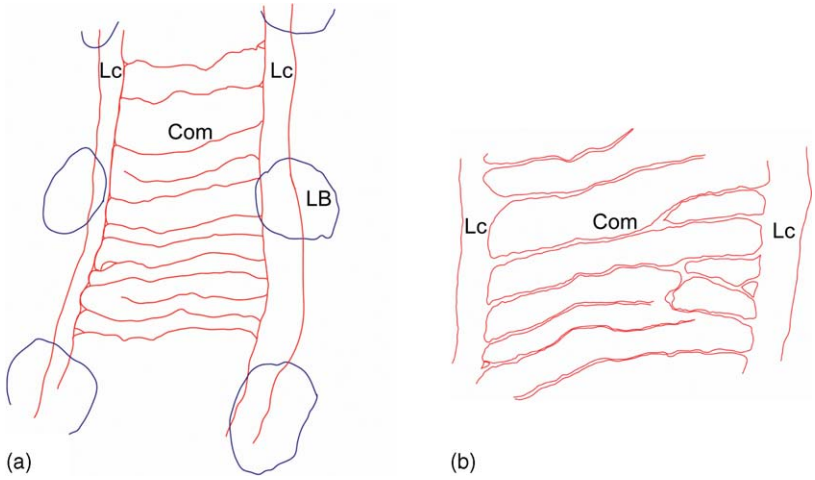


Figure 8 Camera lucida drawings of axons in the VNC of whole-mount stage 7 *A. kaputensis* embryos immuno-stained with anti-HRP antibody (P. M. Whitington and D. Leach, unpublished). a, The axon pattern in two adjacent neuromeres; b, higher-power view of a single neuromere. Lc, longitudinal connectives; Com, commissural axons; LB, base of limb bud.

that the adult shows a more regular arrangement of commissures, with either 9 or 10 being found per neuromere (Balfour, 1883; Feodorow, 1926).

1.20.3.3 Ventral Organ Formation

A pair of prominent structures, called ventral organs, forms in close association with the developing brain of the onychophoran embryo. Their first appearance is at stage 7, when the neuroectoderm just lateral to the ventral midline invaginates to form a pair of grooves (Figure 9a). Eventually, these invaginations detach from the surface to form two vesicles – so-called hypocerebral organs – at the ventral base of the brain (Figure 9b). The function of these organs is unclear, although the ultrastructure of the cells facing their lumen suggests a glandular role (Eriksson *et al.*, 2003, 2005).

Structures called ventral organs have been described in a number of myriapod embryos (e.g., in Symphyla (Tiegs, 1940), Pauropoda (Tiegs, 1947), and Chilopoda (Heymons, 1901)), raising the question of whether these are homologous to the onychophoran ventral organ. Indeed, possession of ventral organs has been claimed to be a synapomorphy of Onychophora and Myriapoda (Anderson, 1973). This issue is complicated by the fact that the term ventral organ has been used to refer to two quite different structures in myriapod embryos (see Sections 1.20.4.1.2 and 1.20.4.1.3).

One clear difference between myriapod and onychophoran ventral organs is that these structures are segmentally repeated in the former group and not in the latter. Pflugfelder (1948) has claimed that ventral organs form in every body segment of onychophoran embryos. However, he uses the term more broadly to refer to the more medial portion of the ventral neuroectoderm. While invagination does takes place within this region in the antennal neuromere, there is no clear indication that the medial neuroectodermal region in other segments undergoes this morphogenetic change or has a different fate to the rest of the neuroectoderm. There would therefore appear to be little justification in claiming that ventral organs exist in segments other than the antennal segment in Onychophora.

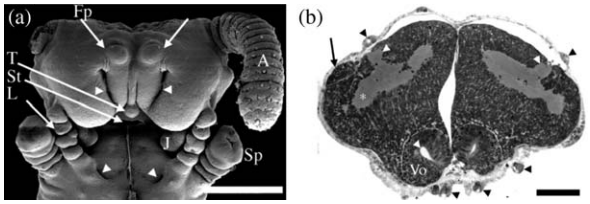


Figure 9 a, Scanning electron microscopy (SEM) micrograph of a stage 7 *E. kanangrensis* embryo. Upper arrowheads show the sites of invagination of the ventral organs. Lower arrowheads show salivary duct openings. A, antenna; L, lips; Sp, slime papillae; St, stomodeum; T, tongue; Fp, frontal process. Scale bar: 200 μm. b, Cross section of a stage 9 *E. kanangrensis* embryo at the axial level of the anterior part of the brain, anterior to the mouth. Ventral organs (Vo) have completely invaginated. Lower white arrowhead indicates the lumen of the ventral organs. Asterisk, antennal glomeruli; upper white arrowheads, antennal tracts; black arrowheads, dermal papillae. Scale bar: 100 μm. Reproduced from 'Head development in the onychophoran *Euperipatoides kanagrensis* with particular reference to the central nervous system', *J. Morphol.*; Eriksson, B. J., Tait, N. N., and Budd, G. E.; Copyright © 2003, Wiley-Liss. Reprinted with permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.

1.20.4 Neural Development in the Myriapoda

As noted above, there is some debate about the evolutionary relationships between the different myriapod taxa – Symphyla, Pauropoda, Chilopoda, and Diplopoda. Nonetheless, I have chosen to discuss their neural development as a group as the pattern of neurogenesis shows similarities in all of these groups. Accounts of myriapod embryology employing classical histological methods (Heymons, 1901; Tiegs, 1940, 1947; Dohle, 1964; Knoll, 1974b; Hertzel, 1984) have been augmented in recent years by a number of studies that have used more modern techniques to examine cell morphology and patterns of cell division within the neuroectoderm, the expression of genes involved in neurogenesis, and the axon morphology of individual neurons (Whitington *et al.*, 1991; Hughes and Kaufman, 2002b; Dove and Stollewerk, 2003; Kadner and Stollewerk, 2004).

1.20.4.1 Neurogenesis

1.20.4.1.1 Thickening of the neuroectoderm In all of the myriapod groups, development of the VNC begins at an early stage, when the first limb buds are just beginning to form. The onset of neurogenesis is marked by a thickening of the ventral ectoderm on either side of the midline, associated with proliferative divisions of the surface ectodermal cells (Figures 10a and 10b). The inner layer of ectodermal cells may retain cytoplasmic connections with the surface (Tiegs, 1940).

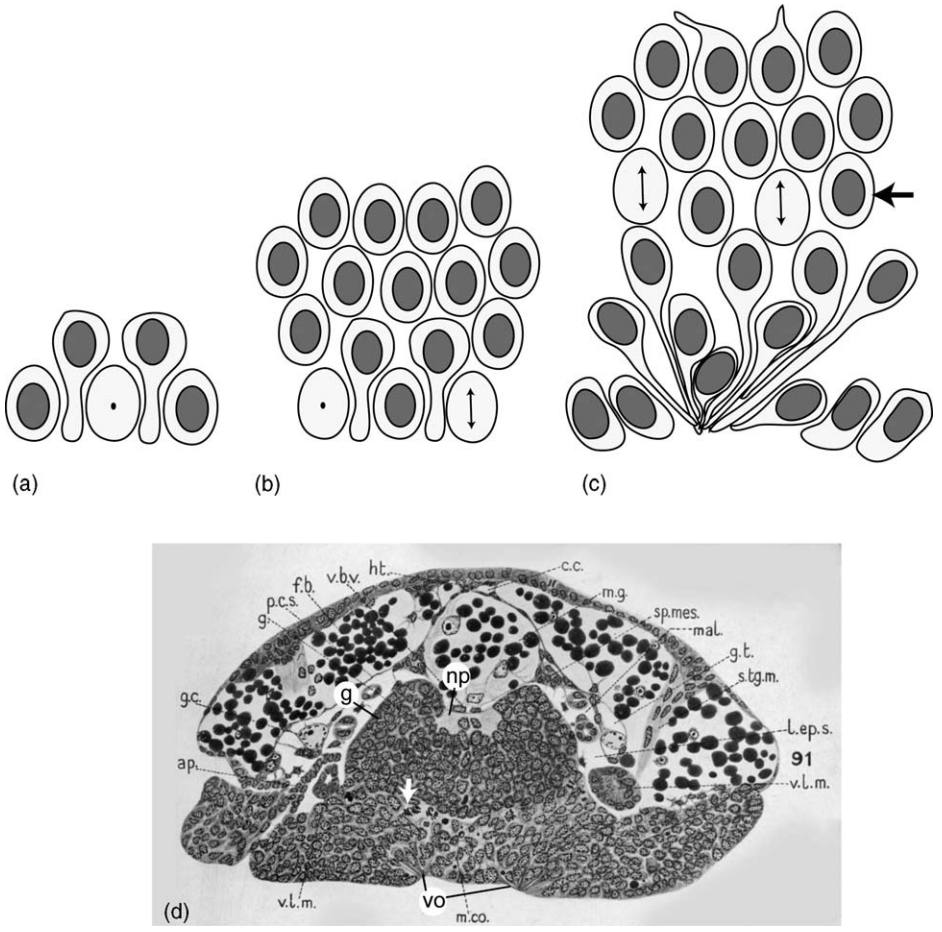


Figure 10 Schematic representations of the neuroectoderm and ganglion anlage of a generalized myriapod embryo at different developmental stages. Lines with double arrowheads indicate orientation of long axis of mitotic spindle within dividing cells. a and b, The neuroectoderm thickens as a result of cell divisions. c, The nuclei of groups of superficial ectodermal cells migrate internally and their apical processes converge at a site or sites on the surface of the neuroectoderm. A mitotically active zone of cells internal to the cells with apical processes (arrow) generates immature neurons. These neurons subsequently send axonal processes to the dorsal surface of the ganglion anlage to form the neuropile. d, Cross-sectional view of the ventral region of an embryo of *Hanseniella agilis* at a somewhat later stage (9 day) to that shown in (c). Vo, ventral organs; g, ganglion anlage; np, neuropile; white arrow indicates the zone of high mitotic activity that lies just external to the ganglion anlage. d, Reproduced from Tiegs, O. W. 1947. The development and affinities of the Pauropoda, based on a study of *Pauropus silvaticus*. *Q. J. Microsc. Sci.* 88, 165–336, with permission from The Company of Biologists.

1.20.4.1.2 Nuclear migration and origin of neural stem cells The second major event of neurogenesis is a migration of nuclei from a superficial to an internal, basal position in certain ventral ectodermal cells (Figures 10c and 10d). These cells retain a connection to the surface by a thin cellular process. The extensions of groups of cells converge at a point on the surface, which is immunoreactive for the anti-HRP antigen (Whitington *et al.*, 1991; Kadner and Stollewerk, 2004) and which stains with rhodamine-phalloidin, presumably because of a high concentration of F-actin in those apical processes (Dove and Stollewerk, 2003; Kadner and Stollewerk, 2004). The surface of the ectoderm flattens at the site of convergence of the cell processes. However, there is no sign of invagination of the ectoderm at this time. This process of nuclear migration has been reported in all myriapod groups: Diplopoda (Dohle, 1964; Dove and Stollewerk, 2003); Chilopoda (Heymons, 1901; Knoll, 1974b; Hertzel, 1984; Whitington *et al.*, 1991; Kadner and Stollewerk, 2004); Symphyla (Tiegs, 1940); and Pauropoda (Tiegs, 1947). It is therefore highly likely to represent a basal character for the Myriapoda.

Earlier studies reported a single site of convergence of cell processes per hemisegment: the group of cells with converging apical processes were referred to as a ventral organ (Figure 10d) (Heymons, 1901; Tiegs, 1940, 1947; Knoll, 1974a; Hertzel, 1984) . More recent studies using anti-HRP and rhodamine-phalloidin staining in Chilopoda and Diplopoda reveal that there are multiple sites of convergence of apical cell processes per hemisegment in these groups. These techniques have not yet been applied to symphylan and pauropodan embryos to clarify the number of sites in these groups.

In the diplopod *Glomeris marginata* a stereotypic pattern of 30–32 sites, arranged in seven rows with four to five sites per row, appears in each head and trunk hemisegment (Figure 11a) (Dove and Stollewerk, 2003). Up to 11 cells contribute processes to each site (Figures 11b and 11c). Individual sites appear in a precise sequence during four waves of production. Somewhat confusingly, Dove and Stollewerk (2003) refer to this process of basal nuclear migration as invagination. There is, however, no evidence from this or other studies that a true invagination of the ectoderm takes place at this stage.

A very similar arrangement of 30 sites per hemisegment is seen in the chilopods *Lithobius forficatus* (Kolbe, 2001; Kadner and Stollewerk, 2004; Figure 11d), *Ethmostigmus rubripes* (Whitington *et al.*, 1991), and *Strigamia maritima* (Chipman and Stollewerk, 2006). However, fewer cells (five to nine) contribute processes to each site in *Lithobius* than in the diplopod *Glomeris* and in *Strigamia* the cellular processes converge on one of the cells in the group, rather than on the apical surface of the ectoderm. A strikingly similar pattern of phalloidin-positive sites is seen in the neuroectoderm of the spider *Cupiennius salei* (Stollewerk *et al.*, 2001). This has been advanced as evidence for a close phylogenetic relationship between the Myriapoda and the Chelicerata (Kadner and Stollewerk, 2004).

It is tempting to conclude that this striking, evolutionary-conserved morphological event is related to the specification of neural fate within the ventral ectoderm. What is the fate of the groups of cells whose nuclei undergo internal migration? Some early workers claimed that these cells are neural stem cells, with the properties of insect neuroblasts, i.e., cells that divide repeatedly in an asymmetric

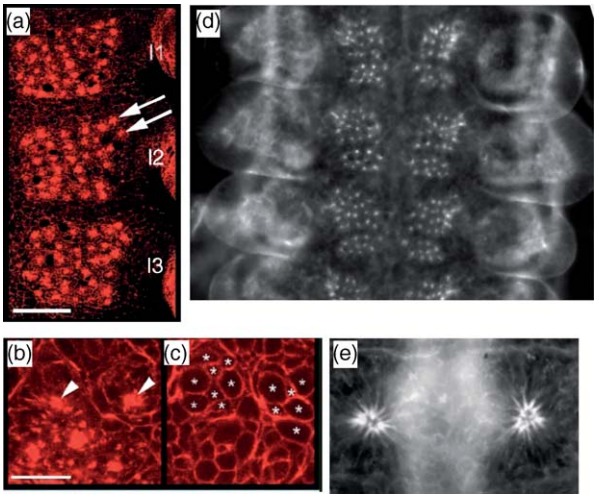


Figure 11 Rhodamine-phalloidin staining of myriapod embryos reveals sites of convergence of apical processes of neuroectodermal cells. a, The regular pattern of these sites (arrows) in three adjacent trunk neuromeres of the diplopod *G. marginata*. b, A higher-magnification view of an apical optical section showing several sites (arrowheads). c, A basal optical section at the level of the nuclei of the cells with apical processes (asterisks). The processes of the cluster of eight cells on the left of (c) converge on the left site marked with an arrowhead in (b). d, Shows the conserved pattern of phalloidin-stained sites in the first and second maxillary and maxilliped segments of *L. forficatus*. e, Invagination of the neuroectoderm later in development draws the superficial phalloidin-stained sites into a circle in the center of the hemiganglion. The longitudinal axon tracts lie out of focus between and internal to these circles of phalloidin staining. Scale bars: 50 μm (a); 10 μm (b and c). a–c, Reproduced from Dove, H. and Stollewerk, A. 2003. Comparative analysis of neurogenesis in the myriapod *Glomeris marginata* (Diplopoda) suggests more similarities to chelicerates than to insects. *Development* 130, 2161–2171, with permission from The Company of Biologists. d and e, Reproduced from Kolbe, S. 2001. Comparisons of Central Nervous System Development in a Centipede and a Spider. Honours thesis, University of Melbourne.

fashion to produce a chain of ganglion mother cells, each of which divides symmetrically to produce a pair of neurons. However, the histological evidence advanced for this idea is not convincing (see figures 37, 48, and 49 in Dohle, 1964). Tieg's detailed histological studies of symphylan (Tiegs, 1940) and pauropodan (Tiegs, 1947) embryos provide no evidence for enlarged cells with the characteristics of insect neuroblasts in the ventral ectoderm of these groups. Nor do they indicate that the cells whose nuclei have migrated internally are a particular focus of mitotic activity. Rather, divisions are concentrated in a layer internal to these cells (Figures 10c and 10d), which abuts the developing ganglion.

To determine the relationship between the sites of phalloidin staining and mitosis in the diplopod embryo, Dove and Stollewerk (2003) employed antiphosphohistone 3 staining. They found that mitotic cells are found adjacent to sites of phalloidin staining or in regions where such staining is later seen (Figures 12a–12c). These dividing nuclei are mainly located at the surface of the ectoderm, rather than internally. They observed that the dividing cells are significantly larger than surrounding cells and suggested that these are neuronal stem cells.

If this presumption is correct, then these observations would argue for a stereotypic pattern of neural stem cells in the neuroectoderm of diplopodan embryos. What remains unclear however is whether the dividing superficial ectodermal cells identified in the Dove and Stollewerk (2003) study generate the nearby cells with convergent apical processes. It is also unclear whether these internal groups of cells are undifferentiated neurons, neural progenitors, or some other cell type. The reported positive anti-HRP immunoreactivity of the sites where the processes of these cells converge is not conclusive evidence for a neural identity, since anti-HRP is not expressed in axons in the centipede CNS later in embryonic development (Whitington *et al.*, 1991).

Confusingly, Dove and Stollewerk (2003) refer to the cells with basal nuclei and convergent cytoplasmic processes as neural precursors, a term normally reserved for progenitor cells that divide to produce neurons. However, it is clear that these authors use the term neural precursors to mean postmitotic, undifferentiated neurons (Stollewerk, 2004, p. 4). Whitington *et al.* (1991) used a different method – BrdU incorporation – to determine patterns of cell division within the neuroectoderm of the scolopendromorph centipede *Ethmostigmus*. They found labeled cells at all positions in the internal–external axis of the neuroectoderm, not just on the apical ectodermal surface (Figure 12d). This observation concurs with Tiegs' observations in Pauropoda and Symphyla. Strongly labeled cells were not conspicuously larger than their presumed, more weakly stained progeny. The latter were also not oriented

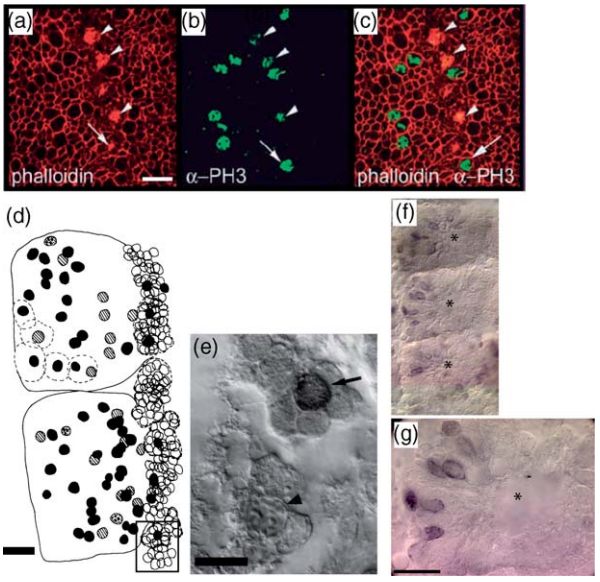


Figure 12 Patterns of cell division in the neuroectoderm of Diplopoda and Chilopoda. a–c, Confocal micrographs of a millipede (*G. marginata*) hemineuromere showing the close relationship between mitotic cells, stained with antiphosphohistone 3 antibody, and rhodamine-phalloidin-stained sites. The arrowheads in the single (a, b) and merged images (c) show where mitotic cells correspond to stained sites, while the arrows show where a mitosis occurs near a stained site. Scale bar: 10 μm. d, Camera lucida drawing of BrdU-labeled nuclei within two adjacent trunk hemineuromeres of a centipede (*E. rubripes*) embryo. Black nuclei are internal, hatched nuclei external, and dotted nuclei in intermediate positions. Medial is to the right and anterior is up. Dashed lines show the borders of clusters of neurons. These clusters are particularly obvious along the medial edge of the ganglion anlage. Scale bar: 20 μm. e, Photomicrograph of the region within the rectangle in (d). A BrdU-labeled nucleus (arrow) and a labeled nucleus in mitosis (arrowhead) are found in the center of the two most posterior clusters in this hemisegment. Scale bar: 20 μm. f, Photomicrograph of three trunk hemineuromeres in a centipede (*L. forficatus*) embryo, showing BrdU-labeled nuclei in purple. This embryo is older than the *Ethmostigmus* embryo shown in (d) as the central region of neuroectoderm has begun to invaginate (asterisks). The neuroectodermal cells lie in clusters, which radiate out from the rim of the invagination. The strongest BrdU-labeled cell within each cluster tends to lie towards the outer edge of the cluster. g, Enlargement of the middle neuromere in (f). Scale bar: 20 μm. a–c, Reproduced from Dove, H. and Stollewerk, A. 2003. Comparative analysis of neurogenesis in the myriapod *Glomeris marginata* (Diplopoda) suggests more similarities to chelicerates than to insects. *Development* 130, 2161–2171, with permission from The Company of Biologists. d and e, Reproduced from *Roux's Arch. Dev. Biol.*, vol. 199, 1991, pp. 349–363, Segmentation, neurogenesis and formation of early axonal pathways in the centipede, *Ethmostigmus rubripes* (Brandt), Whitington, P. M., Meier, T., and King, P., figure 3, with kind permission of Springer Science and Business Media. f and g, Reproduced from Kolbe, S. 2001. Comparisons of Central Nervous System Development in a Centipede and a Spider. Honours thesis, University of Melbourne.

in internally directed columns. These observations argue against the presence of insect-like neuroblasts in this centipede.

However, cells were found to lie in clusters in the developing ganglia, often with a strongly labeled BrdU-positive cell near the center or towards the dorsal side of the cluster (Figures 12d and 12e). A longitudinal column of four to five prominent clusters was found on the medial border of each hemineuromere (Figure 12d). Injections of the dye Lucifer yellow into a single cell in one of these clusters revealed that all of its cells are dye-coupled. These observations are consistent with the idea that each of these clusters represents a clone of progeny arising from a single, central stem cell, although this is yet to be confirmed by direct lineage-tracing methods. It is tempting to conclude that these clusters are equivalent to the groups of internal cells whose processes converge on phalloidin-stained ectodermal sites, but direct evidence for this is also lacking.

To shed further light on neurogenic mechanisms in the Myriapoda, Dove and Stollewerk (2003) examined the expression pattern within the diplopod neuroectoderm of orthologues of the *Drosophila achaete-scute* complex (*asc*) proneural genes and neurogenic genes *Delta* (*Dl*) and *Notch* (*N*). In *Drosophila*, proneural genes define regions within the neuroectoderm where cells acquire the potential to differentiate into neuroblasts. Subsequent cell–cell signaling mediated by *N* and *Dl* causes a single cell within each proneural cluster to adopt a neuroblast fate, while the rest acquire an epidermal progenitor fate (Baker, 2000).

Dove and Stollewerk (2003) found that the *Glomeris achaete-scute* homologue (*GmASH*) is expressed in neuroectodermal cells at the same locations as phalloidin-stained sites, just prior to the appearance of those sites. It is also transiently expressed in the groups of cells whose processes converge on those sites, as is the *Glomeris Delta* homologue (*GmDelta*). Similar observations have been made for homologues of *achaete-scute* (*LfASH*) and *Delta* (*LfDelta*) in the centipede *L. forficatus* (Kadner and Stollewerk, 2004). Assuming that *GmASH*, *LfASH*, *GmDelta,* and *LfDelta* are involved in specification of neural stem cell fate, these findings would support the view that the apical ectodermal cells that express these genes belong to this lineage, but leave unresolved the identity of the internal groups of cells. Direct lineage-tracing experiments are called for to establish the relationship between dividing superficial ectodermal cells, the groups of cells with basal nuclei and post-mitotic neurons.

While the chilopod and diplopod homologues of insect proneural and neurogenic genes are expressed in the neuroectoderm, it is clear that these genes must play different roles in insects and myriapods. In insects, the action of these genes leads to a segregation of neuroblasts from epidermal progenitor cells within the neuroectoderm. In myriapods, epidermis arises from ectodermal cells lateral or medial to the neuroectoderm (see Section 1.20.4.1.3). The role of the proneural and neurogenic genes in the latter groups may be to specify the timing of commitment of a stem cell to the neural lineage and the subsequent divisions of this cell to form neurons, rather than a segregation of neural and epidermal fates.

1.20.4.1.3 Ganglion formation Following the process of internal nuclear migration in myriapod embryos, the neuroectoderm thickens rapidly, and the anlagen of the segmental ganglia begin to take shape. This phase of neurogenesis must involve the production of large numbers of neurons from progenitor cells in the neuroectoderm. However, little is known about the location of the neural stem cells or the nature of the divisions that generate these neurons.

It would seem clear that some neuron progenitor cells are not restricted to the most superficial neuroectodermal layer, but are located quite internally. In Symphyla (Tiegs, 1940) and Pauropoda (Tiegs, 1947), two distinct zones can be recognized in the developing neuroectoderm: the ganglion rudiment and the ventral portion of the neuroectoderm (Figure 10d). Based on the distribution of mitotic figures, the zone of ventral ectoderm that abuts the developing ganglion appears to be a major site of generation of neurons, although divisions also occur at other levels of the neuroectoderm and within the ganglion. This zone is internal to the neuroectodermal cells with basal nuclei and apical processes, which appear to be relatively mitotically inactive.

A distinctive morphogenetic event takes place in the ganglion rudiment of diplopod (Dohle, 1964) and chilopod (Heymons, 1901; Whitington *et al.,* 1991; Kolbe, 2001) embryos: the surface of the ganglion rudiment invaginates to form a vesicle, which becomes trapped inside the ganglion (Figures 12f and 12g). During this process, the superficial phalloidin/anti-HRP-stained sites become drawn into a circle in the middle of the hemiganglion (Figure 11e).

Invagination occurs after the process of internal nuclear migration described in Section 1.20.4.1.2. This fact, and the observation that invagination does not occur in symphylan or pauropodan embryos,

which do show nuclear migration, would suggest that the two processes are not coupled. Confusingly, the vesicle arising from invagination has also been called a ventral organ by some authors (Heymons, 1901). The functional significance of ganglionic invagination is unclear: the vesicle is a transitory structure which becomes flattened and disappears late in embryogenesis (Heymons, 1901). Heymons (1901) claimed that these invaginations are the seat of production (Bildungsstätte) of neurons in *Scolopendra dalmatica*. In *Ethmostigmus* (Whitington *et al.*, 1991) and *Lithobius* (Kolbe, 2001), BrdU-labeled nuclei are often found on the outer edge of clusters of cells that radiate out from the invagination site in the ganglion (Figures 12f and 12g). However, the fact that invagination takes place well after the clusters have begun to form indicates that it is probably incidental to neural generation.

The invagination process described above for diplopodan and chilopodan embryos bears some similarities to the process of ventral organ formation in the antennal segment of onychophoran embryos. However, in the latter group, the invaginated tissue develops into a structure, the hypocerebral organ, which remains separate from the CNS.

The separation of the ganglion rudiment from the ventral surface takes place in different ways in different myriapod groups. As noted above for Symphyla and Pauropoda (Tiegs, 1940, 1947), a mass of tissue lies ventral to the ganglion rudiment from an early stage. Some of these ventral cells contribute to the formation of distinctive organs called eversible sacs, located at the limb bases. A similar organ is formed in the collum segment of pauropod embryos, while in other segments the ventral cells are either absorbed into the ganglion or become incorporated into the epidermis. In leg-bearing segments of pauropod embryos, medial ectodermal cells spread laterally to form the ventral epidermis (Tiegs, 1947). In Chilopoda and Diplopoda, the ventral epidermis arises by the medial migration of ectodermal cells that initially lie lateral to the neuroectoderm. These cells eventually form a continuous epithelium which overgrows and internalizes the ganglia (Dove and Stollewerk, 2003; Kadner and Stollewerk, 2004).

Differences are also seen between different myriapod groups in the degree of separation of left and right sides of the developing ganglia. In scolopendromorph centipedes, there is a wide separation between hemiganglia from the earliest stages of neurogenesis (Heymons, 1901; Whitington *et al.*, 1991), which is maintained for a considerable period of embryogenesis (Figure 13a). The eventual fusion of the hemiganglia in the ventral midline is accompanied by apoptosis of the ventral extraembryonic ectodermal cells.

In other Chilopoda and all other myriapod groups, the hemiganglia are quite close together from the outset of neural development, separated by, at most, a narrow band of midline cells. The split of the germ band in scolopendromorph centipedes therefore appears to be a derived character of this group, perhaps associated with the large size of the egg. It is certainly not a shared-derived (synapomorphic) character of Myriapoda and Onychophora.

1.20.4.2 Formation of Central Axon Tracts

Studies of myriapod embryology using standard histological techniques provide some information about the timing of axon growth (Heymons, 1901; Tiegs, 1940, 1947; Dohle, 1964). The first axons appear on the dorsal surface of the ganglion anlagen following internal nuclear migration and the onset of mitotic activity within the thickened neuroectoderm (Figure 10c). There are no neural cell bodies overlying these dorsal axons. In all groups, axonogenesis, as with other developmental processes, takes place in a rostrocaudal sequence, beginning in head neuromeres, then progressing to more posterior segments (Figure 14a).

The order of formation of different axonal tracts has been revealed in the centipedes *Ethmostigmus* and *Lithobius* by a combination of single-neuron dye injections, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) axonal labeling, and rhodamine-phalloidin staining (Whitington *et al.*, 1991; Kolbe, 2001). In *Ethmostigmus*, a wide bundle of caudally directed axons – the primary tract – descends from the brain to posterior segments well before axon growth begins in any of the trunk neuromeres (Figures 13a–13c). This tract becomes progressively wider as development ensues. Heymon's description of axon growth in *Scolopendra* (Heymons, 1901) suggests that a primary tract also forms in this embryo. A similar tract appears to form in *Lithobius*, but axon growth has been initiated in trunk neuromeres by the time the axons of the primary tract arrive in those segments (Kolbe, 2001).

Within trunk segments of the *Lithobius* embryo, longitudinal, peripheral, and commissural axons all begin outgrowth at around the same time (Figure 13a). In contrast, commissural axon outgrowth in *Ethmostigmus* lags behind longitudinal and peripheral axon growth. Commissural axons only cross the midline when the hemiganglia, which are widely separated when the first neurons send out axons, have come into close proximity

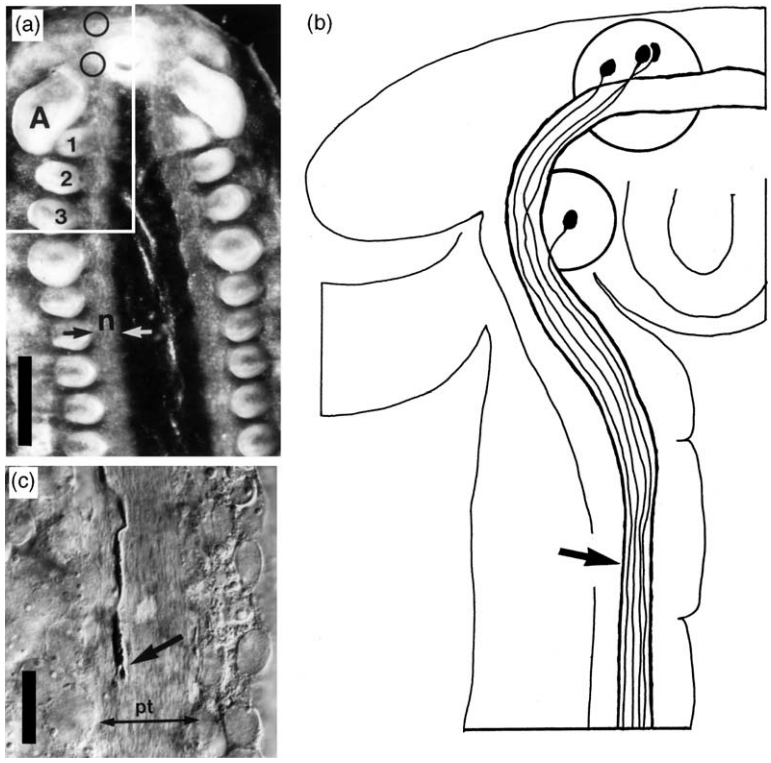


Figure 13 Primary tract formation in the embryo of *E. rubripes*. a, Photomicrograph showing separation of left and right sides of the germ band. Arrows indicate the medial and lateral borders of a hemineuromere (n). A, antenna; 1, mandibles; 2, maxilla 1; 3, maxilla 1. Scale bar: 200 μm. b, Schematic diagram of the region within the white rectangle in (a). Descending axons from neurons located in the circled brain regions form the primary tract (arrow). c, The growth cone (arrow) of a single axon within the primary tract (pt) that has been injected with Lucifer yellow. Scale bar: 20 μm. Reproduced from *Roux's Arch. Dev. Biol.*, vol. 199, 1991, pp. 349–363, Segmentation, neurogenesis and formation of early axonal pathways in the centipede, *Ethmostigmus rubripes* (Brandt), Whitington, P. M., Meier, T., and King, P. figure 6, with kind permission of Springer Science and Business Media.

(Figure 15d). A regular pattern of commissural tracts eventually develops in each trunk neuromere of both *Ethmostigmus* and *Lithobius* (Figures 14b and 14c) embryos (P. M. Whitington unpublished; Kolbe, 2001).

The pattern of axon growth from several early-differentiating neurons in the *Ethmostigmus* CNS has been determined by single-neuron dye-filling (Whitington *et al.*, 1991) (Figures 15 and 16). At early stages, many of these neurons can be individually identified, allowing a comparison with the detailed descriptions of axon growth from identified neurons in insects (Goodman *et al.*, 1984; Thomas *et al.*, 1984; Whitington *et al.*, 1996) and malacostracan crustaceans (Whitington *et al.*, 1993) embryos.

The pattern of axon growth in the centipede appears to be substantially different to that seen in insects and malacostracan crustaceans (Figure 16). However, a caveat to this conclusion is that the axon morphology of only a subset of early differentiating centipede neurons has been determined to date. The large number of cells present in centipede neuromeres at even the earliest stages of axon growth will make an exhaustive description of the pattern of central axon growth a challenging goal. Access to probes for myriapod homologues of *Drosophila* genes that are expressed in specific subsets of neurons would greatly facilitate a comparison between the myriapods and the insects. As an illustration of the potential of this approach, Duman-Scheel and Patel (1999) have used molecular probes to identify putatively homologous neurons in hexapods and crustaceans.

Centipede homologues to several *Drosophila* genes have now been cloned: these include *even-skipped*, *engrailed*, and *wingless* (Hughes and Kaufman, 2002b) and the 10 *Hox* genes (Hughes and Kaufman, 2002a). Unfortunately, mRNA probes have not yet provided the resolution necessary to determine the expression of these genes at the level of single neurons. Nonetheless, examination of figure 4d from Hughes and Kaufman (2002b) suggests that *even-skipped* may be expressed in a small subset of neurons located close to the midline in each hemineuromere, a

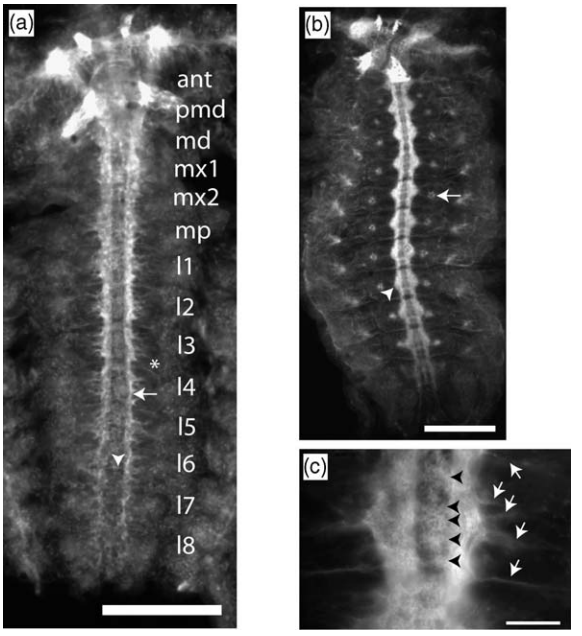


Figure 14 Rhodamine-phalloidin staining reveals patterns of axon growth in *Lithobius* embryos. a, Stage 5 embryo (according to staging scheme of Hertzel, 1984). Axon growth has just begun in the most posterior segments, l7/8, while longitudinal (arrow), commissural (arrowhead), and peripheral (asterisk) axons are present in every neuromere anterior to l6. Segment abbreviations: ant, antennal; pmd, premandibular; md, mandibular; mx1, maxillary 1; mx2, maxillary 2; mp, maxilliped; l1–8, leg-bearing 1–8. Scale bar: 200 μm. b, Late-stage embryo. Each neuromere shows a well-developed neuropile, which stains strongly with rhodamine-phalloidin (arrowhead). The arrow shows the ring of phalloidin staining marking the site of the vesicle that forms by invagination in every hemiganglion. Scale bar: 200 μm. c, Magnified view of a neuromere from the embryo in (b). Arrowheads show the position of commissures. Arrows indicate peripheral nerves. Scale bar: 30 μm. Reproduced from Kolbe, S. 2001. Comparisons of Central Nervous System Development in a Centipede and a Spider. Honours thesis, University of Melbourne.

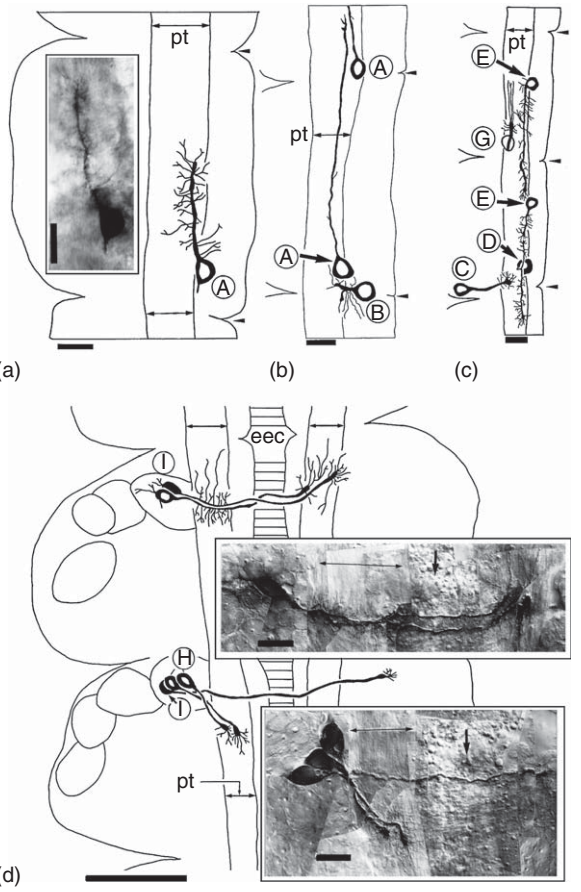


Figure 15 Pattern of axon growth from early-differentiating neurons in trunk neuromeres of stage 5 (a), 6 (b), and 7 (c) *Ethmostigmus* embryos (staging after Whitington *et al.*, 1991). Camera lucida drawings and photomicrographs of neurons injected with Lucifer yellow. Each of the labeled neurons can be individually identified based on soma position and pattern of axon growth. Most of the early axons run longitudinally, fasciculating with axons in the primary tract (pt). The medial edge of the hemineuromere is on the right and anterior is up in each case. Scale bars: 25 μm for the line drawings in (a–c), 20 μm for the micrograph in (a). d, Commissural axon growth in trunk neuromeres of a stage 7 *Ethmostigmus* embryo. The left and right hemineuromeres have approached the midline and are separated by only a thin band of extraembryonic ectoderm (eec). The axon of neuron I has crossed the midline. Scale bars: 100 μm for the line drawings, 20 μm for the micrographs. Reproduced from *Roux's Arch. Dev. Biol.*, vol. 199, 1991, pp. 349–363, Segmentation, neurogenesis and formation of early axonal pathways in the centipede, *Ethmostigmus rubripes* (Brandt), Whitington, P. M., Meier, T., and King, P., figures 7 and 9, with kind permission of Springer Science and Business Media.

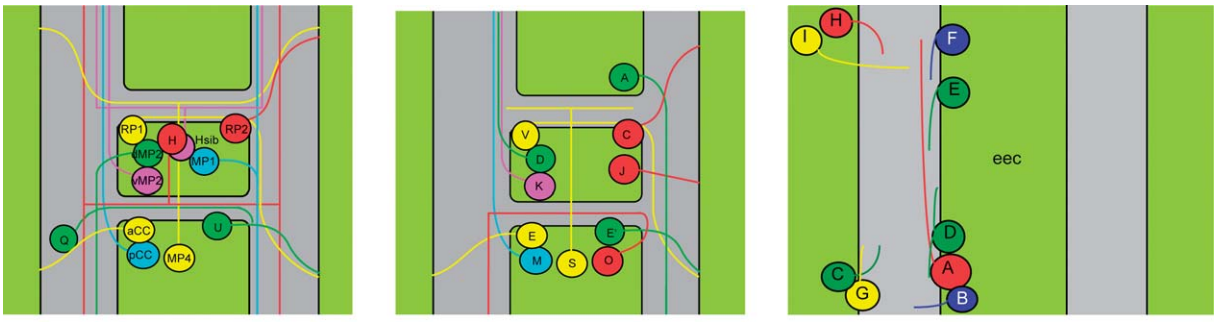
pattern that bears some similarities to insects and crustaceans (Duman-Scheel and Patel, 1999).

1.20.5 Summary and Evolutionary Insights Gained from Studies of Neural Development

While several recent studies have contributed significantly to our understanding of mechanisms for neural development in the Onychophora and Myriapoda, many questions remain. In this final section I will summarize key findings that provide insights into the evolution of the nervous system in these groups and their relationships to other arthropods. In addition, I will highlight some of the unresolved issues in this field and point to future directions for research.

1.20.5.1 Mechanisms for Neurogenesis

Mechanisms for neurogenesis in insects are well understood at cellular and molecular levels, due largely to an extensive body of research in the fly *Drosophila* (reviewed in Goodman and Doe, 1993; Skeath and Thor, 2003). The neural stem cells in



Insect (*Drosophila*, Grasshopper) Malacostracan (crayfish, woodlouse) Centipede (*Ethmostigmus*)

Figure 16 Diagrammatic summary of patterns of early axon growth from identified neurons in the CNS of insect (*Drosophila* and grasshopper), malacostracan crustacean (crayfish *Cherax*, woodlouse *Porcellio*), and centipede (*E. rubripes*) embryos.

this group are neuroblasts, which are formed from the neuroectoderm in a stereotypic pattern due to the action of the proneural and neurogenic genes. This process involves a segregation of neural and epidermal progenitor cell fates from a common sheet of neuroectodermal cells. After delaminating from the surface ectoderm, each neuroblast undergoes several rounds of internal, asymmetric divisions to produce a column of ganglion mother cells, each of which subsequently divides symmetrically to produce a pair of neurons (or in some cases glial progeny). Patterns of neuroblast division are highly stereotypic and result in the production of invariant populations of individually identifiable neurons. Malacostracan crustaceans show a superficially similar mode of neurogenesis, although it is not yet clear whether this represents a synapomorphic character shared by these two groups or an example of evolutionary convergence (Whitington, 1996, 2004; Dohle *et al.*, 2004).

A key issue is to what extent this hexapod/crustacean mode of neurogenesis is shared with other arthropods. It seems clear from the studies reviewed in Sections 1.20.3.1 and 1.20.4.1 that neural stem cells with the morphological features of insect and crustacean neuroblasts are not present in either onychophoran or myriapod embryos. How then are neurons generated in these groups? Is there any evidence that they possess a population of neural stem cells with the functional, if not morphological, properties of neuroblasts?

1.20.5.1.1 Evidence from onychophoran neurogenesis Few answers to these questions can be gleaned from current descriptions of neurogenesis in the Onychophora. The lack of data in this group is especially disappointing as Onychophora might be expected to reveal the basal mode of neurogenesis for the Euarthropoda. Virtually no information is available concerning patterns of cell division in the neuroectoderm of these embryos. We do not know whether cell divisions are randomly distributed across the neuroectoderm or whether they cluster at defined sites: a BrdU or antiphosphohistone study should clarify these issues. Nor is it clear whether neurogenesis involves a segregation of neural and epidermal cell fates within the neuroectoderm.

It seems unlikely that Onychophora display the phenomenon of basal nuclear migration and convergence of apical cellular processes as seen in Myriapoda: even classical histological techniques would have revealed this process, if it were present. Tiegs claimed that a ventral organ is a common feature of neural development in Onychophora, Pauropoda, and Symphyla (Tiegs, 1947). However, this claim does not stand up to close scrutiny as the onychophoran ventral organ is only present in antennal segments and is formed by ectodermal invagination rather than by basal nuclear migration, as in Myriapoda. A similar process of invagination of the neuroectoderm takes place later in certain myriapod groups, but is probably incidental to neural generation (see Section 1.20.4.1.3). One can surmise that the myriapodan mode of neurogenesis was not basal for the Euarthropoda or, alternatively, that it has been modified during onychophoran evolution.

The presence of very large numbers of small globuli cells in the onychophoran nerve cord and the apparent absence of individually identifiable neurons points to a fundamental difference between Onychophora and Hexapoda/Crustacea in mechanisms for neurogenesis. Harzsch *et al.* (2005) have pointed out a trend during arthropod evolution for variably sized groups of neurons with a particular phenotype (e.g., serotonergic) to be replaced with small and stereotypic numbers of individually

identifiable neurons. They suggest that this may be linked to differing modes of neurogenesis in different arthropod groups, with production of neural stem cells by generalized mitotic divisions in the neuroectoderm in basal groups being replaced by segregation of a stereotypic pattern of neuroblasts in more derived groups. An analysis of patterns of cell division and cell lineages within the neuroectoderm of the onychophoran embryo will help to test this hypothesis.

1.20.5.1.2 Evidence from myriapod neurogenesis
Considerably more information is available regarding mechanisms for neurogenesis in the Myriapoda than in the Onychophora. As noted in Section 1.20.4.1.2, all myriapod groups display a basal migration of nuclei in clusters of ectodermal cells, and the convergence of apical extensions from these cells to sites on the ectodermal surface (termed invagination sites by Dove and Stollewerk, 2003; Kadner and Stollewerk, 2004).

The pattern of these apical ectodermal sites is highly stereotypic and conserved between Diplopoda and Chilopoda. It is not clear whether the other two myriapod groups, Symphyla and Pauropoda, show the same pattern of sites. This is a key issue as it would help to clarify whether the presence of the same pattern of ectodermal sites in spider embryos represents a synapomorphy of the Chelicerata and Myriapoda or a convergent character. As noted above, the absence of these ectodermal sites in onychophoran embryos may speak against the idea that this is a basal character of the Euarthropoda.

The stereotypic and evolutionary conserved pattern of phalloidin/anti-HRP-positive sites on the surface of the diplopodan and chilopodan neuroectoderm suggests that this process plays an important role in the specification of neural fate. However, the nature of that role is not entirely clear. The positions of these sites correspond to the location of apical cell divisions as well as the expression of proneural and neurogenic genes. This has led Kadner and Stollewerk (2004) and Dove and Stollewerk (2003) to propose a model in which apical neuroectodermal cells at stereotypic positions become committed to a neural lineage. Repeated divisions of each of those stem cells generate a clone of daughter cells (neural precursors, in their terminology). The nuclei of these daughter cells move to a basal position in the neuroectoderm and these cells later differentiate into neurons. These apical stem cells would therefore have similar properties to insect/crustacean neuroblasts.

While this model is attractive, we currently lack some key data to assess its validity. The identity, spatial pattern, and mode of division of these apical stem cells have not been demonstrated directly. Nor has it been definitively shown that their presumed progeny, the cells with basal nuclei, are indeed immature, postmitotic neurons. Cell lineage studies in the diplopodan or chilopodan neuroectoderm, similar to those carried out in insect and crustacean embryos (Bossing *et al.*, 1996; Schmidt *et al.*, 1997; Gerberding and Scholtz, 2001), are needed to resolve these issues.

Whether or not neural stem cells are located in the apical ectodermal layer, it would seem clear from observations of mitotic figures in Symphyla and Pauropoda and of BrdU-labeled cells in Chilopoda, that more internal regions of the neuroectoderm are additional important zones of cell production. There is some evidence for the existence of neural stem cells in scolopendromorph centipedes. Neurons are found in discrete clusters which generally contain a mitotically active cell in the center or towards one side. All cells in a cluster are dye-coupled, as seen in the clone of cells arising from an individual neuroblast in the grasshopper embryo (Goodman *et al.*, 1979). Whether these mitotically active cells have stem cell properties like insect neuroblasts and whether they generate invariant sets of individually identifiable neural progeny remain to be determined in future lineage-tracing experiments. Certainly, some neurons in the embryonic (Whitington *et al.*, 1991) and adult (Harzsch, 2004; Harzsch *et al.*, 2005) centipede VNC can be identified as individuals, unlike the situation in Onychophora.

As noted in Section 1.20.4.1.2, there is an important difference between myriapods and insects in at least one aspect of neurogenesis: specification of neural fate in the myriapod neuroectoderm does not involve a segregation of neural precursor versus epidermal precursor fate because the ventral epidermis forms from cells originally located medial or lateral to the neuroectoderm.

1.20.5.2 Mode of Formation of Central Axon Pathways

Detailed descriptions at the level of individually identified neurons of the pattern of central axon growth have been carried out in a variety of insect and crustacean embryos. This knowledge provides a solid base for comparative studies in other arthropod groups. Such comparisons are particularly interesting because of the many striking similarities between insects and malacostracan crustaceans in the pattern of pioneering axon growth (Whitington *et al.*, 1993; Whitington, 1995). These similarities, together with

a similar pattern of neural expression of the genes *even-skipped* and *engrailed* (Duman-Scheel and Patel, 1999), suggest that these two groups of arthropod share a common evolutionary Bauplan for the formation of early axon pathways in the CNS. They also raise the question of the extent of conservation of this Bauplan among the Arthropoda. For example, does the insect/crustacean pattern resemble the basal pattern for the Euarthropoda?

1.20.5.2.1 Axon growth in onychophoran embryos

As noted in Section 1.20.5.1.1, the large number and small size of neurons in the onychophoran CNS, even at early stages of ganglion development, make it difficult, if not impossible, to identify cells as individuals. This precludes a comparison with the pattern of axon growth in insect and crustacean embryos at this level. Nevertheless, staining of axon tracts in the CNS of onychophoran embryos using antiacetylated tubulin or anti-HRP antibodies does point to a number of significant differences to the insect/crustacean pattern of axon growth (see Section 1.20.3.2.2). First, axons first appear in the onychophoran embryo at a stage when the ganglion anlage contains large numbers of neurons. Second, commissural axon growth in trunk segments of the onychophoran embryo is quite irregular, contrasting with the stereotypic pattern in insects and crustaceans. Third, the commissural connection in Onychophora is made up of a large number (up to nine) of separate axon fascicles that cover the entire anteroposterior extent of each trunk neuromere, whereas in insects and crustaceans a pair of commissures is found towards the central region of each neuromere. Finally, commissural axon growth in trunk segments of the onychophoran embryo is delayed with respect to longitudinal and peripheral axon growth, whereas commissural axons are among the first to form in insect and crustacean neuromeres. This difference is presumably a consequence of the early separation of hemiganglia in Onychophora.

Given the extent of these differences, further insights into the relationship between the onychophoran and insect/crustacean patterns of axon growth will probably have to await the development of molecular probes that label subsets of neurons expressing homologous genes in these different groups. Such studies will hopefully provide clues as to which aspects of early axon growth reflect the basal pattern for the Euarthropoda and which represent onychophoran specializations.

1.20.5.2.2 Axon growth in myriapod embryos

Classic histological studies show that axon growth begins in all myriapod embryos when there is a relatively large population of cells in the ganglion primordium. In this respect, the Myriapoda resemble the Onychophora and differ from the Hexapoda and Crustacea. Information about the pattern of early axon growth that can be usefully compared to the insect/crustacean pattern is only available for one group of myriapods – the Chilopoda. The best-studied representative, the scolopendromorph centipede *Ethmostigmus*, shows a number of differences to the insect/crustacean pattern.

First, commissural axon growth is delayed with respect to longitudinal and efferent axon growth. As for the Onychophora, this delay can be attributed to the wide separation of hemiganglia at early developmental stages and is therefore likely to be an apomorphic character of the scolopendromorpha: no such delay is evident in the lithobiomorph *Lithobius*, which does not possess a split germ band.

Second, the pioneering axon population in the VNC of *Ethmostigmus* is a bundle of longitudinally projecting axons – the primary tract – which originates from neuron cell bodies in the brain and descends through all segmental neuromeres to the posterior end of the embryo well before axon growth commences in trunk segments. In contrast, the first longitudinal axons to appear in embryonic insect and crustacean neuromeres arise from local, segmental neurons. The precocious development of this primary tract appears to be another specialization of the scolopendromorph centipedes as it is not seen in *Lithobius*. It is certainly not present in onychophoran embryos, since the axonal connection between the brain and the VNC in that group is only made after longitudinal connectives have formed between the trunk neuromeres (see Section 1.20.3.2.2).

Finally, the pattern of pioneering axons in the *Ethmostigmus* embryo, as determined by dye-filling of individual neurons that have begun to enlarge, shows few similarities to the conserved insect/crustacean plan (Whitington *et al.*, 1991). However, it is possible that a population of pioneering neurons with a similar pattern of axon growth to the insects and crustaceans has been overlooked in studies to date. A resolution of this issue is likely to be close at hand now that centipede homologues to *even-skipped* and *engrailed*, genes that show a conserved pattern of expression in insects and malacostracan crustaceans, have been cloned. Existing data on the pattern of expression of transcripts of these genes (Hughes and Kaufman, 2002b) should soon be supplemented by descriptions of expression of their protein products, extending resolution to the level of single neurons.

As should be clear from the above discussions, there is an urgent need for studies of the pattern of early axon growth to be extended to the other myriapod groups, employing modern techniques such as antiacetylated tubulin and anti-HRP immunohistochemistry, labeling of single neurons by dye injection and DiI, and rhodamine-phalloidin labeling of axon tracts.

1.20.5.3 Overall Conclusions

While the picture is still incomplete, it seems clear that neural development in myriapods – including both mechanisms for neurogenesis and patterns of early axon growth – differs substantially from insect and crustacean embryos. The differences in neurogenesis take on a special significance, given the recently described similarities between spider and centipede/millipede embryos in the pattern of putative neural progenitor cells.

On the other hand, there are also considerable differences between the Myriapoda and the Onychophora in both mechanisms for neurogenesis and in patterns of axon growth. One of the challenges for the future will be to determine which, if either, of these modes of development most closely resembles the basal plan for the Arthropoda.

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1.21 Segmental Organization of Cephalic Ganglia in Arthropods

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Glossary

antennal lobes Deutocerebral glomerular neuropils receiving sensory inputs from the antennas.

central complex Prominent median unpaired neuropils in the protocerebrum implicated in locomotor control.

deutocerebrum Neuromere of the (first) antennal segment in mandibulates, which comprises the antennal lobes.

engrailed Segment polarity gene involved in the formation of segmental compartments.

head Anterior-most body part, which in insects encompasses three gnathal and three to four pregnathal segments.

Hox *genes* Homeotic genes that confer segmental identities.

labrum Preoral ectodermal outgrowth of the body wall, probably of appendicular nature.

mushroom bodies Prominent paired neuropil structures in the protocerebrum, each consisting of distinct lobes (alpha-, beta-, and gamma-lobe), the peduncle, and the calyx; these structures are involved in olfactory memory and learning.

neuroblast Neural stem cell.

neuromere Segmental unit of the central nervous system.

ophistosoma Posterior part of the body in certain chelicerates (i.e., arachnids).

orthodenticle Head gap gene involved in the specification and formation of the anterior-most head segments.

prosoma Anterior part of the body in certain chelicerates (i.e., arachnids), including the head segments.

protocerebrum Anterior-most and largest part of the brain comprising several distinct areas of neuropil, among which are the paired optic lobes (visual processing centers), the median central complex, and, in most arthropods, the paired mushroom bodies.

subesophageal ganglion Fused neuromeres of the gnathal (labial, maxillary, and mandibular) segments located below the esophagus.

supraesopahgeal ganglion Fused neuromeres of the pregnathal segments located above the esophagus.

tritocerebrum Neuromere of the intercalary or second antennal segment in mandibulates, which constitutes the posterior-most and smallest part of the brain.

1.21.1 Introduction

Along the anteroposterior (AP) axis cephalic ganglia encompass the supraesophageal ganglion (i.e., the brain) and the subesophageal ganglion. Although (adult) cephalic ganglia are usually morphologically distinct units, condensation of ganglia varies considerably in different arthropods; in certain chelicerates (arachnids), brain and all ventral ganglia (including the subesophageal) are fused to form

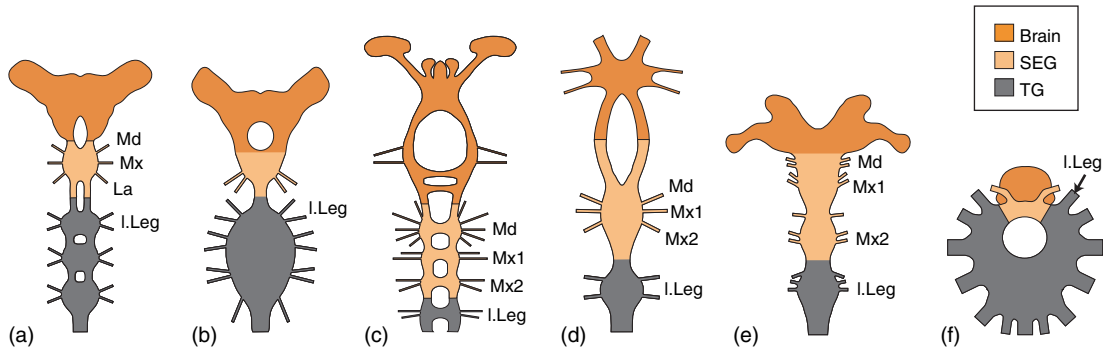

Figure 1 Schematic representations of the gross morphology of the anterior central nervous system (CNS) in different arthropods displaying various degrees of condensation in cephalic ganglia. Brain, subesophageal ganglion (SEG), and thoracic ganglion (TG) are indicated by color code. Insects: a, *Locusta* (Orthoptera); b, *Musca* (Diptera). Crustaceans: c, *Triops* (Notostraca); d, *Homarus* (Decapoda). e, *Thereupoda* (Myriapoda). f, *Limulus* (acquatic chelicerata). Drawings are not to scale. Md, Mx (1, 2), La: mandibular, maxillary (first, secondary), labial neuromere, respectively; I. Leg, neuromere innervating the first leg. Modified from Functional organization of the subesophageal ganglion in arthropods. In: Arthropod Brain; Altman, J. and Kien, J., *ed.* A. P. Gupta. Copyright © 1987, Wiley. Reprinted with permission of John Wiley & Sons, Inc.

a single nerve mass (Bullock and Horridge, 1965) (Figure 1). The ground pattern of the brain in most arthropods distinguishes three main regions, the relative size and pattern of which differ between arthropod taxa (Bullock and Horridge, 1965; Strausfeld, 1998; Mittmann and Scholtz, 2003):

1. The protocerebrum, which generally represents the anterior-most and largest part of the brain and in the adult consists of several distinct areas of neuropils. Among them are the prominent structures of the paired optic lobes (visual processing centers), the median central complex (implicated in locomotor control), and, in most arthropods (except crustaceans), the paired mushroom bodies (involved in olfactory memory and learning).
2. The deutocerebrum, the neuromere of the (first) antennal segment in mandibulates, which contains the antennal lobes, the olfactory processing centers of antennal input.
3. The tritocerebrum, the neuromere of the intercalary/second antennal segment in mandibulates, which constitutes the posterior-most and smallest part of the brain.

The subesophageal ganglion arises from the fusion of the neuromeres of the gnathal segments which innervate the mouth parts: in mandibulates, from anterior to posterior, usually the mandibular, (first) maxillary, and labial/second maxillary neuromere. However, the number of neuromeres fused into the subesophageal ganglion can vary in certain arthropods (Figure 1), especially in crustaceans (e.g., up to six in amphipods; reviewed in Bullock and Horridge, 1965). Whereas segments of the gnathal part of the head and corresponding neuromeres of the subesophageal ganglion are clearly addressable in all arthropods, the segmental pattern of the pregnathal part of the head is highly derived. The segmental organization of the arthropod head is one of the most enigmatic issues and there are numerous theories concerning it (reviewed in Rempel, 1975; Scholtz, 1998; Haas *et al.*, 2001; Urbach and Technau, 2003c). One of the most reliable criteria in defining a body segment is the presence of a corresponding neuromere (Rempel, 1975). Therefore, a key towards an understanding of the segmental organization of the arthropod head lies in the segmental composition of its (embryonic) ganglia, and in particular in that of the brain. This article provides a review of the current status of a long-lasting debate on the segmental pattern of the arthropod head and brain. Embryonic morphological data as well as recent molecular data on key developmental genes (such as *Hox* genes, head gap gene *orthodenticle*, segment polarity gene *engrailed* (*en*), and dorsoventral (DV) patterning genes) will be discussed (see A History of Ideas in Evolutionary Neuroscience, Field Homologies, Metazoan Phylogeny, Basic Nervous System Types: One or Many?, Commissural Organization and Brain Segmentation in Insects, The Evolution of Arthropod Nervous Systems: Insights from Neural Development in the Onychophora and Myriapoda, Origin and Evolution of the First Nervous System).

1.21.2 Identification of Homologous Head (and Brain) Regions in Different Arthropods by the Expression of *Hox* Genes and *orthodenticle*

Analysis of gene expression during embryonic development provides useful tools for a phylogenetic comparison and has contributed significantly to

our understanding of arthropod relationships. Data on the expression of *Hox* genes have recently accumulated, which reliably allow us to deduce homologies between head segments and brain neuromeres among different arthropod groups (insects, crustaceans, myriapods, and chelicerates).

Hox genes of the *Antennapedia* complex (Antp-C; i.e., *labial* (*lab*), *proboscipedia* (*pb*), *deformed* (*dfd*), and *sex comb reduced* (*scr*), *Antp*) define the identities of posterior head segments. The extent of the expression domains of head *Hox* genes varies considerably among different arthropod groups (reviewed in Hughes and Kaufman, 2002a). In the insect and crustacean head, *Hox* expression domains are distinct (segment-specific), usually nonoverlapping, and restricted to one or two segments (at least those of *lab*, *pb*, *dfd*, and *scr*) (Figure 2) (Abzhanov and Kaufman, 1999a, 1999b). In chelicerates, *Hox* domains encompass several segments and are broadly overlapping, probably representing an ancestral state (which is reminiscent of homologous *Hox* genes in vertebrates, although it is not yet known whether this represents conservation or convergence) (Damen *et al.*, 1998; Telford and Thomas, 1998). In myriapods (centipedes), the extent of *Hox* domains appears to reflect an intermediate state between that of insects/crustaceans and chelicerates (Hughes and Kaufman, 2002b) (Figure 2). According to these observations, it seems that evolution of head *Hox* genes involves shrinkage of their expression domains from chelicerates to the insect/crustacean clade. However, the relative positions of anterior boundaries of *Hox* gene expression appear to be evolutionarily stable (in accordance with spatial co-linearity of genes on the chromosome) and therefore are good positional markers.

Some of the results of such a comparison of *Hox* gene expression in different arthropod classes are summarized in Figure 2. Expression of head *Hox* genes in mandibulates (which include the insect/crustacean clade and myriapods) unambiguously supports homology of the three gnathal segments (mandibular, maxillary, and labial), the existence of which has never been seriously questioned. In all arthropods *lab* is the most anteriorly expressed *Hox* gene. In mandibulates, *lab* specifically indicates a head segment anterior to the mandibular one, the intercalary/second antennal segment, as well as a corresponding brain neuromere, the tritocerebrum (Diederich *et al.*, 1991; Rogers and Kaufman, 1997; Abzhanov *et al.*, 1999; Peterson *et al.*, 1999; Urbach and Technau, 2003b). Chelicerates represent the only arthropod class without a distinct head region. Their body exhibits only two major tagmata, the anterior prosoma which includes head segments and a posterior ophistosoma (Figure 2). A homology at least of anterior head segments with those in other arthropods

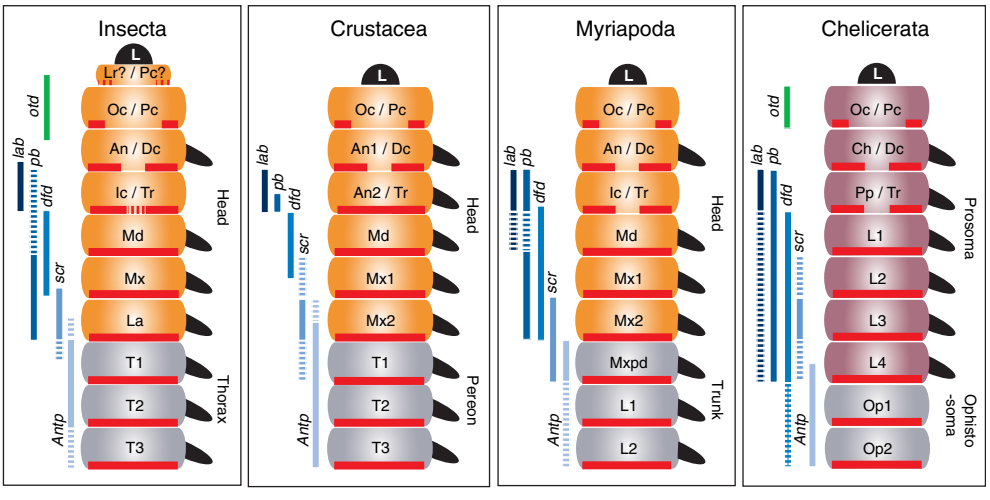

Figure 2 Alignment and proposed homologies between anterior body segments across the arthropod lineages as deduced from expression of *Hox* genes, *otd* and *en*. Schematic presentations of head segments (and corresponding parts of the brain) in mandibulates are highlighted in orange. The prosoma in chelicerates, which includes head segments, is indicated in purple. *en* expression is illustrated as red horizontal bars within the body segments. For each arthropod group, expression domains of *orthodenticle* (*otd*; green vertical bar) and *Hox* genes of the Antp complex (blue vertical bars: *labial* (*lab*), *proboscipedia* (*pb*), *deformed* (*dfd*), and *sex combs reduced* (*scr*)) are indicated on the left, and segmental appendages are indicated on the right. For all genes, regions of weak, transient, or variable expression are indicated by broken bars.

Data on *Hox* gene expression in insects are from Diederich *et al.* (1991), Rogers and Kaufman (1997), Peterson *et al.* (1999), Rogers *et al.* (2002); in crustaceans from Abzhanov and Kaufman (1999a, 1999b, 2000); in myriapods from Hughes and Kaufman (2002b); in chelicerates from Damen *et al.* (1998), Telford and Thomas (1998), Abzhanov *et al.* (1999), Damen and Tautz (1999); for a detailed review and further references on *Hox* gene expression, see Hughes and Kaufman (2002a).

appeared problematic, since it was traditionally thought that they are lacking a homologue of the (mandibulate) first antennal segment (including a deutocerebral part of the brain) (Remane *et al.*, 1975; Weygoldt, 1985). A major conclusion from *Hox* gene expression is that they reveal a conserved mode of head segmentation: expression of *lab*, supported by the anterior extension of *dfd*, suggests that: (1) the pedipalpal segment is homologous to the intercalary/second antennal segment in mandibulates; and (2) the mandibulate (first) antennal segment (and deutocerebrum) is not missing but homologous to the cheliceral segment (Damen *et al.*, 1998; Telford and Thomas, 1998) (Figure 2). Notably, the existence of a chelicerate deutocerebrum has been confirmed by morphological studies (Mittmann and Scholtz, 2003).

Since in all arthropods *Hox* genes are usually not expressed in or anterior to the antennal segment (Hughes and Kaufman, 2002a) (Figure 2), it is useful to include other marker genes in such a phylogenetic analysis, such as the conserved head gap genes. In *Drosophila*, head gap genes have been shown to be directly involved in the metamerization of the anterior head and, in addition, are assumed to confer segmental identity (Cohen and Jürgens, 1990; Schöck *et al.*, 2000). For example, at the blastodermal stage *orthodenticle* (*otd*) is expressed in the ocular and antennal segmental primordia (Cohen and Jürgens, 1990), and later in the part of the protocerebrum deriving from the ocular segment and in a small adjacent part of the deutocerebrum (Younossi-Hartenstein *et al.*, 1997; Urbach and Technau, 2003b). Two *otd* genes have been identified in the beetle *Tribolium*; the expression of *Tc otd-1* closely resembles that of *otd* in *Drosophila* (Li *et al.*, 1996). Interestingly, *otd* expression has also been found in the ocular segment of a chelicerate, the mite *Archegozetes* (Telford and Thomas, 1998). These findings provide evidence that the segmental organization of the anterior head is closely related among arthropods. Further studies will have to show how far the expression (and perhaps function) of other head gap genes, such as *empty spiracles* and *buttonhead* (Younossi-Hartenstein *et al.*, 1997), are evolutionarily conserved.

1.21.3 *engrailed* Expression in the Arthropod Head and Its Implication for the Segmental Organization of the Brain

The segment polarity gene *en* shows a highly conserved pattern of expression in the posterior compartment of each segment (Kornberg *et al.*, 1985), where it determines posterior cell fates. *en* expression has permitted fundamental insights into the segmental composition of the head in a wide variety of arthropods (for review, see Rogers and Kaufman, 1997; Scholtz, 2001; Urbach and Technau, 2003c).

1.21.3.1 Insects

Mainly based on the expression of *en*, it has been suggested that in insects the pregnathal head consists of at least three segments (intercalary, antennal, and ocular), each contributing a neuromere (tritocerebrum, deutocerebrum, and ocular neuromere, respectively) to the brain, a view that is widely accepted (reviewed in Rogers and Kaufman, 1997; Urbach and Technau, 2003c). Some of these studies not only focus on the peripheral head ectoderm, but also embrace structures of the developing embryonic brain, such as neuroblasts (Figure 3) (reviewed in Urbach and Technau, 2003c). Neuroblasts (NBs) are neural stem cells, which delaminate from a specialized ectodermal region, the neuroectoderm. Each NB produces a certain number of progeny cells, which differentiate into specific neuronal and/or glial cell types. The principal pattern of *en*-expressing ectodermal head domains and descending brain NBs appears to be strongly conserved among different insects (except *en* expression in the anterior-most part of the head; see below). The extent of the embryonic primordia of the trito- and deutocerebrum is clearly indicated by small numbers of *en*-expressing NBs deriving from ectodermal *en* stripes at the posterior border of each segment (the intercalary and antennal stripe, respectively) (Figure 3). Sizes of the tritocerebral and deutocerebral primordia are reduced compared with truncal neuromeres. In most insects the number of NBs forming the tritocerebrum is less than half of a truncal neuromere (Figure 3; total numbers of brain NBs in different insects are reviewed in Urbach and Technau, 2003c). In all insects, the ocular neuromere encompasses the largest fraction of brain NBs. Its posterior border (at least partially) is indicated by *en*-expressing NBs detaching from the *en* head spot (Figure 3). In the ocular segment the second head spot, an additional cluster of *en*-expressing neural cells, becomes visible later on. However, the clonal origin of these cells may be different among insects. Expression of *en* has been found in the (clypeo-) labral region of several insects (although absent in others). In *Drosophila*, *en* expression in the dorsal hemispheres and descending brain NBs lends support

for an additional neuromere, the labral neuromere (Schmidt-Ott and Technau, 1992; Urbach and Technau, 2003a). However, its existence and identity are controversial issues, and will be discussed later.

1.21.3.2 Other Arthropods

Unfortunately, *en* expression in the embryonic brain had not been investigated in arthropods other than insects. So far *en* data in other arthropods are limited to the peripheral head ectoderm. The spatial pattern of *en* head domains seems to be largely conserved among mandibulates (crustaceans: Patel *et al.*, 1989; Scholtz, 1995; Abzhanov and Kaufman, 2000; myriapods: Hughes and Kaufman, 2002c). It comprises three segmental gnathal *en* stripes: (1) a continuous intercalary *en* stripe (similar to basal insects); (2) a discontinuous antennal *en* stripe (separated at the midline); and (3) a smaller *en* domain in the central part of each preantennal head hemisphere. The latter presumably represents the counterpart of the *en* head spot in insects. In malacostracan crustaceans, the equivalent *en* domain (the ocular-protocerebral *en* stripe) seems to be enlarged and surrounds the preantennal head ectoderm (including the visual anlagen). Therefore, in crustaceans it is not clear whether this *en* domain indicates the ocular segment or the posterior border of the unsegmented acron (which is traditionally referred to as the eye-bearing part of the head) (Scholtz, 1998). *en* expression in chelicerates, with a mid-stage embryonic head morphology very similar to that of mandibulates, exhibits close correspondences to mandibulates: an *en* stripe exists in each gnathal and in the intercalary segment. Furthermore, the next *en* stripe demarcates the cheliceral segment (which supports homology to the (first) antennal segment), and more anteriorly, a head spot-like *en* domain has been identified, which might indicate a preantennal segment (Damen *et al.*, 1998; Abzhanov *et al.*, 1999), most likely the ocular segment. However, this *en* spot has not been detected in another chelicerate, the mite *Archegozetes*. This has been interpreted as being due to the lack of eyes, since in insects the size of the *en* head spot appears to be correlated with that of the eye (Telford and Thomas, 1998). Taken together, in line with the data from *Hox* gene and *otd* expression (Figure 2), many aspects of the *en* pattern support the existence of at least six head segments to be conserved among arthropods.

1.21.3.3 The Labrum – Insights into an Endless Dispute

Still enigmatic and the subject of ongoing debate is the status of the labrum, and the existence of or affiliation to a corresponding segment and neuromere (Rempel, 1975; Schmidt-Ott and Technau, 1992; Schmidt-Ott *et al.*, 1994; Rogers and Kaufman, 1996, 1997; Popadic *et al.*, 1998b; Scholtz, 1998, 2001; Haas *et al.*, 2001; Urbach and Technau, 2003c). It has not yet been determined whether the labrum represents a metameric appendage, serially homologous to other body appendages, or simply a nonappendicular preoral outgrowth of the body wall. The following body of evidence argues in favor of the labrum being homologous to a segmental appendage.

1. The labrum is often composed of secondarily fused, paired appendages, and labral coelomic sacks have been identified (reviewed in Haas *et al.*, 2001; Urbach and Technau, 2003c).
2. A homeotic transformation of the labrum into a gnathal limb has been reported in a coleopteran (Haas *et al.*, 2001), although this could not be induced in *Drosophila* (Rogers and Kaufman, 1996).
3. Expression of *distal-less* (*dll*) (Panganiban *et al.*, 1995; Popadic *et al.*, 1998b; Thomas and Telford, 1999; Schoppmeier and Damen, 2001) and *dachshund (dac)* (Prpic *et al.*, 2001), usually found in segmental appendages, has been reported in the labrum of several arthropod groups and might support homology (Scholtz, 1998; Thomas and Telford, 1999; Prpic *et al.*, 2001); however, *dll* is likewise expressed in nonappendicular structures (Panganiban *et al.*, 1997; Scholtz, 2001).
4. Support for the appendicular nature of the labrum might come from motoneuronal innervation and pattern of glial and sensory cells (Boyan *et al.*, 2002).

Assuming that the labrum represents a true appendage, a central issue is the question: to which segment does it belong? (For a review of theories on head segmentation and the labral discussion in the older literature, see Rempel, 1975.) One current model of head segmentation (the L-/bent-Y model) suggests that the (insect) labrum represents the appendage of a labro-intercalary segment (Figure 4a) (for details, see Haas *et al.*, 2001). Considering that each segment has only one pair of limblike appendages (Manton, 1977), this view appears to be contradicted by the fact that during embryonic development several insect species

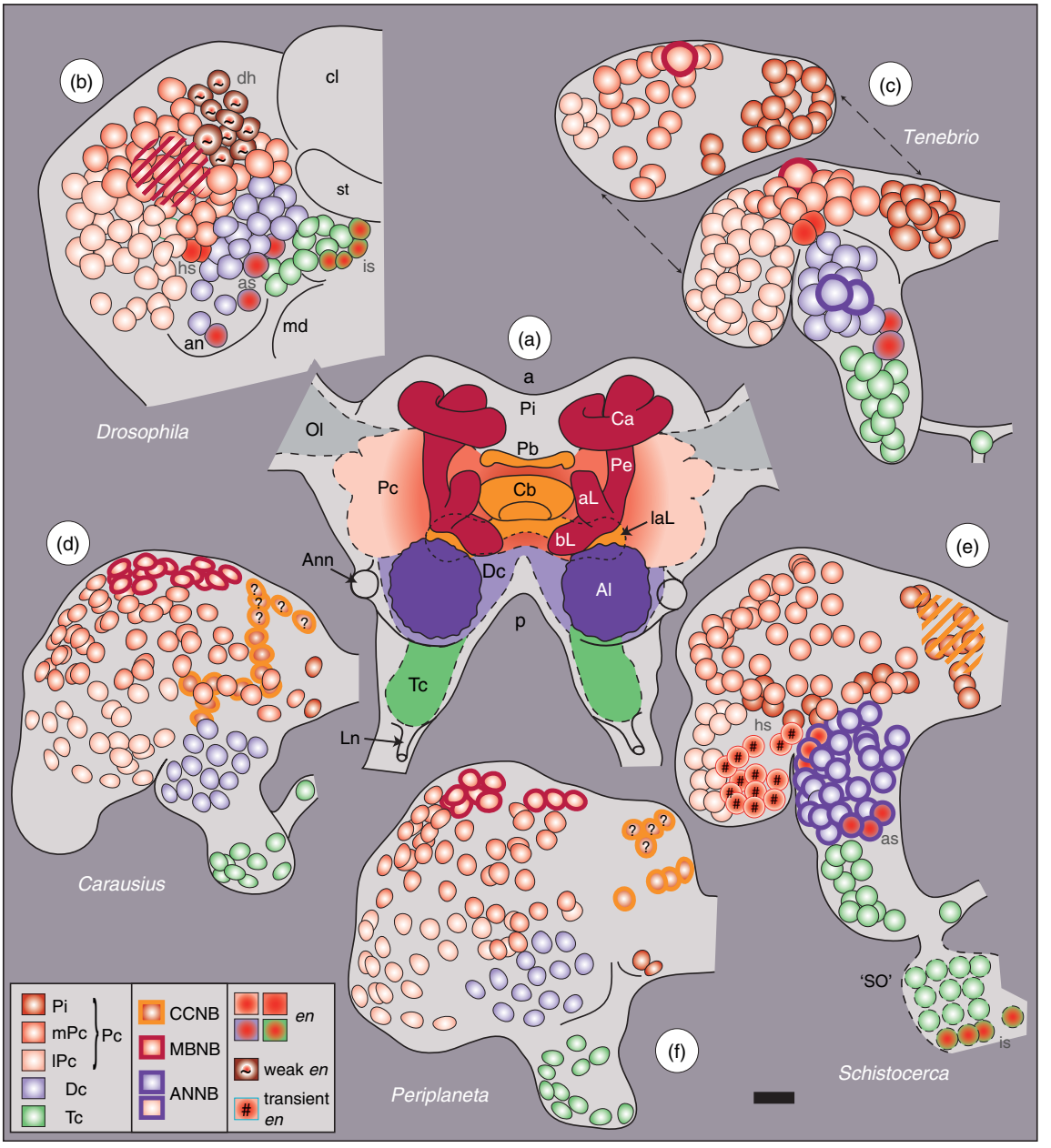

Figure 3 Comparison between different insect species of the spatial arrangement and *engrailed* expression in embryonic brain NBs, and their assignment to specific brain neuropil structures. a, The scheme depicts major neuropil structures of the protocerebrum (mushroom bodies, central complex) and deutocerebrum (antennal lobe, Al) in an adult insect brain. The mushroom body neuropils are built by the axons of thousands of Kenyon cell fibers, which project through the peduncle (Pe) into distinct lobes (aL, alpha-lobe; bL, beta/gamma-lobe). The calyces (Ca) of the mushroom bodies are mainly formed by dendrites of the Kenyon cells. The unpaired median neuropil structures include the central complex (Cc) and the protocerebral bridge (Pb), which are mainly formed by neurons from the pars intercerebralis (Pi). The central complex shows massive connections to the lateral accessory lobes (laL). The optic lobes (Ol) and nerves innervating the ocelli (which occur in some insects) are not shown. Anterior (a) is top and posterior (p) is bottom.

b–f, Semischematic presentations of the NB pattern disclosing the left half of an embryonic brain primordium in a ventral view. The color code indicates the distribution of NBs in the trito- (Tc), deuto- (Dc), and protocerebrum (Pc); the protocerebral NBs are further subdivided into a lateral (lateral protocerebrum, lPc), central (median protocerebrum, mPc), and mediodorsal population (pars intercerebralis, Pi). In embryonic brains of species in (b), (c), and (e) NBs expressing *engrailed* (*en*) are highlighted (see color code, which also distinguishes between strong, weak, and transient *en* expression). NBs that have been assigned to the mushroom bodies (MBNB; encircled in dark red), the central complex (CCNB; encircled in orange), and the antennal lobe (ANNB; encircled in blue) have been indicated. Hatching marks populations of NBs, which include putative progenitors of the respective neuropils (purple hatching indicates putative MBNBs, orange hatching, CCNBs). b, *Drosophila* (Diptera): entire population of brain NBs at about 35% embryogenesis (corresponding to late stage 11; data according to Urbach and Technau, 2003a). The four progenitors of the mushroom bodies in each hemisphere (Noveen *et al.*, 2000) are part of the protocerebral NB (mPC) population which is covered by dark red hatching (unpublished data).

(*Continued*)

Figure 3 (Continued) c, *Tenebrio* (Coleoptera): entire population of brain NBs at 40% embryogenesis. For clarity, the ventral and dorsal half of the brain primordium is shown separately. Data according to Urbach *et al.* (2003). In each hemisphere two NBs of the median protocerebrum form the larval mushroom bodies (encircled in dark red), and two deutocerebral NBs contribute to the larval antennal lobe (encircled in blue). It is not yet known if other deutocerebral NBs also contribute to the antennal lobe.

d, *Carausius* (Orthoptera) (stage D2, adapted from Malzacher, 1968); NB map includes almost all brain NBs; however, progenitors of the mushroom bodies might also form later. According to Malzacher (1968), the mushroom bodies descend from a population of about 15 NBs (group Od; encircled in dark red); NB group 1Ac is suggested to contribute to the central complex (encircled in orange); NBs of group 1Aa (indicated with ?) might additionally participate in the formation of the central complex.

e, *Schistocerca* (Orthoptera); entire population of brain NBs at about 45% embryogenesis. Each NB is indicated by a sphere of equal size with its center corresponding to the position of the NB *in situ*. Data on the NB pattern are according to Zacharias *et al.* (1993).

en expression data are according to Zacharias *et al.* (1993) and Boyan and Williams (2000). A large population of protocerebral NBs expresses *en* (albeit most of them only transiently), which all derive from a *en* hs-like domain. Three *en*-expressing NBs separate from the lPc to join the Dc. Note that the tritocerebral NBs are split in an anterior and posterior group. According to Doe and Goodman (1985), the NBs of the posterior group were first assigned to a fourth gnathal neuromere, the 'SO' neuromere, but have been reassigned to the TC (Boyan and Williams, 2000). Orange hatching indicates population of pars intercerebralis NBs; of these, a subset of four NBs contributes to the central complex (Boyan and Williams, 1997). Apparently all deutocerebral and three (*en*-expressing) protocerebral NBs form the antennal lobe (encircled in blue; Boyan and Williams, 2000).

f, *Periplaneta* (Orthoptera) (stage E2; adapted from Malzacher, 1968). The map encompasses most brain NBs, although at later stages there might be some more (e.g., NBs of the mushroom bodies might form during the second half of embryogenesis).

According to Malzacher (1968), the mushroom bodies derive from NBs including group Od (encircled in dark red); the central complex is argued to derive from group 1Ac (encircled in orange) and perhaps from group 1Aa (indicated by ?). an, cl, md, antennal, clypeolabral, and mandibular appendage, respectively; Ann, antennal nerve; as, antennal *en* stripe; dh, *en* expression in the dorsal hemispheres; hs, *en* head spot; is, intercalary *en* stripe; Ln, labral nerve. Scale bar: b, 18 μm; c, 25 μm; d–f, about 50 μm. Modified from Urbach, R. and Technau, G. M. 2003c. Early steps in building the insect brain: neuroblast formation and segmental patterning in the developing brain of different insect species. *Arthropod Struct. Dev.* 32, 103–123.

transiently exhibit rudiments of intercalary appendages (homologous to the crustacean second antennas) (Malzacher, 1968; Tamarelle, 1984; Fleig and Sander, 1986). However, since in *Tribolium* the labrum has been considered to be composed only of the proximal portions of an appendage (Haas *et al.*, 2001), it has been suggested that the transitory intercalary appendage (due to lack of *dll* expression) represents the distal part of an appendage, which together with the labral part originally formed one complete appendage of a labro-intercalary segment (Hass *et al.*, 2001). Conflicting with this model is that, based on *dac* expression, the labrum represents the distal rather than the proximal part of an appendage (Prpic *et al.*, 2001). Expression of *lab* in the labrum as well as in the second antennal/intercalary segment of a crustacean (Abzhanov and Kaufman, 1999a) and a myriapod (Hughes and Kaufman, 2002b) might support an origin of the labrum from the second antennal segment. However, the crustacean second antenna appears to represent a complete appendage. Moreover, *lab* has not been found in the labrum of the more basal chelicerates, but instead in the pedipalps and all four pairs of walking legs (Damen *et al.*, 1998). In *Drosophila*, another model of head segmentation has been favored (the S-model; Figures 4a and 4b). The expression of segment polarity genes indicates the existence of remnants of four pregnathal segments arranged in an S-shaped loop, the intercalary, antennal, ocular, and labral segment, with the latter at the anterior tip of the head. The labrum is part of the labral segment, and the ocular segment (see also Rogers and Kaufman, 1996; Sharov, 1966) is located between the antennal and labral segments (Schmidt-Ott and Technau, 1992; Urbach and Technau, 2003a, 2003b). Additional evidence provides the deletion pattern of sensory organs in head gap mutants (Schmidt-Ott *et al.*, 1994). Furthermore, this analysis suggests that the optic lobes are of segmental origin, deriving from the ocular segment (Schmidt-Ott *et al.*, 1995). In this model, the ocular segment (including the anlagen of the visual system) covers that part of the head which in the linear model of head segments (Rempel, 1975) represents the unsegmented acron (traditionally referred to as the eye-bearing part of the arthropod head) (Figure 4a). As a consequence, an ocular segment excludes the existence of a nonsegmental acron.

1.21.4 A Neuromeric Model of the Brain in *Drosophila*

As described above, *en* domains in the pregnathal head, in contrast to the trunk, are discontinuous, and thus leave substantial parts of the segmental boundaries in the head and brain unlabeled (Figure 2). A reconstruction of these boundaries in the procephalic ectoderm and in the array of brain NBs deriving from this region was achieved by

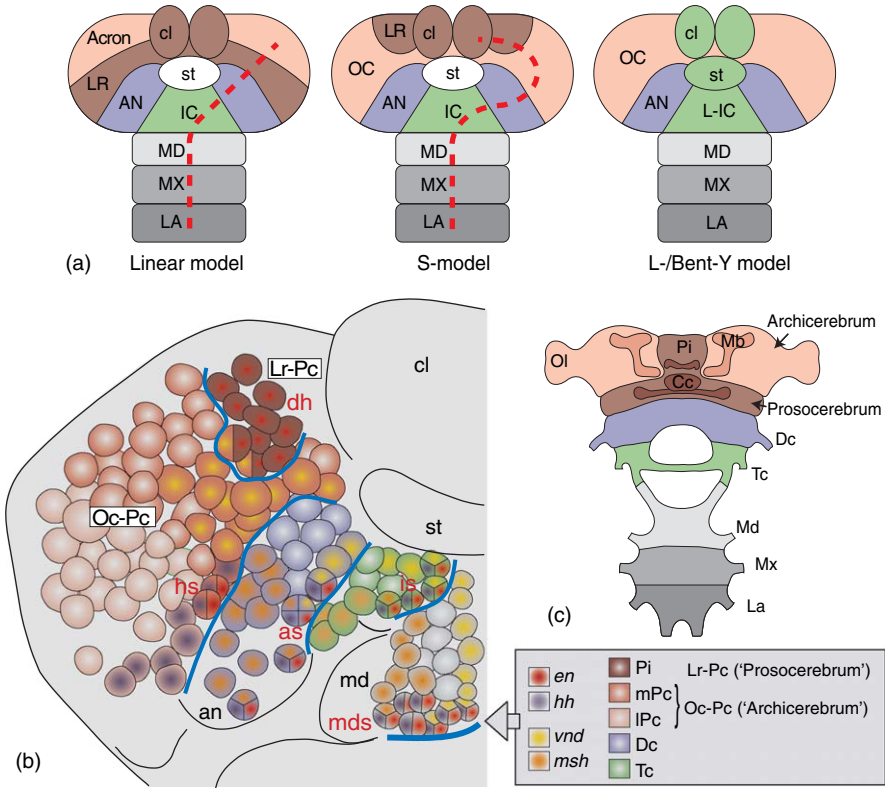

Figure 4 a, Current models on head segmentation in insects. Schemes disclose in a ventral view the different numbers, orientations, and identities of segments in the embryonic insect head.

In the linear model (after Rempel, 1975), six head segments and an acron are arranged rather straight along the anteroposterior axis (as indicated by the red broken line); the labral segment (LR) represents the preantennal segment, and the acron the anterior tip of the head.

In the S-model (after Schmidt-Ott and Technau, 1992), the arrangement of seven head segments follows an S-like loop (as indicated by the red broken line); the ocular segment (OC) represents the preantennal segment, and the labral segment is at the anterior tip of the head. Here, the ocular segment replaces the acron. Compare with the segmental model of the embryonic brain proposed in (b).

The L-/bent-Y model (after Haas *et al.*, 2001) proposes six head segments and reinterprets the ventral territory spanning the intercalary segment (IC), the stomodaeum (st), and the (clypeo-) labral appendages (cl) as the labro-intercalary segment (L-IC). AN, antennal; IC, intercalary; L-IC, labro-intercalary; LA, labial; LR, labral; OC, ocular; MD, mandibular; MX, maxillary segment.

b, Segmental model of the early embryonic brain in *Drososphila*. Semischematic presentation of the brain NB map at about 35% embryogenesis (late stage 11, orientation as in Figure 3). Based on the expression of segment polarity genes (*en*, *hedgehog* (*hh*)) and DV patterning genes (*muscle segment homeobox* (*msh*), *ventral nervous system defective* (*vnd*); as indicated by the color code), the embryonic brain is proposed to consist of four neuromeres (data for pregnathal NBs according to Urbach and Technau, 2003a). Blue lines indicate the borders between the respective neuromeres. As indicated in the color code, from posterior to anterior the brain anlagen encompass the tritocerebrum (Tc), the deutocerebrum (Dc), the ocular (Oc-Pc) and labral (Lr-Pc) part of the protocerebrum. The ocular part comprises the largest fraction of brain NBs (including NBs of the median (mPc) and lateral protocerebrum (lPc), which may correspond to the archicerebrum; the much smaller labral part of the protocerebrum (consisting of NBs of the pars intercerebralis (Pi)) may correspond to the prosocerebrum.

as, is, mds, antennal, intercalary and mandibular *en* stripe, respectively; dh, *en* expression in the dorsal hemispheres; hs, *en* head spot; an, cl, md, antennal, clypeolabral, and mandibular appendage, respectively; st, stomodaeum. a, Modified from Haas, M. S., Brown, S. J., and Beeman, R. W. 2001. Pondering the procephalon: The segmental origin of the labrum. *Dev. Genes Evol.* 211, 89–95.

c, Model of the segmental composition of the adult insect brain and subesophageal ganglion after Rempel. According to this model, the archicerebrum encompasses the optic lobes (Ol) and mushroom bodies (Mb), and the prosocerebum (in Rempel's view, the preantennal neuromer) encompasses the neurosecretory cells of the pars intercerebralis (Pi), and the components of the central complex (Cc). Dc, Tc; deuto-, tritocerebrum; Md, Mx, La, mandibular, maxillary, and labial neuromere, respectively. Modified from Rempel, J. G. 1975. The evolution of the insect head: An endless dispute. *Quaest. Entomol.* 11, 7–25.

analysis of the expression of a large collection of molecular markers, including segment polarity genes and DV patterning genes, which are differentially expressed along the DV as well as the AP axis (Urbach and Technau, 2003a, 2003b). These data support the view that the pregnathal *Drosophila* head is composed of four segments, each contributing a neuromere to the brain. The expression

patterns define the boundaries between trito-, deuto-, and protocerebrum, and provide indications that the protocerebrum is composed of two neuromeres belonging to the ocular and labral segment (Schmidt-Ott and Technau, 1992; Urbach and Technau, 2003a) (Figure 4b). The ocular neuromere comprises more than 50% of all brain NBs. By contrast, the putative labral neuromere consists of a small fraction of about 10 *en*-expressing NBs, thus, being confined to its posterior segmental compartment. These NBs derive from the anterior-most part of the procephalic neuroectoderm (*en*-expressing domain of the dorsal hemispheres) which has been suggested to coincide with the posterior boundary of the labral segment (Schmidt-Ott and Technau, 1992; Urbach and Technau, 2003a). The segmental character of both protocerebral neuromeres seems to be less conserved compared with the trito- and deutocerebrum. This is reflected by the orthogonal expression of segment polarity and DV patterning genes, which is principally conserved in the posterior segments of the pregnathal head and brain, but becomes obscure towards anterior sites. It is further reflected by the identification of NBs in the trito- and deutocerebrum which have serially homologous counterparts in the neuromeres of the ventral nerve cord (reviewed in Urbach and Technau, 2004).

1.21.5 Clues to the Segmental Origin of Some Major Neuropil Centers

1.21.5.1 Mushroom Bodies

Mushroom bodies have been described in all arthropods, except crustaceans (reviewed in Strausfeld *et al.*, 1998). They represent a paired protocerebral brain structure involved in higher brain function, like olfactory learning and memory (reviewed in Heisenberg, 1998; Menzel, 2001). In insects, they consist of a large number of neurons (Kenyon cells), whose fibers form typical substructures, the calyx, peduncle, and lobes (Figure 3a). Mushroom bodies derive from specific NBs (MBNBs) which are already formed in the embryo (Figure 3). The number, arrangement, and proliferation behavior of embryonic (and postembryonic) MBNBs, as well as the final size and complexity of the adult mushroom bodies, show enormous variabilities among insects (reviewed in Strausfeld, 1998; Farris and Sinakevitch, 2003; Urbach and Technau, 2003c). MBNBs reside in the posterior dorsal brain cortex either in small numbers (e.g., two in coleopterans; Figure 3c), or in larger groups (up to 100, e.g., in certain Blattoidea). In *Drosophila*, molecular markers specifically expressed in MBNBs (Noveen *et al.*, 2000) indicate that these NBs are part of the ocular neuromere (Urbach and Technau, 2003c) (Figure 3b).

A traditional view is that the protocerebrum in arthropods consists of the archicerebrum and prosocerebrum (Remane *et al.*, 1975). The archicerebrum is argued to be that part of the brain that derives from the acron, comprising the optic lobes and mushroom bodies (Figure 4c) (reviewed in Rempel, 1975). Since the acron has been regarded as equivalent to the ocular segment (as discussed above), the ocular neuromere would be equivalent to the archicerebrum (Schmidt-Ott and Technau, 1992; Urbach and Technau, 2003a). This would be in agreement with the view that the mushroom bodies are part of the ocular neuromere (or archicerebrum).

1.21.5.2 Central Complex

Another conserved protocerebral neuropil center associated with higher brain function in arthropods is the central complex (Figure 3a) (Utting *et al.*, 2000; Loesel *et al.* 2002; reviewed in Strauss, 2002). In most insects this prominent unpaired structure develops its typical shape during postembryonic stages. Information about the embryonic origin of the central complex is still lacking, except in orthopteran insects, in which the central complex is formed by about 13–20 NBs on either side in the pars intercerebralis (Figures 3d–3f) (Malzacher, 1968; Boyan and Williams, 1997). In *Drosophila*, likely counterparts might be the *en*-expressing labral NBs deriving from the dorsal hemispheres (Figures 3a and 4a). Similar to the situation in orthopterans, their progeny is later found in the larval pars intercerebralis and might participate in the formation of the central complex (Urbach and Technau, 2003a, 2003c). Under the assumption that a labral neuromere remnant is existent, this would be in harmony with the traditional view that the prosocerebrum, corresponding to the labral part of the protocerebrum, includes the central complex and cells of the pars intercerebralis (Figure 4b) (reviewed in Rempel, 1975).

1.21.5.3 Antennal Lobes

The antennal lobes in insects are important centers in the olfactory pathway (reviewed in Stocker, 2001; Jefferis *et al.*, 2002). Similar olfactory deutocerebral centers have been reported in crustaceans (reviewed in Strausfeld and Hildebrand, 1999). Specialized deutocerebral precursor cells of enlarged size and/or specific proliferation behavior have been identified in insects (Figure 3) and crustaceans, the progeny of which contribute to the developing antennal/olfactory lobes (reviewed in Beltz and

Sandeman, 2003; Urbach and Technau, 2003c). These olfactory centers have been traditionally attributed to the deutocerebrum, although not much is known about their embryonic segmental origin. In insects, antennal lobes derive from embryonic deutocerebral NBs (Figure 3) (Nordlander and Edwards, 1970; Truman and Bate, 1988; Stocker *et al.*, 1995; reviewed in Urbach and Technau, 2003c). However, at least in orthopteran insects there are indications that the antennal lobes may not be pure deutocerebral derivatives but also include components descending from the protocerebrum (Boyan and Williams, 2000) (Figure 3e).

1.21.6 Conclusions

Comparison of the expression of key developmental control genes provides fundamental insight into the development and phylogeny of the arthropod head and its ganglia. *en*, *otd*, and *Hox* gene expression data suggest that the sister groups of insects and crustaceans share the same mode of head segmentation with the more basic myriapods and chelicerates, and might further emphasize the monophyletic origin of arthropods (Damen *et al.*, 1998; Popadic *et al.*, 1998a; Abzhanov and Kaufman, 1999; Hughes and Kaufman, 2002a, 2002b). In all arthropods the head appears to consist of at least six segments. Nevertheless, the metameric organization of the anterior head, including the ocular/acronal and labral region (as well as of the descending protocerebrum), is not yet clear and remains open to ongoing discussions. A subdivision of the brain into three main regions, the trito-, deuto-, and protocerebrum, appears to be conserved among insects and crustaceans, but this is less clear in other arthropod taxa (Bullock and Horridge, 1965; Damen *et al.*, 1998; Telford and Thomas, 1998; Eriksson and Budd, 2000; Mittmann and Scholtz, 2003). Moreover, it is uncertain how far particular brain centers in insects have homologous counterparts in other arthropods: homology of the central body in mandibulates and chelicerates has been suggested. Mushroom bodies are missing in crustaceans, but may be homologous to structures found in chelicerates, and homology of the antennal lobes in different arthropods is unresolved (e.g., Breidbach and Wegerhoff, 1993; Strausfeld *et al.* 1993, 1998; Strausfeld, 1998; Strausfeld and Hildebrand, 1999; Utting *et al.*, 2000; Harzsch and Glötzner, 2002; Loesel *et al.*, 2002). In the brain of onychophorans (which are proposed to be very basal to arthropods), several neuropils with similarities to brain structures in euarthropods have been described (Loesel, 2004), but it is not yet known if they represent homologous structures. A study on the cephalic nerves in onychophorans points out that the relationship of head segments in onychophorans and other arthropods is far from clear (Eriksson and Budd, 2000). The key towards solving these and other questions related to brain evolution in arthropods lies in the comparative analysis of embryonic brain development, including a growing array of (evolutionary conserved) developmental control genes, which will be a challenging task for further research.

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1.22 Commissural Organization and Brain Segmentation in Insects

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Glossary

Abd-B, *Abd-B*	Abdominal B gene, protein.
Antp, *Antp*	Antennapedia gene, protein.
cell adhesion glycoprotein	A member of the immunoglobulin superfamily of cell adhesion molecules which promote adhesivity between cells. Such molecules have an extracellular (N-terminal) gobular domain held in place by disulfide bonds and a C-terminal domain anchored in the cell membrane via either a transmembrane amino acid sequence in the protein itself or a lipid. Such molecules promote cell–cell adhesion by binding to one another (homophilic binding) without requiring calcium.
CNS	Central nervous system.
Com, *Com*	Commissureless gene, protein.
conserved	A structure, molecule, or amino acid sequence which is maintained in a recognizable form between different species throughout evolution.
DC	Deutocerebrum.
Dfd, *Dfd*	Deformed gene, protein.
DUM	Dorsal unpaired median
ems, *EMS*	Empty spiracles gene, protein.
expression	Gene activity resulting in the production of its protein (and detectable for example via an antibody against the protein).
gmc	Glial cells missing gene.
homeobox gene	A gene which contains a homeobox, a 180-basepair region, which codes for the homeodomain of about 60 amino acids that is able to bind to DNA and therefore can act as a transcription factor.
homo	Homothorax gene.
HRP	Horseradish peroxidase.
inductive factor	A molecule which is produced by one group of cells and acts as a signal in that it influences the development of a second, neighboring, group of cells.
jing	*jing* gene.
lab, *Labial*	Labial gene, protein.
MaC	Mandibular commissure.
mutational analyses	Deduction of gene function in development by studying possible phenotypes caused by the mutation of a gene. Mutation of a gene can be the result of either its absence (deletion) or the inactivation/malfunction of its encoded protein (mutation).
Mx	Maxillary neuromere.
neuromere	The nervous system component of an embryonic segment.
null mutant background	The genotype in which both alleles of a gene are absent or inactivated.
otd, *Otd*	Orthodenticle gene, protein.
PC	Protocerebrum.
PCC	Protocerebral commissure.
pcXX, XXI	Posterior commissures XX, XXI of the brain.
pioneer (neuron)	A neuron and its axonal projection which together are responsible for guiding subsequent neuronal processes to their targets in the developing nervous system.
repo	Reversed polarity gene.
stomodeum	The mouthlike modification of the anterior end of the gut (in embryos) formed by an invagination of the ectoderm.
TC	Tritocerebrum.
TCC	Tritocerebral commissure.
temporal dynamic	Time-dependent morphological changes in the nervous system.
transcription factor	A protein that is able to bind to DNA and therefore can either activate or suppress the function of a gene.

Ubx, *Ubx*	Ultrabithorax gene, protein.
VG 1,3	Ventral giant cells 1, 3 of the deutocerebrum.
VNC	Ventral nerve cord.

1.22.1 Commissural Organization and the Segmental Body Plan

The evolution of a bilateral body form and bilaterally coordinated behavior required the development of a central nervous system (CNS) in which information could be exchanged across the midline. The most obvious neural adaptation in this regard is the presence of serially iterative transverse commissures linking the left and right hemispheres of all bilateral nervous systems (Bullock and Horridge, 1965). Many cellular and molecular studies have focused on the proper establishment of such commissures during development (Kaprielian *et al.*, 2001), often in conjunction with the expression patterns of genes which identify specific brain regions or segment borders (Hirth and Reichert, 1999). One such marker is the segment polarity gene *Engrailed* which encodes a transcription factor and is expressed in cells located within the posterior compartment of a given body segment (Nüsslein-Volhard and Wieschaus, 1980; Hidalgo, 1996). Its role in positional specification is remarkably conserved in both invertebrates and vertebrates (Patel *et al.*, 1989a, 1989b; Lans *et al.*, 1993; Eizema *et al.*, 1994; Fleig, 1994; Scholtz, 1995; Wray *et al.*, 1995; Itasaki and Nakamura, 1996; Rétaux and Harris, 1996; Rogers and Kaufman, 1996; Marie and Bacon, 2000; see Segmental Organization of Cephalic Ganglia in Arthropods, Cognition in Invertebrates, Aggression in Invertebrates: The Emergence and Nature of Agonistic Behavioral Patterns).

Recent molecular data, including *Engrailed* expression in grasshopper (Figure 1a) and *Drosophila* (Figure 1b), suggest that the insect brain comprises three neuromeres: (1) the proto cerebrum (PC), corresponding to the ocular and preantennal segments; (2) the deuto cerebrum (DC), corresponding to the antennal segment; and (3) the tritocerebrum (TC), corresponding to the intercalary segment (Diederich *et al.*, 1991; Rogers and Kaufman, 1996; Hartmann and Reichert, 1998; Hirth *et al.*, 1998; Peterson *et al.*, 1998; Haas *et al.*, 2001a, 2001b; Boyan and Williams, 2002). In the early embryonic ventral nerve cord (VNC) of insects, *Engrailed* expression has the appearance of a regular series of stripes which extend into the posterior epithelium of the associated appendage (Figure 1a; Patel *et al.*, 1989a). In the brain, this regular pattern is broken by the presence of the foregut (or stomodeum), which bisects the head anteroposteriorly and bilaterally (Figure 1a). Traditionally, the stomodeum has been held to divide the brain between the deutocerebral and tritocerebral neuromeres (Snodgrass, 1935; Bullock and Horridge, 1965). PC and DC have been considered the preoral neuromeres, while the TC has been considered postoral in its anatomical organization. New molecular data discussed below suggest that this interpretation requires revision. The most obvious evidence that the foregut affects the organization of head neuromeres is seen in the pattern of *Engrailed* expression, which in the brain is highly irregular compared to that in the VNC in that it comprises a mix of partial stripes and spots bearing a pronounced temporal dynamic (Patel *et al.*, 1989a; Cohen and Jürgens, 1991; Fleig, 1994; Boyan *et al.*, 1995; Rogers and Kaufman, 1996; Marie and Bacon, 2000; Boyan and Williams, 2002).

The foregut appears to have a profound influence not only on the gross morphology of the brain, but also as an inductive factor on the organization of axons within it (Page, 2002). Whereas in the VNC each ganglion possesses a transverse anterior (a-type) and a posterior (b-type) commissure (Figure 1c; Goodman and Bastiani, 1984; Thomas *et al.*, 1984; Whitington, 1995), in the head, the early embryonic mandibular, tritocerebral, and protocerebral neuromeres contain only a single commissure. More extreme still, the embryonic DC appears not to possess a commissure at all (Figures 1d and 1e). These differences in axonal organization may relate to where, topologically, the stomodeum bisects the brain. Molecular data provide an insight into this problem, and into the identity (a-type or b-type) of the various cephalic commissures.

The Fasciclin (Fas) I antigen belongs to the family of cell adhesion glycoproteins and is primarily expressed by pioneer axons early in the embryonic development of the insect nervous system (Snow *et al.*, 1987, 1988). Immunocytochemical data show that the expression of Fasciclin I in commissural fibers is segmentally iterative along the VNC (Figure 1f; Bastiani *et al.*, 1987). Careful inspection reveals that this expression appears to be consistently stronger in the a-type commissure than in the b-type commissure of each neuromere (Figure 1f). More interestingly, this strong Fasciclin I expression within the a-type commissure appears to be consistently localized to a single fascicle at this embryonic stage (Figure 1g). With regard to the tritocerebral commissure (TCC), only a single clearly defined Fasciclin I immunoreactive fascicle is observed (Figure 1h). Preorally, and at the equivalent stage of embryogenesis, double immunolabeling with anti-horseradish peroxidase

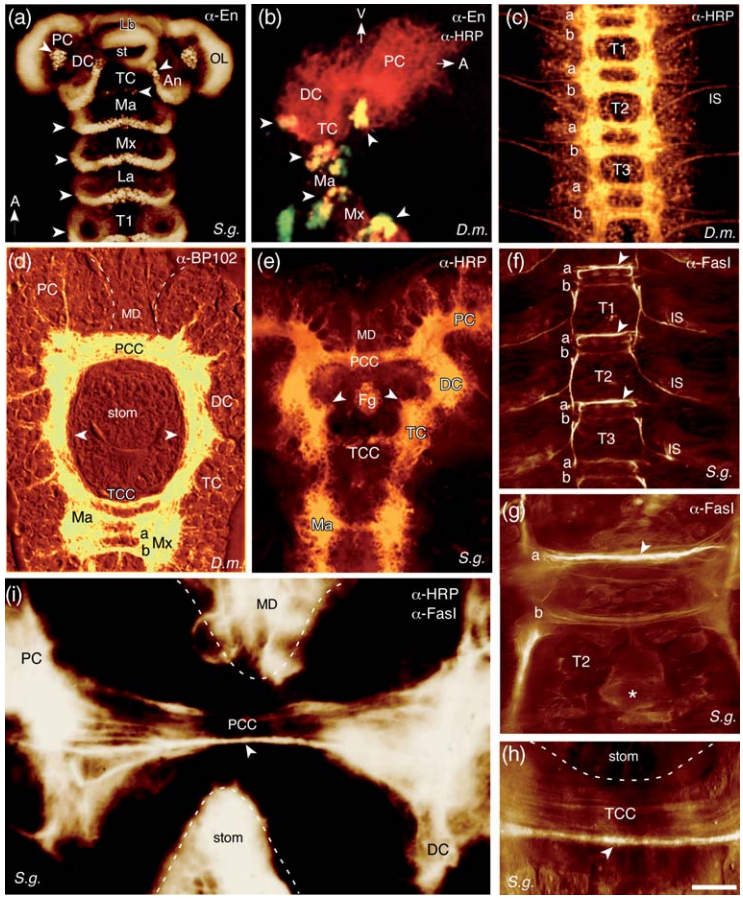

Figure 1 Segmental organization of commissures in the embryonic insect nervous system. a, Immunocytochemical labeling with the anti-En antibody reveals pattern of *Engrailed* expression in the head of the grasshopper *Schistocerca gregaria* (*S. g.*) at 28% of embryogenesis. Wholemount embryo, ventral view. *Engrailed* expression (white arrowheads) is present in stripes which extend into the posterior epithelium of the appendage associated with each segment. The brain comprises three neuromeres: (1) in the PC, *Engrailed* expression in neuroblasts is associated with the optic lobe (OL); (2) in the deutocerebrum (DC), *Engrailed* expression extends into the posterior antennal (An) epithelium; and (3) in the tritocerebrum (TC), *Engrailed* expression is associated with the labrum (Lb). Anterior (A) is to the top in all figures except (b). b, Laser confocal image of the anterior CNS of a wild-type stage 12 *Drosophila melanogaster* (*D. m.*) embryo viewed laterally following double labeling with anti-*Engrailed* antibody (yellow/green) and neuron-specific anti-HRP antibody (red/orange). En-expressing cells appear as clusters (white arrowheads) in lateral view. Ventral (v) is to the top. c, Laser confocal image following neuron-specific anti-HRP immunostaining reveals the structure of the ventral nerve cord in wild-type stage-15 *Drosophila* embryo. Ventral view. Ladder-like axonal tracts with anterior (a-type) and posterior (b-type) commissures and longitudinal connectives, along with the serially iterated intersegmental nerves (IS) are seen. Prothorax = T1; mesothorax = T2; metathorax = T3. d, Section through a stage-16 brain of *Drosophila melanogaster* (*D. m.*) embryo stained with the axon-specific Mab BP 102 reveals the orthogonal scaffold of fibers around the stomodeum (stom). Anteriorly (preorally) lies the protocerebral commissure (PCC); posteriorly (postorally), lies the tritocerebral commissure (TCC) and three commissures (S1, mandibular; S2a, anterior maxillary; S2b, posterior maxillary) of the subesophageal ganglion. White arrowheads indicate the location where a deutocerebral commissure would be expected. e, Confocal image of the brain of the grasshopper at 40% of embryogenesis following neuron-specific anti-HRP immunocytochemistry. Note the prominent PCC preorally and the TCC postorally. White arrowheads indicate the location where a deutocerebral commissure would be expected. f, Segmental organization of axon pathways in thoracic neuromeres of the ventral nerve cord of the grasshopper viewed ventrally and in wholemount following anti-Fasciclin I immunocytochemistry at 40% of embryogenesis. Each neuromere possesses an anterior (a-type) and posterior (b-type) commissure, longitudinal tracts, and an intersegmental nerve (IS) to the periphery. Note that the antibody staining appears consistently stronger in the anterior commissure (white arrowheads). g, Higher-power micrograph of the mesothoracic neuromere from another preparation to that in (c) reveals that Fasciclin I expression in the anterior commissure (a) is restricted to a single fascicle (white arrowhead). The immunoreaction in the b-type commissure is much weaker. The lineage (white asterisk) of the dorsal unpaired median (DUM) neuroblast of T2 is visible posteriorly. h, Photomicrograph of the tritocerebral commissure in the embryonic grasshopper (40% stage) following anti-Fasciclin I immunocytochemistry. A single strongly immunoreactive fascicle (white arrowhead) is visible within the commissure. i, Photomicrograph of the brain of the grasshopper at 40% of embryogenesis as seen in wholemount and viewed ventrally following double immunolabeling with neuron-specific anti-HRP and anti-FasI antibodies. Note the single prominent Fasciclin I-positive fascicle (white arrowhead) within the PCC. MD, median domain; Fg, frontal ganglion. Scale bar (in h): 400 μm (a); 10 μm (b, c); 20 μm (d); 200 μm (e, f); 50 μm (g, h); 100 μm (i). b, Modified from Hirth, F., Therianos, S., Loop, T., Gehring, W. J., Reichert, H., and Furukubo-Tokunaga, K. 1995. Developmental defects in brain segmentation caused by mutations of the homeobox genes *orthodenticle* and *empty spiracles* in *Drosophila*. *Neuron* 15, 769–778, Elsevier. c, Modified from Leutzinger, S., Hirth, F., Gerlich, D., *et al.* 1998. Equivalence of the fly *orthodenticle* gene and the human *OTX genes* in embryonic brain development of *Drosophila*. *Development* 125, 1703–1710. d, Reproduced from Reichert, H. and Boyan, G. 1997. Building a brain: Developmental insights in insects. *Trends Neurosci.* 20, 258–264, with permission from Elsevier.

(anti-HRP) and anti-FasI shows (Figure 1i) that the protocerebral commissure (PCC) is already well developed, comprising over 100 axons (Boyan *et al.*, 1995). Within the PCC, a single large fascicle can be seen expressing the FasI antigen. The pattern in both PCC and TCC is therefore identical to that found in the a-type commissure of each neuromere in the VNC (Figures 1f and 1g) implying that the protocerebral and tritocerebral neuromeres have, like the mandibular neuromere (Figure 1d), 'lost' a specific b commissural component, whether via fusion, splitting, or axonal redirection.

1.22.2 Genes, Commissures, and Segments

1.22.2.1 The Preoral Brain

Developmental genetic analyses have so far identified three genes that are involved in the formation of the preoral brain commissure (PCC). These are *orthodenticle* (*otd*), *commissureless*, and *jing*. The homeobox gene, *otd*, has been shown to play a major role in anterior brain development in insects and vertebrates alike (Hirth *et al.*, 1995; Acampora *et al.*, 1998; Hirth and Reichert, 1999; Reichert and Simeone, 1999). In *otd* null mutants of *Drosophila*, the entire protocerebral neuromere is missing (Figures 3a and 3c; Hirth *et al.*, 1995). The mutation appears to have no gross morphological defects in DC and TC development, but disruption of anterior brain structures leads to the complete absence of all protocerebral commissural axons. In addition, the *otd* mutant phenotype implies that commissural axons from other neuromeres of the brain fail to cross the preoral midline in the mutant brain, probably due to missing pioneer cells which have been shown to be of protocerebral origin (Boyan *et al.*, 2003). In the VNC, the *otd* null mutant is characterized by a condensation of ganglia and a failure to form discrete metameric commissures (Figure 3c). Overexpression of the *otd* gene rescues the commissural organization of the PC and re-establishes the wild-type plan of a-type and b-type commissures in the VNC (Leutzinger *et al.*, 1998).

In *commissureless* mutants, commissures in the VNC do not form (Figures 2a, 2b, 3a, and 3b; Seeger *et al.*, 1993; Tear *et al.*, 1996). Also missing are the tritocerebral (TCC) and mandibular (MaC) commissures. However, the preoral PCC, though reduced, is still present in the brain (Therianos *et al.*, 1995). The Commissureless protein might therefore not be necessary for the initial pioneering of the PCC. The survival of the PCC in the *commissureless* null mutant points to additional molecular mechanism(s) to those acting in the VNC as being responsible for pioneering this commissure. The foremost candidate is probably the *jing* zinc finger transcription factor. In homozygous *jing* mutant embryos the PCC is not pioneered and never forms, while the TCC is pioneered but subsequently does not form properly (Figure 3f). *jing* appears to be essential for the formation of the primary axon scaffold of the brain by regulating the differentiation of Repo-, Castor-, and Sim-positive cells such as midline glia (Sedaghat and Sonnenfeld, 2002). These findings are consistent with the data from *otd* and *com* mutants in pointing to molecularly distinct mechanisms underlying PCC and TCC formation, respectively.

The *commissureless* mutants provide important additional information as to the origin of the pioneering fibers contributing to the PCC. Pioneer axons early in embryogenesis express Fasciclin II, a member of the NCAM family of glycoproteins (Bastiani *et al.*, 1987; Grenningloh *et al.*, 1991). FasII immunocytochemistry in the *commissureless* mutant reveals that fibers from the PC and two locations within the DC contribute to the PCC (Figure 2c), indicating that not only protocerebral but also deutocerebral commissural axons survive the mutation. These data provide evidence that in the wild type, embryonic deutocerebral fibers cross the midline in association with the PCC, thereby contributing to preoral axon pathways in the developing *Drosophila* brain. This appears to also be the case in the early embryonic nervous system of the grasshopper. Examination of axon pathways following Lazarillo immunocytochemistry shows that pioneer neurons from the DC project growth cones anteriorly to the PC where they fasciculate with axons of identified Lazarillo immunoreactive cells (the LC cells) (Figure 2d). These embryonic deutocerebral cells subsequently direct axons across the midline via fascicle XX (Figure 2e) which develops into posterior commissure XX (pcXX) of the adult brain (Figure 2f).

1.22.2.2 The Postoral Brain

In the grasshopper, the DC is also the origin of pioneer neurons which project axonal growth cones posteriorly toward the TC (Figure 2d). En route they fasciculate with the anteriorly directed growth cones of tritocerebral neurons. Further investigation at a later developmental stage identifies the deutocerebral cells as being a bilaterally symmetrical pair of Fasciclin I-immunoreactive neurons with axonal growth cones that pioneer a fascicle within the TCC (Figure 2g). The growth

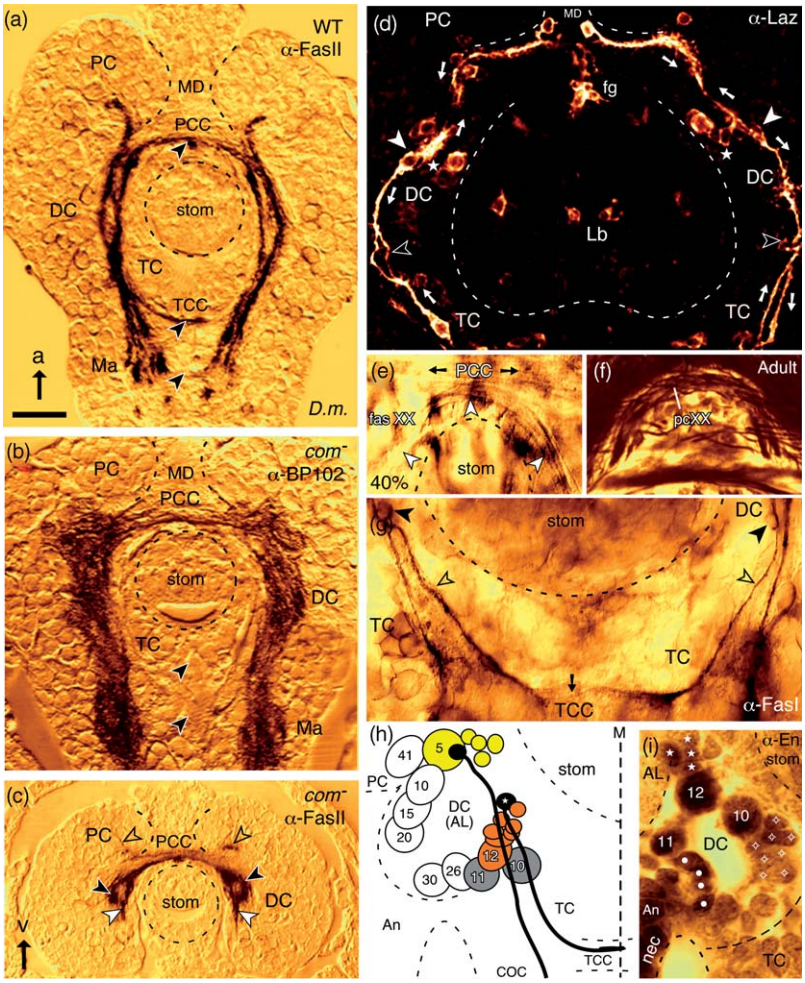

Figure 2 Pathway choices made by pioneering deutocerebral cells in the brain of *Drosophila* (a–c) and grasshopper (d–i). a, Section through the brain of a stage 16 wild-type (WT) *Drosophila* embryo in the plane of both circumesophageal connectives following Fasciclin II immunocytochemistry. Fasciclin II-expressing axons are seen in preoral (PCC) and postoral (TCC, MaC) commissural fascicles (black arrowheads), as well as in longitudinal circumoesophageal fascicles. Arrow points to anterior and applies to all figures. b, Section through the brain of a *Drosophila commissureless* mutant embryo following axon-specific anti-BP 102 immunocytochemistry. Note that the PCC is reduced but still present, while all other postoral commissures, such as the TCC and MaC, are missing (black arrowheads indicate normal location of TCC, MaC). c, Section through the brain of a stage-16 *Drosophila commissureless* mutant embryo following Fasciclin II immunocytochemistry reveals the segmental origin of fibers contributing to the reduced PCC. A single fascicle from the PC (open black arrowhead), and two from the DC (black and white arrowheads), cross in the PCC in this particular section. No fibers from the DC project across the postoral midline in this section and the TCC itself is missing. d, Confocal image following anti-Lazarillo immunocytochemistry at 32% of embryogenesis reveals direction of axonal outgrowth of pioneer neurons in the brain. Some deutocerebral pioneers (white stars) grow out anteriorly toward the PC, while others differentiating at the same site (white arrowheads) grow posteriorly toward the tritocerebrum where they fasciculate with anterior-growing TC pioneers. Sites of fasciculation are indicated by open white arrowheads, while the direction of axogenesis is indicated by white arrows. e, Photomicrograph from wholemount showing commissural axons of deutocerebral origin in embryonic axon fascicle XX (white arrowheads) at 40% of embryogenesis. Note that fas XX joins the PCC at the brain midline. f, Photomicrograph from frontal section through an adult brain showing posterior commissure XX following Bielschowsky histology. pcXX contains axons of deutocerebral origin. Its location and shape suggest it is the adult equivalent of embryonic fas XX. g, Photomicrograph of the medial tritocerebrum viewed dorsally and in wholemount at 34% of embryogenesis following anti-FasI immunocytochemistry. Deutocerebral Fasciclin I immunoreactive pioneers (black arrowheads) from the border of the antennal lobe direct axons (open arrowheads) posteriorly and medially toward the midline of the tritocerebral neuromere where they fasciculate (black arrow) and so pioneer a fascicle of the TCC. h, Ontogeny of deutocerebral pioneer neurons. Lineage analysis (semischematic not to scale) reveals that the bilaterally symmetrical Fas I-expressing pioneer neurons of the TCC shown in (g) above derive from neuroblast 12 of the deutocerebrum. At 34% of embryogenesis the lineage (brown) of NB12 comprises a ganglion mother cell (GMC) and six progeny, one of which (white star) directs an axon posteriorly and medially into the TCC. i, Pioneer neurons and their mother cells express the Engrailed protein, proving their deutocerebral origin. Photomicrograph at 30% of embryogenesis reveals that neuroblast 12 of the deutocerebrum is Engrailed immunoreactive, as are all its progeny (white stars). Neighboring neuroblasts 10, 11 of the DC along with their progeny are also Engrailed immunoreactive. Axogenesis has not yet commenced in these lineages. AL, antennal lobe; An, antenna; coc, circumesophageal connective; nec, neuroepithelial cells. Scale bar (in a): 12 μm (a); 15 μm (b); 8 μm (c); 70 μm (d); 10 μm (e); 50 μm (f); 70 μm (g); 20 μm in (h, i). Reproduced from Boyan, G. S., Reichert, H., and Hirth, F. 2003. Commissure formation in the embryonic insect brain. *Arthropod Struct. Dev.* 32, 61–77, with permission from Elsevier.

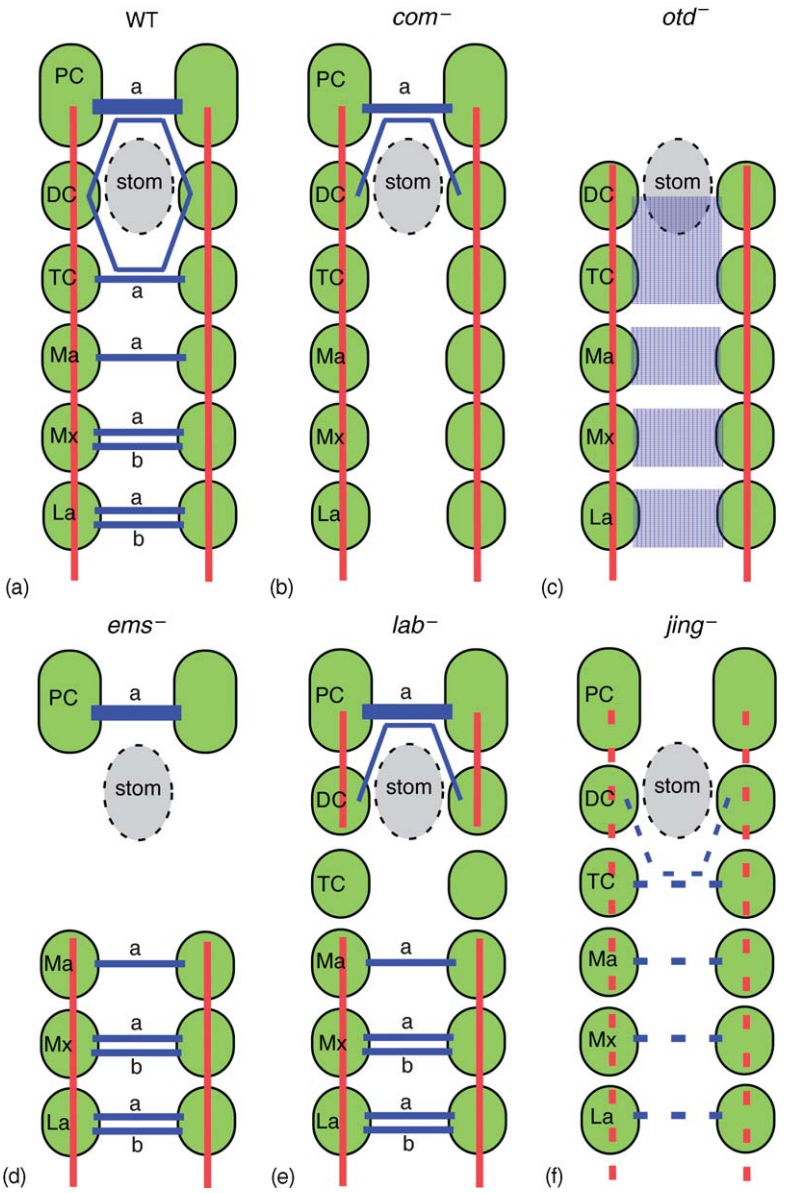

Figure 3 Summary diagrams illustrating the *Drosophila* primary axon scaffold of the embryonic wild type and *com, otd, ems, lab,* and *jing* mutant brains. Commissures are blue; longitudinal fiber tracts are red; neuromeres are green. a, In the wild-type (WT) nervous system, anterior head neuromeres PC, TC, and Ma possess a single a-type commissure, while posterior Mx and La head neuromeres possess two commissures like those neuromeres of the VNC (a-type, b-type). The commissural fibers of the DC utilize the preoral PCC and the postoral TCC to cross the midline. The stomodeum therefore bisects the brain within the DC itself. b, In the *commissureless* (*com*) mutant, all commissures of the VNC are missing, but the PCC survives in reduced form, as does the preoral contribution from the DC. c, In the *orthodenticle* (*otd*) mutant, the entire PC, including the PCC, is missing. Remaining neuromeres are fused along the midline and discrete commissures cannot be discerned (blue hatching). d, In the *empty spiracles* (*ems*) mutant, the DC and TC including the TCC are missing, leading to a gap between the PC and the postoral CNS. The commissural organization in the remaining neuromeres is intact. e, In the *labial* (*lab*) mutant, the tritocerebral commissure, commissural fibers of the DC normally utilizing the TCC, as well as tritocerebral longitudinal connectives, are missing. f, In the *jing* mutant, the protocerebral commissure is missing as well as commissural fibers of the DC utilizing the preoral PCC. Remaining commissures are partially present but malformed (dashes), as are the longitudinal fiber tracts.

cones from each bilateral homologue subsequently meet at the midline of the CNS. Lineage analysis confirms these cells as deriving from deutocerebral neuroblast 12 at the posterior border of the antennal lobe (Figure 2h). This neuroblast is one of three prominent Engrailed-expressing neuroblasts making up the posterior compartment of the grasshopper DC (Figure 2i); its progeny, including the TCC pioneers, are also Engrailed immunoreactive at this stage.

The data thus far show that deutocerebral fibers pioneer commissural fascicles of the preoral (PCC) and postoral (TCC) brain, implying that the stomodeum lies topologically within the deutocerebral neuromere itself. These findings are supported by analyses carried out in *Drosophila* mutants. Thus, mutational inactivation of the *empty spiracles* (*ems*) gene affects the formation of the DC and TC. In *ems* mutants no gross morphological defects are observed in the protocerebral neuromere, and the PCC itself is preserved (Figure 3d), but the TCC does not form and the longitudinal connectives between brain and VNC are interrupted (i.e., not continuous) (Hirth *et al.*, 1995). Mutations in homeobox genes such as *extradenticle* (*exd*) and *homothorax* (*hth*), also strongly perturb the formation of the primary axon scaffold of the brain (Nagao *et al.*, 2000). In *exd* null mutants the formation of the PCC is normal, but delayed, and subsequently shifted (topologically) posteriorly according to neuraxis. While the PCC still forms in the *exd* mutant, the TCC is missing (Nagao *et al.*, 2000). The absence of the TCC in *exd* mutants may be attributed to the fact that *exd* appears to be involved in the regulation of *labial (lab)*, which itself is required for the formation of the TCC (Figure 3e; Hirth *et al.*, 2001; Boyan *et al.*, 2003). *labial* belongs to the Hox gene family of transcription factors whose spatial and temporal expression domains show striking similarities along the anteroposterior axis of the CNS in flies and mice (Hirth and Reichert, 1999; Reichert and Simeone, 1999). Expression of the *labial* gene is restricted to the developing TC and functional analyses have shown that the Lab protein is required for the specification of segmental neuronal identity during embryonic brain development in *Drosophila* (Hirth *et al.*, 1998, 2001). Thus, in *labial* null mutants, tritocerebral cells are generated and positioned correctly, however these cells fail to express neuronal markers and marked axogenesis defects occur leading to the absence of the commissure (TCC) and connectives in this neuromere (Hirth *et al.*, 1998). Indeed, *lab*::Gal4 driven UAS::*taulacZ* reporter gene expression which mimics normal Labial expression in wild type and *lab* null mutant *Drosophila* embryos (Figure 4a), demonstrates that the *labial* gene is essential for the formation of the TCC (Hirth *et al.*, 2001).

Interestingly, in addition to the expression in the endogenous labial domain, ectopic *lab*::Gal4 driven UAS::*taulacZ* reporter gene expression can also be seen in putative deutocerebral cells which direct axons posteriorly into the TCC. These molecularly ectopic deutocerebral cells occupy an equivalent location to the identified deutocerebral pioneers of the TCC in the grasshopper (Figure 2g), and provide additional evidence that the foregut bisects the brain within the DC itself. Neuron specific anti-HRP immunolabelling reveals that the TCC is missing in the *lab* loss-of-function mutant embryonic brain (Figures 3e and 4b), suggesting that the Labial protein is essential for the formation of the TCC. This is further supported by the fact that the TCC can be rescued by transgenic expression of the Lab protein in a *lab* null mutant ($lab^{-/-}$) background (Hirth *et al.*, 2001). Moreover, *lablacZ* reporter gene expression together with anti-β-tubulin immunoreactivity reveals that commissural fibers in the TCC originate from two sources: (1) from the labial domain in the TC, and (2) from ectopic neurons of the labial domain located in the DC (Figure 4c). The axon projections from these putative deutocerebral neurons bear a striking similarity to those of the Fasciclin I expressing pioneers of the TCC in the grasshopper (cf. Figure 2g). These cells are missing in the *lab* null mutant (Figure 4d), as is the TCC (Figure 3e). UAS::*taulacZ* reporter gene expression in combination with anti-β-Gal and anti-Fasciclin II immunolabelling in the *lab* null mutant showed that these pioneers require the Labial protein in order to cross the midline and form the TCC (Hirth *et al.*, 2001). Thus, in addition to the data obtained from otd^{-} and com^{-} mutant analyses, the *exd* and *lab* mutant phenotypes provide further genetic evidence for distinct molecular mechanisms underlying PCC and TCC formation, respectively.

Does the TCC, then, develop like other postoral commissures of the suboesophageal ganglion and the VNC? Recently, it has been shown that genes of the Hox group such as *Dfd*, *Antp*, and *Ubx* whose expression domains are restricted to neuromeres posterior to the TC, are capable of rescuing the TCC in the *lab* null mutant (Hirth *et al.*, 2001 and reviewed in Boyan *et al.*, 2003). Only the most posteriorly expressed *Abd-B* could not rescue the TCC. Nevertheless, there appears to be a molecular distinction between postoral commissures within the head such as TCC and MaC and the commissures of the VNC. The distinction lies in the fact that only in the tritocerebral (via *labial*) and mandibular (via *Deformed*) cephalic neuromeres is commissure formation itself regulated by Hox genes. The TCC and the MaC are in this respect molecularly more akin to one another than they are to the more posteriorly situated commissures of the VNC. An interesting study in this regard proposes that the TCC and MaC, which are ultimately unitary in their respective neuromeres in the grasshopper and in *Drosophila* (Figures 1d and 1e) actually derive from a single

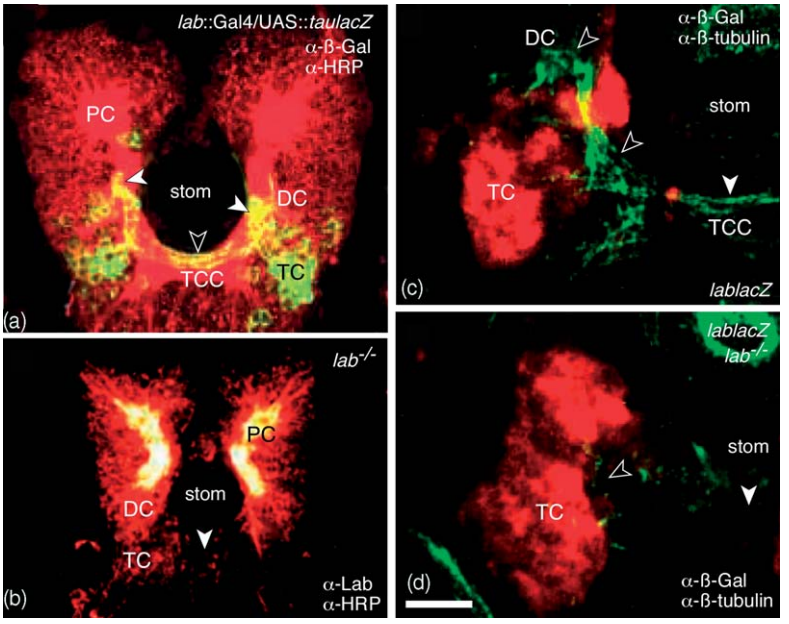

Figure 4 Genetic regulation of tritocerebral commissure (TCC) formation in the embryonic *Drosophila* brain. Reporter gene expression reveals dependence on the Lab protein for the formation of commissural axons in the TCC. a, Laser confocal reconstruction of a stage-15 embryo in frontal view reveals P{w$^+$ *lab*::Gal4}K5J2- driven UAS::*taulacZ* reporter gene expression in the wild-type brain. Double immunolabeling with anti-HRP (red) and anti-β-Gal (green). Co-expression is yellow. UAS::*tauLacZ* reporter gene expression is seen in axons of cells from the TC (the endogenous tritocerebral Lab expression domain) which project into the TCC, as well as ectopic reporter gene expression in deutocerebral cells (white arrowheads) which direct axons (open arrowhead) into the TCC. b, Laser confocal microscopy of stage-15 *lab* loss-of-function mutant embryonic brain following anti-HRP immunolabeling. White arrowhead indicates missing tritocerebral commissure. c, Laser confocal reconstruction of a stage-14 wild-type brain with a *lab-lacZ* reporter construct following double labeling with anti-β-Gal (red) and anti-β-tubulin (yellow/green). Commissural fibers (white arrowhead) in the TCC originate from the labial domain in the TC, and from β-tubulin-expressing neurons of the DC (open arrowheads). d, Laser confocal reconstruction from a stage 14 of a *lab* mutant brain with *lab-lacZ* reporter construct. The TCC (white arrowhead indicates normal location) is absent, as are the axon projections from deutocerebral cells (open white arrowhead; cf. (d)). Scale bar (in d): 10 μm (a, b); 4 μm (c); 5 μm (d). Modified from Hirth, F., Loop, T., Egger, B., Miller, D. F. B., Kaufman, T. C., and Reichert, H. 2001. Functional equivalence of Hox gene products in the specification of the tritocerebrum during embryonic brain development of *Drosophila*. *Development* 128, 4781–4788; Boyan, G. S., Reichert, H., and Hirth, F. 2003. Commissure formation in the embryonic insect brain. *Arthropod Struct. Dev.* 32, 61–77.

commissure that subsequently splits into an anterior a-type and a posterior b-type fascicle (Page, 2004). According to this hypothesis, the TCC can be considered as equivalent to an a-type commissure, and the MaC as equivalent to a b-type commissure, of a given VNC neuromere. The splitting of the commissure is proposed to be a function of Repo-expressing glia cells which appear between the diverging axon fascicles. However, the proposed role of these glial cells in *Drosophila* awaits further confirmation, for example by examining TCC and MaC formation in embryos mutant for glial cell formation like the *glial cells missing* (Hosoya *et al.*, 1995) or *repo* (Halter *et al.*, 1995) mutants. In the grasshopper, no evidence for a splitting of a common commissure in the formation of the TCC and MaC is seen. Nevertheless, both our data (Figures 1f–1h) and those of Page (2004) suggest that the TCC at least develops like an a-type commissure of the VNC.

1.22.3 The Split Deutocerebrum: Evidence from Identified Neurons of the Adult Brain

Several lines of evidence suggest that deutocerebral neurons project commissural axons along pre- and postoral routes to cross the embryonic brain midline (Figure 3a). These are supported by histological data and cobalt backfills from the circumoesophageal connectives in the adult grasshopper brain (Figures 5a–5f) which show populations of identified deutocerebral cells, some with axons in preoral, others with axons in postoral, commissures. The bilateral ventral giant 3 (VG3) cells of the posterior deutocerebral midline direct axons into commissure pcXXI of the preoral adult brain (Figures 5b, 5e, and see Boyan *et al.*, 1993, for an atlas of brain commissures). Each neuron projects an axon into the respective contralateral

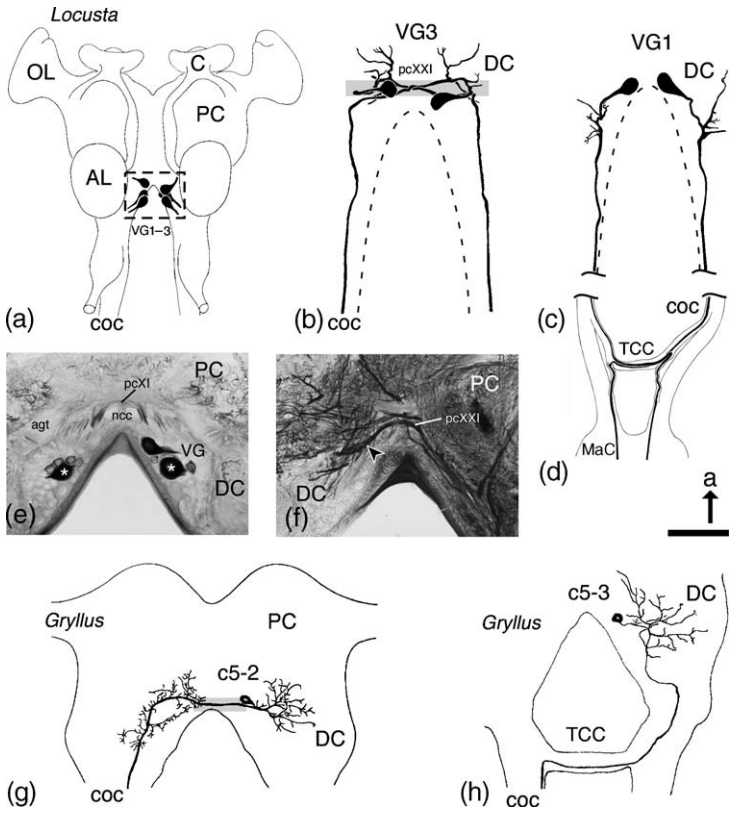

Figure 5 Commissural interneurons of the deutocerebrum in the adult brain of the grasshopper, *Schistocerca gregaria* (a–e), and cricket, *Gryllus bimaculatus* (f and g). a, Semi-schematic drawing (not to scale) showing the ventral cluster of cell bodies (VG1–3) at the posterior midline of the deutocerebrum in the adult locust. Dashed rectangle indicates the cell body group investigated in (b–e). b, Drawing from sections following Wigglesworth histology shows the bilaterally paired VG3 interneurons of the deutocerebrum with crossing segments in posterior commissure XXI (pcXXI), and axons descending contralaterally via the circumoesophageal connectives (coc) to the ventral nerve cord. Note that pcXXI is located preorally in the brain. Dashed outline indicates 'crotch' of the brain as in (a). c, Drawing from sections following Wigglesworth histology shows the bilaterally paired VG1 interneurons of the deutocerebrum with axons descending ipsilaterally into the circumoesophageal connectives, but (d) then crossing the midline postorally via the tritocerebral commissure (TCC) of the TC. e, Photomicrograph from section following cobalt backfilling from a circumoesophageal connective shows cell bodies of the VG cell cluster in the deutocerebral brain midline of the locust. White asterisks label the bilaterally paired VG1 cell bodies. One of the commissures (pcXI) containing axons of deutocerebral origin is indicated. agt, antennal glomerular tract; ncc, nerve roots of the corpora cardiaca. f, Photomicrograph (as in (e)) shows axons (black arrowhead) of deutocerebral origin crossing the brain midline preorally in pcXXI. g, Drawing of identified contralateral descending interneuron c5-2 in the brain of the cricket, *G. bimaculatus*, following retrograde labeling via the cervical connective (see Staudacher, 1998 for details). This neuron has its cell body in the equivalent ventral cluster to that described for the locust above, and the crossing segment is probably also in a preoral posterior commissure (shaded gray). h, As for (g) but of ipsilateral descender c5-3. This neuron is equivalent to VG1 in the locust (see (c)) and crosses the midline postorally via the TCC. Anterior is to the top in all figures. Scale bar: 100 μm (b–e); 270 μm (f, g). a–f, Modified from Williams, J. L. D. 1975. Anatomical studies of the insect central nervous system: A ground-plan of the midbrain and an introduction to the central complex in the locust, *Schistocerca gregaria* (Orthoptera). *J. Zool.* (*Lond.*) 176, 67–86. g and h, Reproduced from *Cell Tissue Res.*, vol. 294, 1998, pp.187–202, Distribution and morphology of descending brain neurons in the cricket *Gryllus bimaculatus*, Staudacher, E. With kind permission of Springer Science and Business Media.

circumoesophageal connective and then into the VNC. The VG1 cells of the same deutocerebral cluster (Figures 5a, 5c, and 5d), by contrast, first project axons ipsilaterally into the circumoesophageal connectives, and these axons then cross the midline postorally in the commissure of the tritocerebral neuromere (Figure 5c).

These data are supported by studies undertaken in other insects. In the cricket, for example, an identified interneuron of the deutocerebral neuromere projects a commissural process preorally across the brain midline (Figure 5f), while another interneuron projects an axon contralaterally via the postoral TCC (Figure 5g). These neurons may well be homologues of those described in the grasshopper (Figures 5b and 5c; see Staudacher, 1998). Consistent with these findings, Strausfeld (1976) describes deutocerebral B interneurons and CentP

neurons in Diptera which project dendrites into antennal glomeruli via a preoral commissure.

1.22.4 Conclusions

1. The commissures of the insect CNS are organized as an iterative array as far anteriorly as the maxillary (Mx) neuromere of the head. In more anterior neuromeres (mandibular, tritocerebral, deurocerebral, and protocerebral), the typical pattern of anterior and posterior commissures breaks down: PC, TC, and mandible possess only single commissures (Figure 1) which might represent the anterior or a-type commissure of a typical neuromere in the VNC (Figure 3a).
2. In the grasshopper embryo, pioneers from the DC project growth cones anteriorly where they cross in a preoral posterior fascicle XX (Figure 2d). Other deutocerebral pioneers project growth cones posteriorly where they fasciculate with tritocerebral ascending axons before pioneering a fascicle of the TCC (Figures 2g and 4c). In the adult (Figure 5), some identified deutocerebral interneurons in grasshopper, cricket, and fly contribute to preoral (e.g., grasshopper pcXXI), others postoral (TCC), commissures. The foregut therefore bisects the brain topologically within the DC itself and not, as traditionally thought, between DC and TC (Figure 3a).
3. Genetic experiments in *Drosophila* provide further evidence for a molecular distinction between the commissures in the head and VNC (Figures 3b–3f). In *otd* (Figure 3c) and *jing* (Figure 3f) mutants the PCC is missing, as are axons from deutocerebral neurons which normally contribute to the preoral brain commissure. Pre- and postoral deutocerebral axons are also missing in the *ems* mutant which specifically affects the formation of the DC and the TC (Figure 3d). In *lab* and *exd* mutants the TCC is absent (Figures 3e and 4b) due to missing tritocerebral, and postoral deutocerebral, commissural axons (Figures 4c and 4d). In *com* mutants, only part of the PCC and preoral fibers from the DC survive (Figures 2c and 3b). The *com*, *otd*, *jing*, *lab*, and *exd* mutant phenotypes suggest that distinct molecular genetic mechanisms underlie commissure formation in the anterior insect brain as opposed to the VNC.

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1.23 Evolution of Color Vision and Visual Pigments in Invertebrates

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Glossary

color vision	The ability to discriminate among stimuli on the basis of hue, independently of brightness or any other cue.
microspectrophotometry	Measuring absorption spectra of very small objects, typically of single photoreceptor cells. The technique is useful for finding the spectra of visual pigments *in situ*.
opsin	The protein on which all visual pigments are based.
trichromatic	Based on three spectrally different photoreceptor classes.
visual pigment	The molecules used in all animals that absorb light and begin the process of seeing.

1.23.1 Introduction

1.23.1.1 Visual Pigments: Definition and Properties

Vision in all animals is based on the same set of molecules, light-absorbing compounds called visual pigments. These pigments capture incoming photons of light, transducing light's energy into the biochemical language of cells and thereby beginning the process of seeing. Although visual pigments are homologous across all animals, even occurring in some plants, their diversity among invertebrates is bewildering and leads in many cases to visual capabilities and systems of color vision quite unlike those of humans.

All visual pigments are built by combining a protein, opsin, with one of four different chromophores, each an aldehyde of a molecule in the vitamin A family. Opsins vary greatly in their amino acid sequences, and this, together with the potential to bind different chromophores, produces an enormous range of different visual pigment types. The primary influence that visual pigments have on visual sensitivity and color vision is via the spectral region in which each pigment absorbs light. The shapes of the spectral absorption curves of all visual pigments are similar, so it is possible to characterize each pigment by specifying the wavelength at which it absorbs light most effectively. Spectral properties of visual pigments are measured either in solution, using classical spectrophotometric techniques, or in individual photoreceptor cells using microspectrophotometry. Figure 1 shows an example of the absorption spectrum of a visual pigment within an invertebrate photoreceptor, illustrating both typical data and the fit of these data to an idealized visual pigment spectral template. As with all visual pigments, this spectrum has a smooth, nearly symmetrical peak dropping to complete transparency on the long-wavelength limb and to a shoulder on the short-wavelength side.

1.23.1.2 Color Vision: Definition and Properties

Color vision is the ability to discriminate among stimuli on the basis of hue, independently of

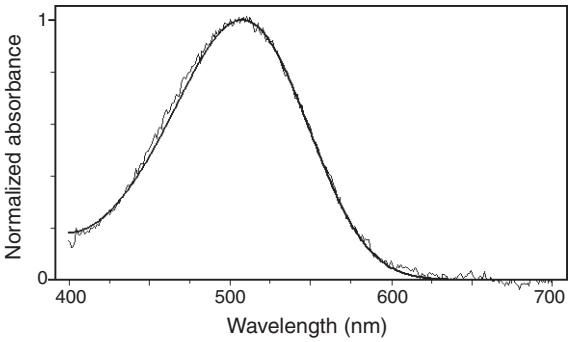

Figure 1 The major visual pigment in photoreceptors of the hermit crab, *Petrochirus diogenes*. The jagged, thin curve is the original data, collected using microspectrophotometry, and the smooth, dark curve is the ideal visual pigment template curve that best fits the data. The best-fit curve peaks at 508 nm, drops to near transparency by ~625 nm on the long-wavelength limb, and decreases more gradually at shorter wavelengths.

brightness or any other cue. To do this, at least two receptor classes differing in their spectral sensitivities must contribute to vision in an animal. By sampling two separate spectral regions, and with the assistance of appropriate neural processing, a visual system can extract spectral (i.e., color) differences from stimuli. The great benefit of color vision is that it adds both visibility and perceptual uniqueness to visualized objects, making it easier both to discriminate them (i.e., distinguish them from surrounding objects or backgrounds) and to identify them (i.e., know what they are, based on their color). Color vision is important for orientation and navigation, for identifying conspecifics, for detecting predators and/or prey, and for recognizing visual signals produced by other organisms.

The simplest, and by far the most common, technique for producing multiple spectral classes of receptors is to use different visual pigments in independent receptor types. Thus, like humans – whose color vision is based on sets of red-, green-, and blue-sensitive cones, each with a different visual pigment – most species of invertebrates have color-vision systems founded on two or more visual pigments with different absorption maxima (see The Comparative Biology of Photopigments and Color Vision in Primates).

1.23.1.3 Establishing the Presence of Color Vision in a Species

1.23.1.3.1 Genetic and physiological approaches It is rarely easy to know whether or not a given animal species possesses color vision. Typically, one first determines the number of receptor classes that are present in the retina. Often a screen of the genome provides a first estimate of the number of visual pigment genes, but this is often misleading for estimating the diversity of potential color receptors for several reasons. Different visual pigments may be devoted to different visual tasks (e.g., in receptors specialized for use in bright or dim light). Also, not all opsin genes may be expressed, or they may be expressed at different life stages. To avoid some of these problems, retinal mRNAs encoding for opsins can be analyzed, but this still does not discriminate color from non-color receptor classes. At higher levels of inquiry, receptor types can be classified by their structure or by their physiological properties (e.g., using single-cell recording techniques), or they can be characterized using microspectrophotometry.

1.23.1.3.2 Behavioral approaches All these molecular biological or physiological approaches assess only the potential of the visual system, not its actual performance. Different receptors may operate in different light regimes (analogously to vertebrate rods and cones), or they may exist in different regions of the eye and provide no input to a color-vision system. They could also contribute to what are known as 'wavelength-specific behaviors', wherein different spectral receptor classes mediate different behaviors, but there is no recognition of color as such. For example, water fleas (*Daphnia*) swim differently depending on the predominant wavelength of light in the environment, producing color-specific 'dances' that rely on the excitation of particular receptor types but do not apparently involve hue discrimination by comparing receptor inputs (Smith and Baylor, 1953). Therefore, the presence of color vision in a given species must ultimately be established by using behavioral tests, challenging an animal to learn to associate a given color with a reward. This approach has been used successfully with a number of invertebrate species, often revealing surprisingly sophisticated capacities (see Cognition in Invertebrates). Unfortunately, the failure to train an animal to color cues can never disprove that it has color vision, but if the species has only a single visual pigment class, it is generally safe to infer its absence.

1.23.2 Survey of Visual Pigment Type and Color Vision in Invertebrates

1.23.2.1 General Principles

With the existence of millions of invertebrate species, it is impossible to generalize or to cover all possible invertebrate visual systems. More importantly, there are almost certainly visual systems with extremely odd or unique properties that have not even been discovered yet. However, since at this point all known visual pigments are homologous and have similar absorption properties, little needs to be said about their diversity other than that it

exists. Spectral differences among species are evidenced primarily as shifts in the wavelength of maximum absorption, although secondary effects produced by the identity of the chromophore that is bound to the opsin also play a role in the shape and position of the absorption spectrum. Visual pigments also vary among species and across phyla in their interactions with the biochemical machinery of the receptor cell, but these properties are not thought to affect color vision.

1.23.2.2 Invertebrates other than Arthropods

The simplest metazoans, hydras, have at least one opsin-based visual pigment (Musio *et al.*, 2001), but, lacking eyes, they do not 'see' and thus could not possibly have color vision (though color-specific behavior remains possible). It is an unfortunate fact that, with the exception of the arthropods and cephalopod mollusks, little is known about the visual systems of most invertebrate phyla other than the basics of eye structure. Color vision has arisen repeatedly in well-studied phyla, so it probably exists widely throughout the invertebrates. Molecular techniques, combined with physiological and behavioral approaches, will hopefully be employed to address some of the gaps in our knowledge of the huge diversity of animals that remain virtual mysteries (see Gene Expression in the Honeybee Mushroom Body and Its Gene Orthologues).

Cephalopod mollusks (octopus, cuttlefish, and squid) have large and well-developed eyes and sophisticated visual behavior, making them major invertebrate model systems in vision science. Opsin genes of several species have been sequenced and visual pigments have been spectrally characterized. Unexpectedly, cephalopods in general are color-blind, having vision based on a single visual pigment class (see Figure 2, *Sepia officinalis* – the common cuttlefish). There is indirect evidence that one nocturnal, deep-sea species, the Japanese firefly squid *Watasenia scintillans*, has multiple visual pigments and receptor types (Seidou *et al.*, 1990), but this is a difficult species to study behaviorally; having color vision certainly could be useful to such a species for recognizing bioluminescent signals in the dark of the sea. In general, though, cephalopods may be the only animals with highly complex eyes that have bypassed color vision, probably in favor of other systems of visual analysis.

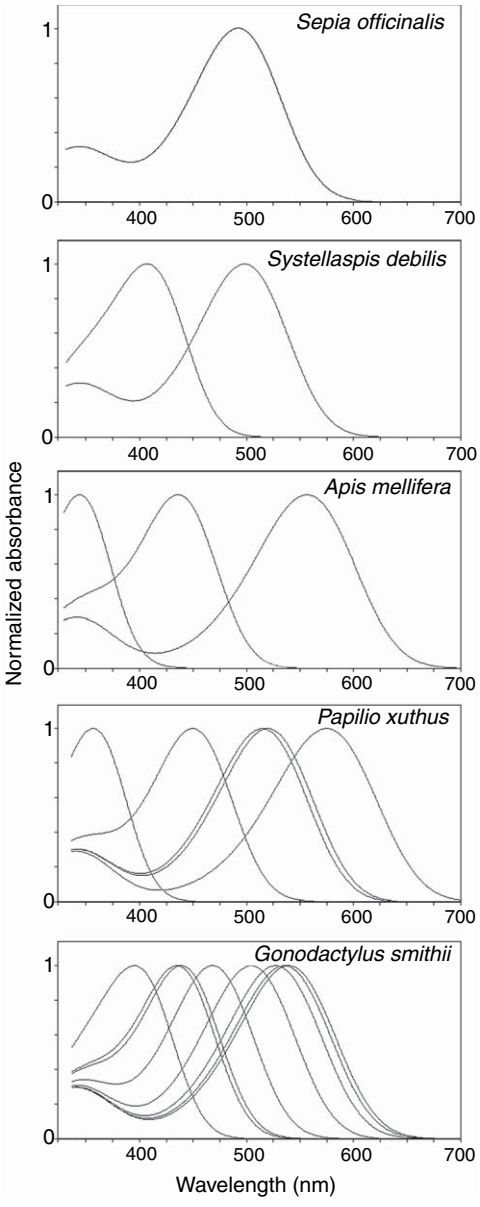

Figure 2 Spectra of visual pigments in representative color-vision systems of a variety of invertebrates, mostly arthropods. The top panel shows the single-receptor system of the cuttlefish, *Sepia officinalis*, with one visual pigment peaking at 492 nm. Next is the visual system of a deep-sea shrimp, *Systellaspis debilis*, with two pigment classes, peaking at 410 and 498 nm. The center panel illustrates the trichromatic system of the honeybee (pigments at 344, 436, and 556 nm). Complex color vision in the Japanese swallowtail butterfly, *Papilio xuthus*, is illustrated in the fourth panel (pigment peaks at 360, 450, 515, 520, and 575 nm). The bottom panel shows the eight-receptor color system of the mantis shrimp, *Gonodactylus smithii* (pigments at 400, 424, 454, 474, 496, 521, 541, and 546 nm).

1.23.2.3 Arthropods

By far the greatest diversity of visual pigment classes exists in arthropods. Their pigments peak at wavelengths from deep in the ultraviolet (<340 nm) to well into the red (600 nm or longer), and the visual pigments of some species (e.g., the horseshoe crab *Limulus polyphemus* and the crayfish *Procambarus clarkii*) have historical significance for understanding visual pigment function. More impressively, arthropods have the greatest diversity in systems of color vision among animals. The insects and

crustaceans in particular have taken color vision to its most complex forms yet described, with at least eight primary color classes present in some species. The honeybee, *Apis mellifera*, has served as a major model system for studying the principles of color vision, and in fact bees have the best-known system next to that of humans.

Examples of arthropod sets of visual pigments that contribute to color-vision systems are illustrated in the lower four panels of Figure 2. The simplest possible system includes two receptor types, each with its own pigment; this is exemplified in the deep-sea shrimp *Systellaspis debilis* (Cronin and Frank, 1996). It is present in many common crustaceans, including most crabs, lobsters, and shrimps. The central panel illustrates the trichromatic (literally, 'three-color') color-vision system of the bee, with ultraviolet-, blue-, and green-sensitive receptor types. Next is the five-receptor system of a swallowtail butterfly, which differs from that of the bee primarily by adding two receptor types with visual pigments absorbing maximally near 500–520 nm (in the green). Finally, the most elaborate color systems known exist in the mantis shrimps. An example from *Gonodactylus smithii* is illustrated in the bottom panel of Figure 2, with eight classes of color receptors present peaking at wavelengths from 400 to near 560 nm. Like other mantis shrimps, this species also has five spectral classes of ultraviolet photoreceptors, which may also play roles in color vision, and other receptor classes devoted to spatial, motion, and other aspects of vision; altogether this species and its relatives have at least 16 visual pigment classes used in vision, the largest number known among animals (Cronin and Marshall, 2004).

Increasing the number of visual pigments, however, does not by itself lead automatically to improved color vision. Visual pigment spectra are broad, and packing many of these spectra into a restricted range simply leads to lots of overlap with no increased ability to discriminate colors. This is well illustrated at the bottom of Figure 2, where many spectra of pigments in the retina of a mantis shrimp are piled on top of one another. Mantis shrimps, as well as many other arthropods, deal with this complication using filters lying directly in the path of incoming light or placed alongside the receptor, that modify the light traveling inside it. All these consist of some sort of photostable pigment, often a carotenoid, that generally transmits only in a restricted spectral range. The pigment trims the spectrum of light, sharpening the sensitivity of the underlying visual pigment and improving spectral discrimination. Figure 3 shows an example of receptors containing two different visual pigments that are tuned with different filters, producing a pair of receptor classes with very narrow spectral sensitivities. This tactic for improving color vision is seen in butterflies, fireflies, click beetles, crabs, locusts, and no doubt many other insects and crustaceans.

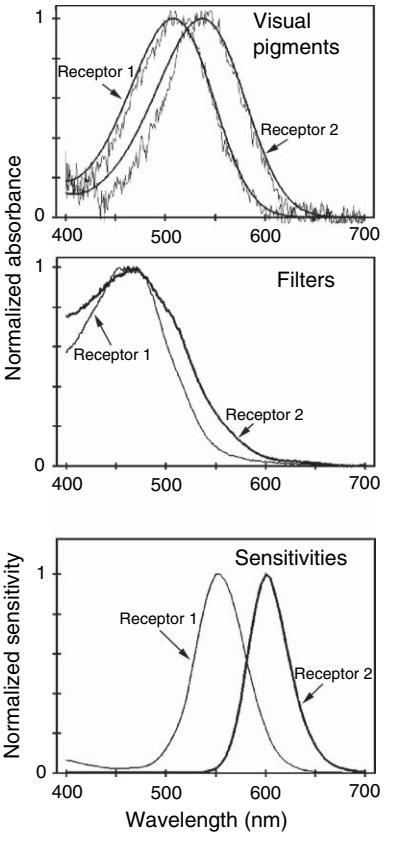

Figure 3 The use of filter pigments to sharpen spectral sensitivities, here showing an example from the mantis shrimp *Haptosquilla trispinosa*. In the illustrated receptor pair, visual pigments (the thin, jagged lines show original data, while the thick, smooth lines are idealized visual pigment spectra) in two receptors, peaking at 508 nm ('receptor 1') and 537 nm ('receptor 2'), are tuned by overlying filter pigments with absorption spectra illustrated in the center panel. The filters absorb short-wavelength light, producing a pair of receptors with spectral sensitivities that are much narrower and shifted to long wavelengths. The presence of these and other filters allows many similar visual pigments (as in the lower panel of Figure 2) to produce a series of separately tuned photoreceptors, providing multidimensional color vision. Many invertebrates use similar filters to tune photoreceptor sensitivities.

1.23.3 Theories of Evolution of Visual Pigments and Color Vision

1.23.3.1 Visual Pigment Tuning: Sensitivity and Contrast Hypotheses

Spectral properties of visual pigments are sensitive to the presence of specific amino acids at critical locations in the opsin molecule; consequently, evolutionary alterations can produce highly diverse sets

of pigments. How visual pigments are optimally tuned has long been of interest to vision scientists. To be effective, the visual system should allow important objects to stand out against their backgrounds, and two hypotheses have been advanced to predict how this might be done. The 'sensitivity hypothesis' predicts that visual pigments should be tuned to match the light present in an animal's environment, thereby providing the greatest possible response to any illumination. The 'contrast hypothesis' predicts that vision should be tuned to maximize the stimulation difference between object and background. The hypotheses are related, in that in many dim-light environments, the only difference between foreground and background is in their brightness, favoring sensitivity. Nevertheless, in colorful environments, it is not always obvious how contrast should be optimized, especially for a color-vision system. Thus, while these hypotheses can predict the spectral properties of visual pigments that evolve in animals with a single photoreceptor class, they are not very useful for explaining how color vision evolves.

1.23.3.2 Visual Ecology and Natural Scenes

A second approach to understanding the evolution of color vision is to model how visual systems should adapt to the scenes that animals view. Each species uses its eyes in a limited selection of all possible visual environments, whether in forests, meadows, lakes, coral reefs, the deep ocean, or wherever it is found; thus, its visual system should be adapted for optimal function in the scenes it is most likely to view. This approach requires careful documentation of environmental illuminants and colors (spectral reflectances) and heavy mathematical treatment of how these interact with each other and with a selection of visual systems. Nevertheless, it has been very successful for predicting how simple color-vision systems, based on two or three color receptor classes, can be optimized.

1.23.3.3 Role of Color Vision in Object Identification, Feeding, and Signal Detection

Animals use their visual systems both for general tasks like orientation and object avoidance and for species-specific needs like food finding, predator detection, and signal interpretation. Thus, while there could be overall environmental control (via lighting, natural scenes) of the evolution of visual pigments and color vision, there must also be special evolutionary significance for specific tasks directly related to survival, like seeing food and early recognition of the presence of a predator (see Evolutionary Neuroethology – A Case Study: Origins and Evolution of Novel Forms of Locomotion in Hippid Sand Crabs (Malacostraca, Decapoda, Anomala). Furthermore, there must be co-evolution of signals and visual systems, so that signals are effective. Several studies, based on different models of visual function, have demonstrated how flower colors and honeybee color vision have co-evolved. Here, however, it appears that it is the evolution of flower colors, rather than of the receptors of the bees, that optimizes signaling. Our current understanding of visual evolution and the evolutionary tuning of color vision suggests that visual systems evolve under the influence of environments, and signals evolve under the influence of visual systems.

1.23.4 Summary and Conclusions

The invertebrates, with their enormous phyletic diversity, make it nearly impossible to generalize except to say that nearly any conceivable method of visual pigment tuning or system of seeing and analyzing color is likely to be found among them. Consequently, the greatest advantage that these animals offer to vision scientists is the chance to explore well-selected questions concerning visual evolution in a well-suited biological system. Invertebrates will continue to serve as means of answering new questions as they arise and as sources for finding unexpected evolutionary directions of visual systems.

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1.24 A Tale of Two CPGs: Phylogenetically Polymorphic Networks

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Glossary

central pattern generator	An ensemble of neurons that generates a rhythmic motor pattern.
notaspid	Marine gastropod in the order Notaspidea, a sister order to the nudibranchs.
nudibranch	Marine gastropod in the order Nudibranchia, characterized by a lack of a shell.
Nudipleura	Clade consisting of Nudibranchia and Notaspidea.
opisthobranch	Marine gastropod in the subclass Opisthobranchia, characterized by a reduced shell, mantle, and gills.

1.24.1 Introduction

> It was the best of times, it was the worst of times, it was the age of wisdom. . .(Charles Dickens)

It is the best of times for understanding neural circuits from a functional perspective; much has been gained from the approach of studying specialized neural circuits in invertebrate species with large neurons and simple behaviors. With this approach, neuroscientists have had tremendous success in unraveling neural circuits and mechanisms underlying behaviors. Invertebrate circuits, and in particular opisthobranch nervous systems, have provided important insights into motor pattern generation (Getting, 1989a), and learning and memory (Pittenger and Kandel, 2003). Thanks to work on invertebrate circuits, there is now a recognition that individual neurons and the circuits that they form can be multifunctional; that is, a single anatomically defined group of neurons can be dynamically reconfigured to serve multiple functions (Harris-Warrick and Marder, 1991; Kupfermann and Weiss, 2001; Marder, 1994; Marder and Calabrese, 1996).

It is the worst of times because success in understanding neural circuits has occurred in the absence of understanding their origins. It has been noted previously that the architecture of modern neural circuits may not reflect functional optimization, but may be the result of historical circumstances (Arbas *et al.*, 1991; Dumont and Robertson, 1986). Despite the new awareness that neural circuits are multifunctional within a given animal, there is little known about how phylogenetically plastic central circuits are. Is it possible that species-specific behaviors arise from the same circuit being differentially modulated or are there species-specific neural circuits? If there are different circuits underlying different behaviors, how did they orginate?

This tale of two central pattern generators (CPGs) in two nudibranch mollusks, serves to illustrate some concepts related to the evolution of species-specific behaviors. The two species of this tale are *Tritonia diomedea* and *Melibe leonina* (Figures 1a and 1f). Both of these sea slugs swim using whole body flexions. Their nervous systems contain homologous neurons, yet they use different groups of neurons to produce their respective swimming behaviors. Comparing the behaviors and their neural mechanisms in these two species shows that distinct functional neural circuits arose through a differential partitioning of a common nervous system to produce species-specific behaviors. Further comparisons with a third species, *Pleurobranchaea californica*, suggest that analogous behaviors can be controlled by

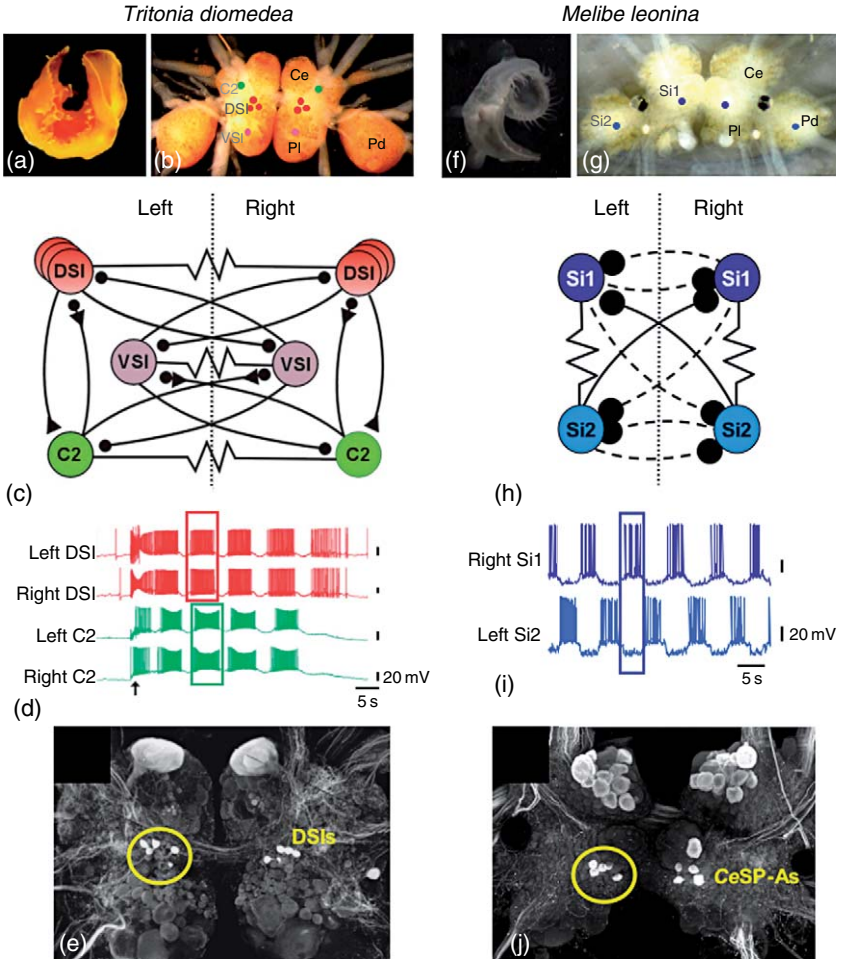

Figure 1 Comparison of the swim central pattern generators (CPGs) in two nudibranchs: *T. diomedea* (a–e) and *Melibe leonina* (f–j). a, *Tritonia* swims by flexing its body in the dorsal and ventral directions. b, A photomicrograph of a *Tritonia* brain, which consists of the cerebral (Ce), pleural (Pl), and pedal (Pd) ganglia. The locations of the three CPG neurons are indicated: cerebral neuron 2 (C2), the three dorsal swim interneurons (DSIs), and ventral swim interneuron-B (VSI). c, The synaptic connectivity of the swim CPG. Excitatory synapses are indicated as triangles, inhibitory synapses are balls, and electrical synapses are shown as resistors. All neurons are electrically coupled to their contralateral counterparts. d, Simultaneous intracellular microelectrode recordings from the left and right DSIs and C2s show that the contralateral counterparts burst in phase with each other during the swim motor pattern, which was elicited at the arrow by brief electrical stimulation of a peripheral nerve. e, Serotonin immunohistochemistry shows the locations of the three DSIs as part of a group of five serotonergic neurons in the posterior portion of the cerebral ganglion. f, *Melibe leonina* swims by flexing from side to side. g, The locations of the swim CPG neurons are superimposed on a photomicrograph of a *Melibe* brain. Swim interneuron 1 (Si1) is located in the cerebral ganglion and swim interneuron 2 (Si2) is in the pedal ganglion. h, The synaptic connectivity of the *Melibe* swim CPG shows that there is mutual inhibition of the contralateral Si2s. i, Simultaneous intracellular microelectrode recordings of the right Si1 and left Si2 show the alternation in firing between the left and right sides that is characteristic of the swim motor pattern. j, Serotonin immunohistochemistry shows the location of the cerebral serotonergic posterior (CeSP-A) neurons, which are homologous to the DSIs in *Tritonia*. a, Reproduced with permission from William Frost. f, Reproduced with permission from Win Watson.

homologous neurons, indicating that a similar partition of the common nervous system could have arisen independently. In other words, similarity in behavior may have arisen through parallel evolution.

1.24.2 The Behaviors

Although the swimming behaviors of *Tritonia* and *Melibe* are both whole body flexions, they differ in several important respects. First, *Tritonia* rarely swims; it is a benthic nudibranch that uses swimming as an escape response to contact with a predatory sea star, *Pycnopodia helianthoides* (Willows and Hoyle, 1968; Willows, 1968). In contrast, *Melibe* swims more readily than *Tritonia*; simply displacing it from the substrate is a sufficient stimulus to cause the animal to swim, although the animal will also swim in response to contact with

seastar tubefeet (Lawrence and Watson, 2002). The animals also have different postures during swimming; *Tritonia* flattens its body in the coronal plane and flexes its body rhythmically in the dorsal and ventral directions, whereas *Melibe* flattens its body in the sagittal plane and rhythmically flexes in the lateral directions (side-to-side) (Willows, 2001). There are other important differences in these swimming behaviors. *Tritonia* swims for relatively short bouts (2–10 flexion cycles, lasting up to a minute), but *Melibe* can swim for extended periods of time (many minutes). There does not appear to be any directionality to the *Tritonia* swim; the primary objective seems to be to cause the animal to rise off the bottom to allow it to be swept away by water currents. For *Melibe*, the swim has some directionality; the animal proceeds in a foot-first direction (Lawrence and Watson, 2002). Clearly, these behaviors, although analogous in being whole body flexions, are species-specific in many ways.

1.24.3 Polymorphic Neural Circuits

1.24.3.1 The *Tritonia* Swim CPG

The CPG underlying the *Tritonia* swim motor pattern represents one of the earliest success stories in research on functional neural circuits. In the mid-1960s, researchers were looking for invertebrates with large neurons that performed simple behaviors so that the neural circuits underlying those behaviors could be deciphered. During this period, Kandel *et al.* (1967) began working on the gill and siphon withdrawal circuit of *Aplysia* and Willows and Hoyle (1969) began studying the *Tritonia* swim response. Willows was the first to show that behavioral acts could be correlated with the activity of single neurons and that stimulation of single neurons could elicit behaviors (Willows and Hoyle, 1968; Willows, 1968). Over the course of several years, Willows, Peter Getting, and colleagues determined the basic components and synaptic connections of the *Tritonia* swim CPG (Taghert and Willows, 1978; Getting, 1976, 1977, 1981; Getting *et al.*, 1980) (Figure 1c). Getting created one of the earliest realistic simulations of a CPG using the components of the *Tritonia* swim circuit and demonstrated the feasibility that the known network components could produce the behavioral output (Getting, 1983, 1989b).

Later work added to the knowledge about the *Tritonia* swim CPG. The trigger and gating elements were identified (Frost and Katz, 1996; Frost *et al.*, 2001) and mechanisms of intrinsic neuromodulation were uncovered (Katz *et al.*, 1994; Katz and Frost, 1996; Sakurai and Katz, 2003). Thus, studying the functions of neurons in the *Tritonia* swim CPG as a dedicated circuit has proved to be very fruitful.

1.24.3.2 Multifunctional Neuronal Circuits

Although the early work was guided by the principle of determining the functions of neurons in dedicated circuits, the work in *Tritonia* was the first to uncover the idea that neurons could be multifunctional. The names given to the neurons that comprise the CPG and the motor system in general reflect the bias that these neurons were part of a dedicated circuit: dorsal swim interneuron (DSI) and ventral swim interneuron (VSI) (Figures 1b and 1c). Subsequently, however, it was suggested that the neurons might have roles in other behaviors such as defensive withdrawal. Thus, the concept of 'polymorphic' or multifunctional networks was originated (Getting and Dekin, 1985). It was later shown, for example, that the DSIs, which are part of the swim CPG and fire rhythmic bursts of action potentials during the swim motor pattern (Figure 1d), also play a role in accelerating crawling when the animal is not swimming (Popescu and Frost, 2002). It is now clear that neurons in other neuronal circuits also can serve many different functions and that neuronal circuits can be reconfigured to produce different behavioral variations (Lieske *et al.*, 2000; Marder and Calabrese, 1996; Meyrand *et al.*, 1994).

It has been suggested that the multifunctionality of neuronal circuits could aid the evolution of species-specific behaviors (Arbas *et al.*, 1991; Katz, 1991; Katz and Harris-Warrick, 1999; Tierney, 1995). If a single neural circuit can produce many outputs, then evolution of species-specific behaviors could arise by differential modulation of a generic circuit.

This has been hypothesized to occur in the feeding circuit of gastropods. It was suggested that homologous neurons form a circuit that is differentially modulated or controlled to produce either stereotyped biting or more variable bite patterns (Benjamin, 1983). In such a case, the same neurons would participate, but their roles would change. For example, there is a giant serotonergic cerebral cell in all gastropods that is involved in feeding, but this neuron has different roles in different species (Pentreath *et al.*, 1982; Rosen *et al.*, 1989; Yeoman *et al.*, 1994a, 1994b).

1.24.3.3 Comparing the *Melibe* and *Tritonia* Swim CPGs

Because the swimming behaviors of *Tritonia* and *Melibe* are so different, they could serve as a test of

the hypothesis that species-specific behaviors arise from the same neural network producing a different output. The architectures of the circuits reflect the fundamental difference between the swimming behaviors in the two animals. *Melibe* swims by alternately flexing from side to side, so the left and right CPG counterparts are mutually inhibitory, assuring alternation (Thompson and Watson, 2005) (Figures 1h and 1i). In *Tritonia*, the left and right sides are co-active, so each CPG neuron is electrically coupled to its contralateral counterpart (Getting, 1981) (Figures 1c and 1d).

The neurons that comprise the *Melibe* swim CPG are not homologous to the neurons in the *Tritonia* swim CPG. The *Melibe* CPG consists of two cell types: swim interneuron 1 (Si1) and swim interneuron 2 (Si2) (Thompson and Watson, 2005). Si2 is located in the pedal ganglion (Figure 1g). It interacts with its contralateral counterpart through one of the pedal–pedal connectives. Thus, if this connective is cut, the swim motor pattern is halted. In contrast, none of the *Tritonia* CPG neurons is found in the pedal ganglion (Figure 1b) and the swim motor pattern in this animal can continue with the pedal–pedal connectives cut. These observations suggest that the two behaviors, although roughly analogous because they involve whole body flexions, evolved independently and have a different neural basis. They do not share a neural circuit that is differentially modulated. Therefore, in this case, species-specific behaviors are not produced by differential modulation of a common neuronal circuit.

1.24.3.4 A Common Nervous System

Although the neurons that comprise the swim CPGs in *Tritonia* and *Melibe* are not homologous, there are homologous neurons in each nervous system. For example, homologues of the serotonergic DSIs in the *Tritonia* swim CPG have been identified in all opisthobranchs that have been examined so far, based on their soma location and serotonin immunoreactivity (Katz *et al.*, 2001) (Figure 1e). We recently identified DSI homologues in *Melibe* (Newcomb and Katz, 2003) (Figure 1j). Because *Melibe* does not swim with dorsal–ventral flexions, we could not name these neurons DSIs, so we chose an anatomical descriptor, the cerebral serotonergic posterior (CeSP-A) neurons. It is likely that homologues of all of the neurons in the *Tritonia* swim CPG will be found in *Melibe* and, conversely, Si1 and Si2 homologues will be found in *Tritonia*. These animals, by virtue of their common descent in the nudibranch lineage share a common nervous system design.

Despite the common neurons, are the nervous systems partitioned differently to produce different functional circuits? The CeSP-As resemble the DSIs in their physiological properties and synaptic connectivity; however, they are not rhythmically active during the *Melibe* swim motor pattern and are not members of the swim CPG in this animal (Newcomb and Katz, 2003). Thus, the same neurons have come to serve different functions and participate in different functional circuits.

Despite the differential partitioning of homologous neurons, there may be common functions that reflect the common heritage of these nervous systems. Although the CeSP-As are not rhythmically active during the *Melibe* swim motor pattern, spiking activity in a CeSP-As is sufficient to elicit a swim motor pattern in a quiescent preparation. Similarly, in *Tritonia*, spiking in a DSI is sufficient to elicit a swim motor pattern (Fickbohm and Katz, 2000; Katz *et al.*, 2004). Thus, the CeSP-As, although not part of the swim CPG in *Melibe*, are able to activate it, suggesting a conserved aspect of their function.

These observations suggest that different functional circuits were partitioned from a common nervous system to produce species-specific circuits that underlie species-specific swimming behaviors (Figure 2). The changes that underlie the differential partitioning could be as subtle as differences in the properties of neurons or strengths of synapses as has

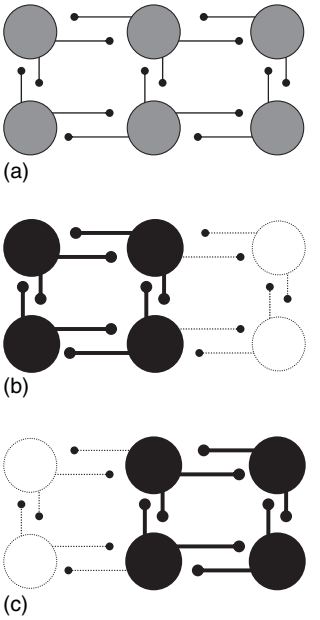

Figure 2 A polymorphic nervous system (a) can be differentially partitioned into different functional circuits (b and c) by changes in cellular and synaptic properties.

been observed in the multifunctional circuits of the crustacean stomatogastric ganglion (Combes *et al.*, 1999; Meyrand *et al.*, 1991, 1994).

1.24.4 Homoplasy and Parallel Evolution

1.24.4.1 Pleurobranchaea

Although the functional circuits for the swim CPGs in *Tritonia* and *Melibe* are different, the hypothesis that they were partitioned from a common nervous system can be tested by examining the neural basis of swimming in closely related species. This has been approached in only one animal that is closely related to the nudibranchs, *Pleurobranchaea californica* (Gillette and Jing, 2001; Jing and Gillette, 1999, 2000). *Pleurobranchaea* is in the order Notaspidea, which together with Nudibranchia, forms a monophyletic clade that has been called 'Nudipleura' (Waegele and Willan, 2000; Wollscheid-Lengeling *et al.*, 2001) (Figure 3). Like *Tritonia*, *Pleurobranchaea* swims with dorsal–ventral body flexions. The swim CPG in *Pleurobranchaea* contains homologues of the DSIs and cerebral neuron 2 (C2), as well other neurons not identified in *Tritonia*. Furthermore, the DSI homologues (called As1–3) and the C2 homologue (called A1) behave the same as they do in *Tritonia* (Jing and Gillette, 1999). Thus, the CPGs of these animals with similar behaviors are also similar.

The similarities in the behavior and the neural circuit underlying swimming in *Pleurobranchaea* and *Tritonia* led Jing and Gillette (1999) to hypothesize that dorsal–ventral (DV) swimming was the ancestral condition for nudibranchs. However, examining the rest of the nudibranch lineage reveals that DV swimming is rare and that some of the intermediary lineages lack this behavior (Figure 3). Thus, a more conservative hypothesis is that DV swimming in *Tritonia* and *Pleurobranchaea* arose independently (homoplasy).

1.24.4.2 Parallel Evolution

Homoplasy can result from convergent or parallel evolution (see The Evolution of Parallel Visual Pathways in the Brains of Primates). Convergent evolution is when nonhomologous structures come to have analogous functions. If the two behaviors arose independently, yet still share a common neural basis, then it would suggest that homologous neurons independently came to have the same functions in these animals: parallel evolution.

Parallel evolution is well recognized in genetics. For example, parallel changes in genes have been shown to play a role in the evolution of plumage patterns in birds (Mundy *et al.*, 2004) and loss of

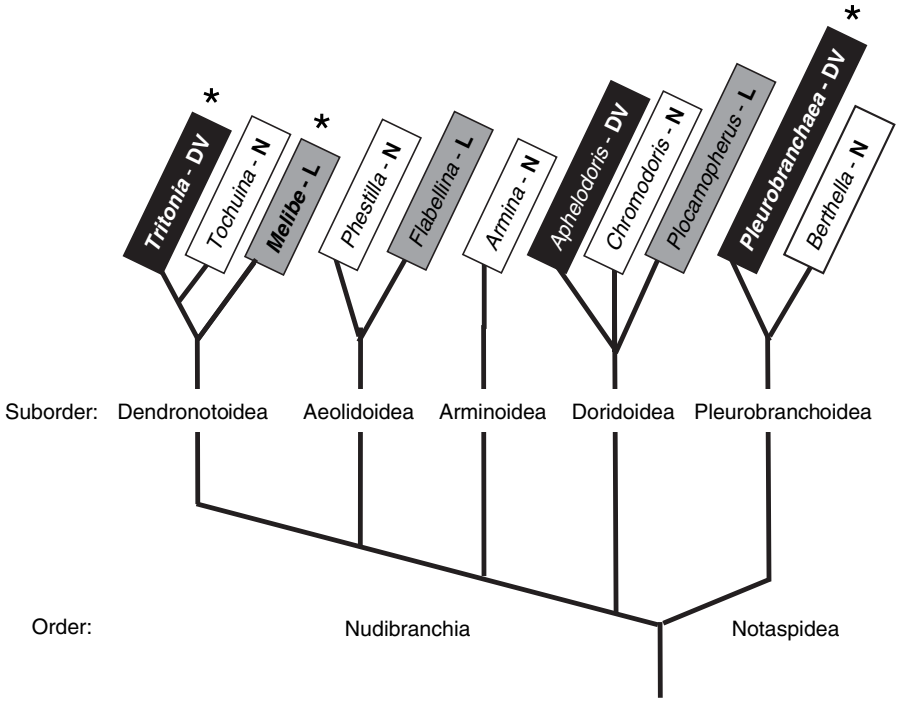

Figure 3 A dendrogram of the Nudipleura clade showing representative species with different modes of swimming. *Tritonia*, *Pleurobranchaea*, and *Aphelodoris* are dorsal–ventral (DV) swimmers. *Melibe*, *Flabellina*, and *Plocamopherus* are lateral swimmers (L). There are nonswimmers (N) in each of the lineages. The species discussed in this article are indicated by an asterisk (*).

armor in stickleback fish (Cresko *et al.*, 2004). However, there is less awareness of the potential for parallel evolution in the nervous system.

The implications of parallel evolution are very important. If the same components of the nervous system can come to serve the same function independently, it means that it is easier to evolve species-specific behaviors from phylogenetically polymorphic nervous systems than if the circuits had to be constructed *de novo* (see Primate Brain Evolution in Phylogenetic Context). There may be many stable configurations of multifunctional circuits that can be selected for, allowing an array of different species-specific behaviors to evolve from a generic network of neurons.

The importance of parallel evolution can be illustrated with the example of pair-bonding behavior in mammals. It has been shown that vole species that pair-bond have vasopressin receptors in the nucleus accumbens, whereas promiscuous vole species do not (Wang *et al.*, 1997; Young and Wang, 2004). The same pattern of receptor distribution occurs in pair-bonding species in other rodent lineages and in primate lineages (Young, 1999), suggesting independent evolution. This demonstrates that the same neural circuitry has come to have the same function in social interactions in disparate species through similar changes in receptor distribution. Thus, the evolution of pair bonding required just a small change in the promoter region of the vasopressin gene to cause species-specific behavior, yet the basic neural circuitry is shared with all mammals and is repeatedly reused for the same function in different lineages.

1.24.5 Conclusions

There is good evidence that neural circuits within an animal are multifunctional; a single circuit can produce different outputs and individual neurons can switch their functions at different times. This has led to the idea that evolution of species-specific behaviors can occur through differential modulation of a common circuit. However, examination of the *Tritonia* and *Melibe* swim CPGs shows that these circuits are not composed of homologous neurons. Thus, in this case, species-specific behaviors are performed by different sets of neurons. However, homologous neurons are present in both species. Therefore, we suggest that evolution of species-specific behaviors occurred through a differential partitioning of a polymorphic common nervous system (Figure 2). Furthermore, the neural circuits underlying swimming in *Tritonia* and *Pleurobranchaea* are remarkably similar despite the phylogenetic distance between these lineages. Rather than this being due to an incredible coincidence, it seems more likely that the same partitioning of the polymorphic network arose twice. Such parallel evolution has important implications for understanding the evolution of species-specific behaviors. To end this tale of two (or three) CPGs and to paraphrase Dickens poorly: it is a far, far better circuit that can evolve from a polymorphic network, than could arise alone.

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http://NeuronBank.org – NeuronBank: It is a knowledgebase of identified neurons and synaptic connections in 'Tritonia' and other repecies.

http://scilib.ucsd.edu – Science and Engineering Library at the University of San Diego: provides a full bibliography of nudibranchs.

http://www.seaslug.com – Seaslug.com: has many useful links for opisthobranchs.

http://seaslugforum.net – The Sea Slug Forum: has descriptions of sea slugs and observations of their behaviors.

1.25 Evolution of Visceral Control in Invertebrates

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Glossary

chemosensory	Neurons that respond to chemicals.
conditional burster	A neuron that requires the presence of a neuromodulatory substance in order to produce bursting activity.
fictive pattern	The pattern recorded in *in vitro* preparation that mimics the pattern recorded in the intact animal.
immunohistochemical	A general histological technique for staining a protein by making an antibody to it and then reacting that antibody with another one made from a different host that has a fluorescent marker attached to it.
intrinsic burster	A neuron that can produce bursting–spiking activity when completely isolated from other neurons.
ligand	A chemical substance that binds and activates a postsynaptic membrane.
myogenic	The ability of muscle tissue to contract without innervation.
neurogenic	Requiring input from neurons.
neuromodulator	A chemical that binds to specialized postsynaptic receptors and activates a second messenger system.
neuropile	The central part of an invertebrate ganglion, location of synaptic connections.
neurosecretory	Release of a chemical transmitter or modulator by a neuron into the blood.
pacemaker	A neuron that fires regular periodic bursts that can entrain other nerves or muscles.
plateau potentials	Membrane potentials that are maintained at a depolarized level.
soma	The cell body of a neuron containing the nucleus.
trophic	Supplying nutrient or growth factors to a cell.
voltage-gated channel	An ionic channel in the membrane that is sensitive to the transmembrane voltage.

1.25.1 Introduction

The evolution of visceral control in invertebrates is a specially useful topic because it not only helps illuminate the evolution of nervous systems in general, but also is particularly important to the field of comparative neurophysiology. This is because the neural circuits that have evolved to control the viscera are among the most fully described neural circuits available – and therefore of great heuristic value. These circuits are different from those investigated in the vertebrate central nervous system (CNS) because they describe detailed synaptic connectivity among identified neurons. From a neurophysiological viewpoint, it is likely that the general principles underlying the function of neural

circuits in mammals will be drawn from invertebrate studies. Given the small number of neurons involved in controlling invertebrate behavior including the viscera, this may seem somewhat surprising. However, it is reasonable because the basic elements or 'building blocks' (Getting, 1989) of small circuits – voltage- and ligand-gated ionic channels, chemical and electrical synapses, neuromodulatory environment, etc. – are also present in the brains of higher animals where the principal difference is the pattern and massive increase of synaptic connectivity. The unresolved question, that applies to comparative neurological studies and that bear on the question of evolution, is how exactly does the increased numerical and synaptic complexity that occurs during evolution give rise to behavioral events that cannot be explained by a simple scaling-up of circuits found in invertebrate nervous systems? Or put another way, does the massive increase in numbers of neurons give rise to special computational processes that are fundamentally different from invertebrate computational processes?

How have the basic functional components of the circuits controlling invertebrate viscera evolved? It is commonly assumed that selection acts on behavior and that alterations in cell or circuit properties that occur by random mutation and which act to enhance behavior, will become permanently incorporated into the genome. For example, consider a small change in a single membrane conductance, say a minor shift in the activation or inactivation curve for that conductance in a particular neuron. Or perhaps the strengthening of a synaptic connection that alters a behavior in an almost imperceptible way can over time become permanent if the behavioral change confers a selective advantage. However, these changes are rather obvious and the effects of evolution on the nervous system are in fact more complicated. Dumont and Robertson (1986) have suggested four fundamental factors that are involved:

- adaptive effects that optimize behavior,
- developmental constraints,
- historical influences, and
- architecture imposed by the design of the organism.

All of these will determine what the final evolutionary outcome will be and these factors are constantly changing.

One way to study evolutionary change is to compare homologous systems (and the behaviors they produce) between species. Generally we consider the changes in behavior to be overt and measurable. However, new cell properties or connection patterns may also be covert. For example, changes may occur that increase the stability or robustness of a circuit without directly affecting behavioral performance. In addition, some alterations may lead to an increase in behavioral flexibility in response to transient or permanent sensory perturbations. Other changes may provide the necessary connectivity for multifunctionality, allowing a single circuit to generate various stable spatiotemporal patterns when exposed to different neuromodulators. This ensures that entirely *de novo* circuits need not be constructed and only new neuromodulators can be made available.

Since the evolution of ion channel proteins, neurotransmitters, gap junctions, etc., have produced common phyla-spanning functional elements. I will briefly consider the evolution of those elemental factors that are used by all regions of the nervous system including visceral components. These include neuronal structure and function, voltage and chemically sensitive ion channels, gap junctions, neuromodulators and receptors, second messenger systems, and finally muscle and neuromuscular connections. I will illustrate how these independent elements have been incorporated into functional visceral mechanisms; in particular, how different circuits have been constructed to ensure reliability and flexibility for the viscera they control. Finally, I will discuss five highly specialized visceral control systems in their present form, which exemplify the evolutionary culmination of processes that have assembled the available components into reliable circuits. These pathways and species are indicated in red in the invertebrate cladogram (Figure 1), which also gives the reader some indication of the very large number of invertebrate classes for which virtually nothing is known in terms of visceral control mechanisms.

Note that the arthropod, annelid, and molluskan groups represent very divergent evolutionary relationships that nevertheless utilize remarkably similar cellular and biochemical mechanisms. In one, the stomatogastric system of crustaceans, which has led to detailed studies on a large number of species, it is possible to compare the relationships between structural and neural analogues involved in control mechanisms in some detail.

The main visceral functions of all animals are responsible for the physiological processes that sustain life – respiration, circulation, and digestion. Processes such as the control of heart pumping and digestive processing must work reliably and constantly throughout the life of the animal. The control of the heart rhythm in the leech and lobster and the control of feeding and digestion in the marine mollusk *Aplysia*, the snail, and the lobster have been examined in great detail. All of these have visceral control systems that use fundamental feedback

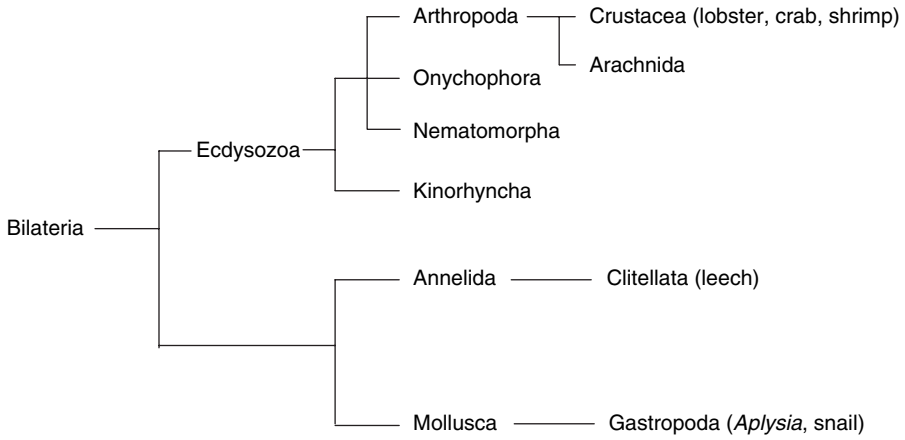


Figure 1 Summary of relationships between Bilateria summarized for the Tree of Life Web Project indicating one current scheme for tracing lineages.

principles to maintain homeostasis. Control over rhythm-generating neural networks is by local and descending inputs that integrate sensory information and issue the proper motor commands. Nervous systems involved in visceral control have to produce rhythmic patterns that are made up of similar design elements. These elements have evolved at very early stages of evolution but have been assembled much differently under a variety of selective environmental pressures (see Evolution of Color Vision and Visual Pigments in Invertebrates, Cognition in Invertebrates, Aggression in Invertebrates: The Emergence and Nature of Agonistic Behavioral Patterns).

1.25.2 Evolution of the Functional Components

A remarkable feature of the invertebrate nervous systems, which have been studied most intensively over the last 50 years or so, has been the recognition that all use the same general 'building blocks', a term suggested by Getting (1989). This term meant that all of the components – ion channels, synaptic mechanisms, and basic patterns of connectivity, are found in all of the nervous systems that have been described thus far, but through evolutionary processes have become arranged differently so as to meet the particular demands of each animal. If so many diverse metazoa, from coelenterates to arthropods, use the same basic components, then these elements must have evolved in their basic form prior to their appearance in functional circuits. It is clear that the similarity between particular channels, for example, spans phyla and although they all could have evolved in parallel to meet the same needs, it is probable that they at least started with the same basic protein structure.

1.25.2.1 Neuron Structure and Physiology – Identified Neurons

Neurons that are contained within visceral neuronal circuits are typical of invertebrate neurons found throughout all animal phyla. Each neuron can be organized into soma, dendritic, and axonal compartments that are electrically contiguous (Figure 2). Neuronal somata in invertebrates can be quite large. In some molluska, they can be close to 1 mm in diameter and be observed with the naked eye. Because their numbers are considerably fewer than the numbers estimated to be present in the 'higher' nervous systems of vertebrates, it has been suggested that they may perform more complicated functions. However, this does not appear to be the case. In general, invertebrate neurons are almost as computationally complicated as their vertebrate counterparts, and their large size probably has a different explanation. The cell somata are usually heavily wrapped in glia and free of synaptic contacts. Their role appears to be largely trophic and from an integrative viewpoint they act mainly as an electrical sink. A large neurite connects the soma to a heavily branched neuritic tree that contains both pre- and postsynaptic terminals and where connections to other neurons are made. Depending on the electrical properties of the principal neurite connecting soma to neuritic tree, some of the subthreshold currents generated in minor processes can be observed in the soma. More importantly, however, these currents will be summed at the spike initiation zone and propagated down the neuron's axon to an effector organ or to a neuron at some distant location. Many invertebrate neurons have been shown to have local circuit interactions with synaptic transmission occurring without spikes, that is, with transmitters being released as a function of

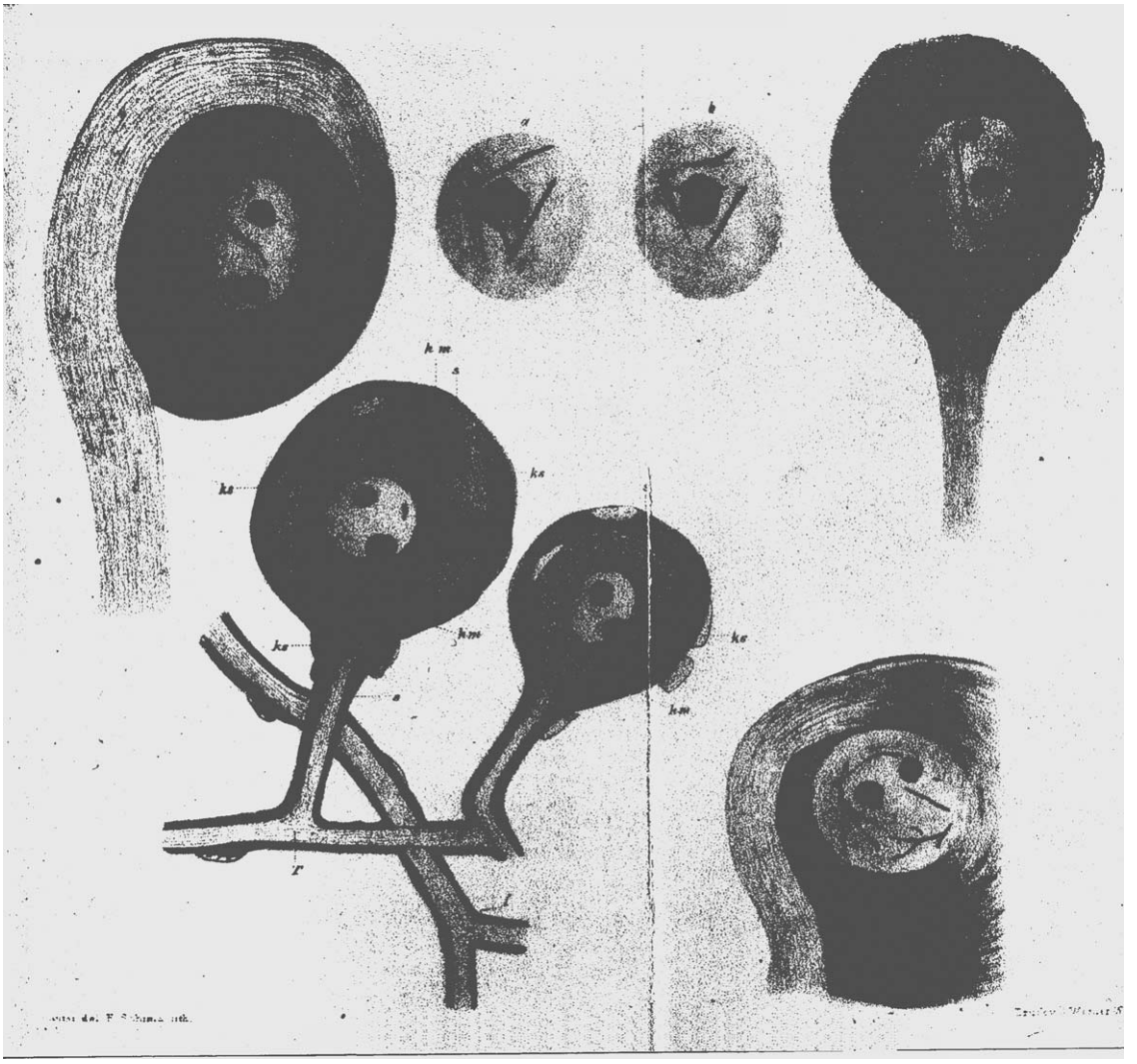


Figure 2 Drawings of visceral neurons from the STG of crayfish by Sigmund Freud illustrating cell somata and primary neurites.

presynaptic depolarization (Roberts and Bush, 1981). Starting from the primitive condition in which single neurons had both sensory and motor functions (Parker, 1919), neurons have evolved in both functional and structural complexity. Neurons have been deleted by degenerative mechanisms as a result of a missing target and their trophic influence suggests that trophic factors played a major role in keeping neurons intact over time. Changes in neuronal shape or function were likely due to evolutionary changes in gene expression (Arbas *et al.*, 1991). Segmental homologues were likely influenced by segmentation genes that tailored cell functions to organs that differed from one segment to another. The study of segmental homologues (Mittenthal and Wine, 1978; Shafer and Calabrese, 1981) has been specially useful in suggesting the range of transformations that might have occurred over the evolution of a particular species.

Of all of the criteria that confer identifiability to invertebrate neurons, the three-dimensional arrangement of neuronal branches is the least consistent (Glasser *et al.*, 1977). Nevertheless, the function of identified neurons is constant from animal to animal, which suggests that mechanisms exist that balance channel and other biophysical properties so as to insure functional consistency. In addition, mechanisms must exist to insure constant function despite membrane protein turnover and changes resulting from synaptic plasticity. These mechanisms have only recently begun to be explored.

1.25.2.2 Voltage-Gated Ion Channels

Many of the same ionic channels present in neurons today are found in protists, life forms that began

their evolutionary history millions of years ago. The simplest of these are the mechanosensitive channels found in bacteria and eukaryotes. Their simplicity lies in the fact that they open and close in response to pressure and do not require voltage sensors or ligand-binding sites. Among the K, Na, and Ca channels, K appears to be the oldest. The K channel is made up of a tetramer of identical subunits while Na and Ca channels are made up of four K-like domains. The evolution of these channels therefore probably began with a K channel structure but Na channels have recently been cloned from a jellyfish, the most primitive organism with nerves.

Which voltage-gated channels were the first to evolve, K, Cl, or Ca? One might suspect Ca because of its role as a second messenger. Studies on unicellular organisms however suggest K channels that are stretch-activated in bacteria and yeast came first. There are seven channels found in paramecia alone including a Ca-activated K channel as well as a voltage-activated Ca channel similar to those found in mollusks, annelids, and chordates. No Na channels have been found in unicellular animals and apparently did not arise until the metazoa. The Na channel genes probably diverged early, before the branching off of arthropods and vertebrates. The K channels became the most diverse functionally and could be gated by depolarization, hyperpolarization, and divalent cations as well as being modulated by second messengers.

As nervous systems evolved to control the viscera, a large palette of channels was already available to control the intrinsic properties of individual neurons and the properties of their synaptic connections. That is why the different channel types are ubiquitous in different phyla and in different specific circuits. Their expression probably became fixed as selective pressures gave advantage to behaviors that had circuits with the best combination of conductances to ensure the success of each animal.

A fundamental concept is that for voltage-gated ion channels, it is the membrane voltage that affects the performance of a neuron in a circuit. One can trace the molecular evolution of channel proteins in a systematic way, but it is the effect of the different channels or combination of channels on neuronal behavior that is selected for. It is therefore likely that the expression of the proteins making up the net conductance characteristics of each identifiable neuron be highly specified and controlled by the genome. For the rhythmicity observed in invertebrate viscera, neuronal properties are quite similar to those found in other parts of the invertebrate nervous system and vertebrate nervous system as well. Certainly inward Na and Ca currents were necessary for depolarization and outward K currents necessary for repolarization of the membrane. Additional currents that could fine-tune neuronal membrane electrical activity could add to the effectiveness of each neuron as a component in a system of neurons. For example, the $I_{K–Ca}$ channel will increase membrane repolarization and add to the effects of the delayed rectifier action of I_K. If it was advantageous to have a neuron repolarize even faster, then I_H would prove effective. If it was necessary to delay the onset of firing, then the transient I_A current would have a selective advantage. All of these currents have been described for neurons in visceral nervous systems and have started to be employed at the molecular level to study the role of each channel in circuit function (Baro *et al.*, 1997).

1.25.2.3 Neuromodulators and Receptors

The large role of neuromodulation in determining behavior has become increasingly apparent with the development of techniques to locate putative neuromodulatory substances within visceral nervous systems. In particular, the ability to make antibodies against particular peptides, amines, etc., and their subsequent amplification and visualization by immunohistochemistry has demonstrated a large array of neuromodulators for even small ganglia. The anatomical localization can suggest experiments with agonists and antagonists of these substances when applied directly to the nervous tissue or when released by stimulation of the appropriate nerves to further characterize the physiological role these substances might have. The effects, which can be observed in *in vitro* experiments, in many cases appear to be more numerous than the particular visceral structure actually requires. For example, the pyloric system of the crustacean stomatogastric system has over 30 neuromodulatory substances associated with it despite the fact that its function is limited to a rather straightforward pumping and filtering action. Because the synaptic connectivity has been conserved during evolution, it has been suggested that neuromodulators had a greater effect on producing variability of motor programs than has change to the neural circuits (Katz and Tazaki, 1992).

1.25.2.4 Muscle and Neuromuscular Factors

The control of invertebrate viscera is mainly comprised of intrinsic and extrinsic neural mechanisms

along with neuromodulatory agents acting either as hormones or local transmitters. The visceral organs we consider are those involved in alimentation and circulation. Both utilize various muscle cell types that evolved from specialized cells containing contractile proteins and strongly resembling the myocytes found in today's sponges. These cells were not innervated and each acted independently under local contact, stretch, or chemical control and would not be considered true muscle by most. The first true muscle probably evolved independently in ancestors of today's coelenterates and whose contractile fibers were arranged in parallel to form longitudinal muscle or at right angles to form circular muscle. The two could act antagonistically to cause shape changes or bending so that if coordinated could cause pumping or peristaltic gut movements (Figure 3). This type of musculature has been retained by most soft-bodied animals including Colenterata, Platyhelminthes, Annelida, and Mollusca.

We can consider, as a starting point for all muscles, a soft-bodied tubular animal similar to present-day *Hydra* with a pair of antagonistic sheets of fine nonstriated contractile cells. Over time we can postulate that these were arranged into locally organized clusters with intrinsic activity and superimposed reflex actions mediated by local neurons. One can also suggest that they could function cooperatively because of the development of connections between the local neurons and through-conducting giant neurons innervating them collectively. The local connections eventually formed a nerve net but their connections with axons of larger and larger diameter permitted the synchronous activity of the whole when necessary. The gut has today retained many of these early features as did other organs that evolved out of the body tube by being pinched off when necessary to provide other functions such as storage, churning, secretion, and transport of blood or other body fluids. Each has its own local nervous system in the form of a nerve net, a plexus, or a ganglion (or some combination thereof).

The gut muscle cells of invertebrates other than arthropods are nonstriated, resemble vertebrate smooth muscle, and are assembled in a similar manner (Hoyle, 1983). Arthropod gut muscle, however, is striated although it has some properties of vertebrate smooth muscle as well. In insects there appears to be a direct innervation of gut musculature, so if there is a peripheral innervation as well, this suggests multiple innervation of individual muscle cells (Cook and Holman, 1979). The anterior parts of the guts, crops, gizzards, etc. are innervated by a medial nerve that is connected to a frontal ganglion that is

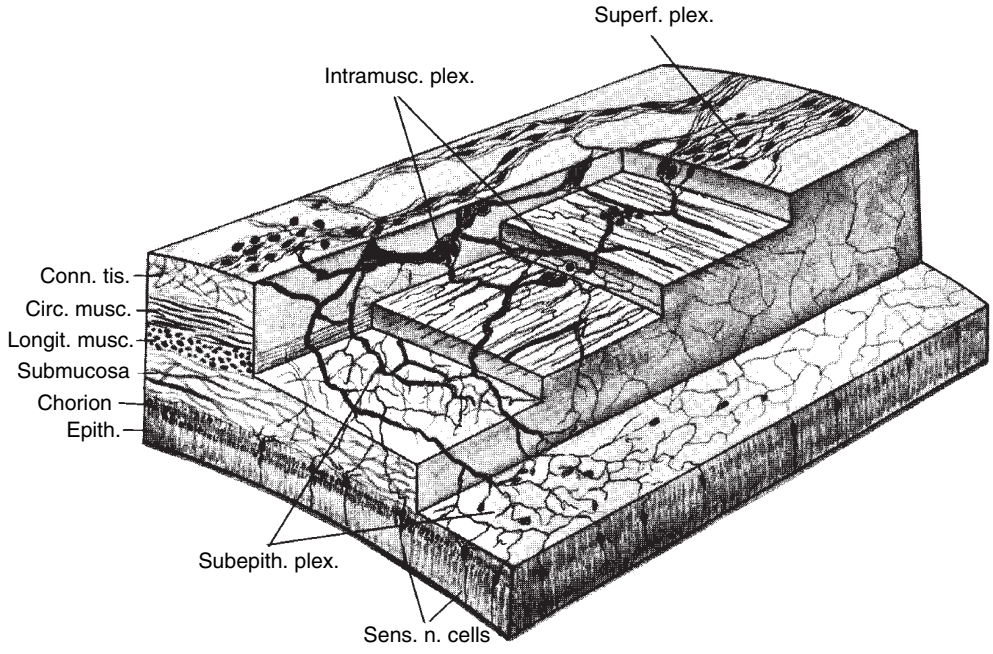


Figure 3 Localized visceral control exemplified by this semischematic drawing of the arrangement of nerves and muscles in the wall of the intestine of *Sepia* (Mollusca) (Alexandrowicz, 1928). Intramusc. plex., intramuscular plexus; Superf. plex., superficial plexus; Conn. tis., connective tissue; Circ. musc., circular muscle; Longit. musc., longitudinal muscle; Epith., epithelium; Subepith. plex., subepithelial plexus; Sens. n. cells, sensory nerve cells.

at the junction of two nerves from the forebrain. In crustacea, a small stomatogastric ganglion (STG) forms along the medial nerve that supplies patterned input to the striated muscles of the stomach. Gut physiology in phyla other than arthropod varies enormously. All show a basic tonus and for some this is all that is observed. In many others one can observe peristaltic and longitudinal slow waves that are in general somewhat slower than their vertebrate counterparts. Prosser *et al.* (1965) described gut activity in a variety of invertebrates and found that actional potentials occurred spontaneously in the esophagus at a mean frequency of ~1 Hz. However, these were highly erratic and asynchronous, and fluctuating tension measurements could not be correlated with the electrical activity. Occasionally, waves, spikes, and tension become synchronized, which indicates that there is an overall control mechanism that can superimpose itself on local activity. Blocking the intrinsic nerves with a local anesthetic led to a tendency toward synchronization of spikes and waves, suggesting that it was local neural inhibition that led to the asynchrony.

In all of the invertebrate guts that have been investigated, a local stimulus causes a contraction that is conducted decrementally away from the point where it is elicited and is reduced significantly after only a few centimeters of conduction. The range of velocities of propagation is narrow, from 4 to 6 cm s^{-1} for *Thyone* to 12 cm s^{-1} for *Loligo* (Prosser *et al.*, 1965). Procaine blocks conduction in all animals tested except for the echinoderm *Thyone*, which suggests that, like vertebrates, conduction is along muscle cells. Since chordates are closely related to echinoderms, intestinal cell-to-cell conductance is characteristic of the echinoderm–chordate line and phylogenetically very old.

1.25.2.5 Generation of Functional Patterns by Defined Circuits

In most primitive invertebrates, neurons that participate in controlling rhythmic visceral activity are arranged in diffuse nerve networks in which neurons are interconnected close to their effector organs and in a position to respond quickly to sensory stimuli (Figure 4). But a phylogenically early step was the clustering of neurons into ganglia that controlled segmental functions or visceral organs (Figures 5 and 6). Generally, but not always, sensory neurons remained peripherally located while ganglia contained interneurons and motorneurons. All invertebrate phyla except for Cnidaria and Ctenophora (Field *et al.*, 1988) have developed

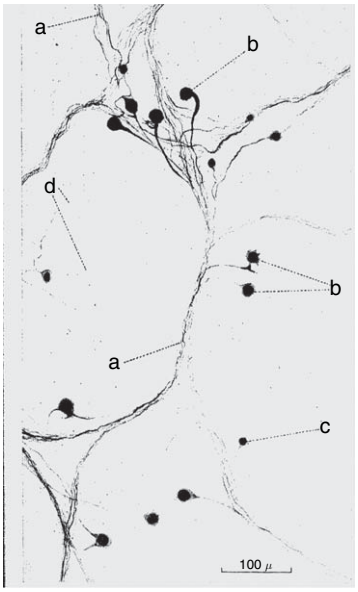


Figure 4 A peripheral nerve plexus in the wall of the posterior stomach of the snail *Helix*. a, nerves; b, cell bodies; c, small cells; d, plexus. Modified from Abraham, A. 1940. Die innervation des darmkanals der gastropoden. *Z. Zellforsch.* 30, 273–296.

formal ganglionic structures, that is, condensations of neurons and their processes into discrete structures (ganglia) linked by axon tracts (connectives) (Leise, 1991). Individual neuron cell bodies, often identifiable, surround a dense neuropile core made up of the neurites of each cell (Figure 7). Synaptic connections are made within the neuropile, often on small varicosities distributed along on the fine neurite processes (King, 1976).

The control of visceral structures became concentrated within these distributed ganglia and because they represented individual local circuits that could be studied independently of the rest of the CNS, they represent the best-described neural circuits in existence. The architecture of each ganglion varies considerably from species to species. The neuropiles of some ganglia have distinct morphological boundaries for separate sensory and motor control purposes (Altman, 1981). Where the neuropile has been investigated more fully, for example, in the case of the STG, some of the motor neuron arborizations do appear to be compartmentalized (Christie *et al.*, 1997).

The condensation of neural functions into larger and larger ganglia appeared to be an evolutionary trend in invertebrates for good reason. Advanced arthropods and mollusks evolved massive brains but seem to have done so independently (Bullock and Horridge, 1965). The condensation of neurons would of course have a significant effect on

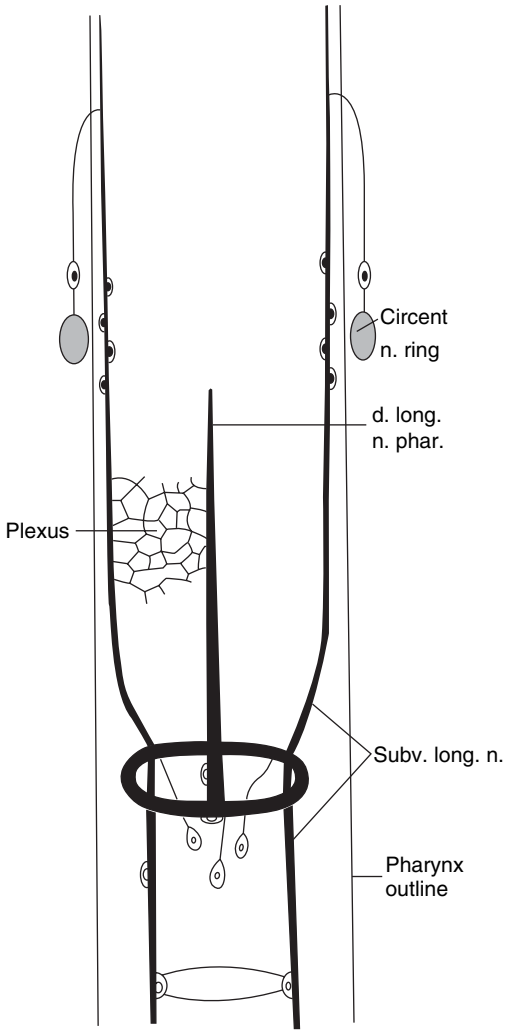


Figure 5 Schematic representation of the visceral nervous system of the nematode. Note the start of clustering of neurons and nerve fibers in addition to the pharyngeal plexus. (Goldschmidt, 1910, unpublished). n., nerve; d. long. n. phar., dorsal longitudinal pharyngeal nerve; Subv. long. n., subventral longitudinal nerve.

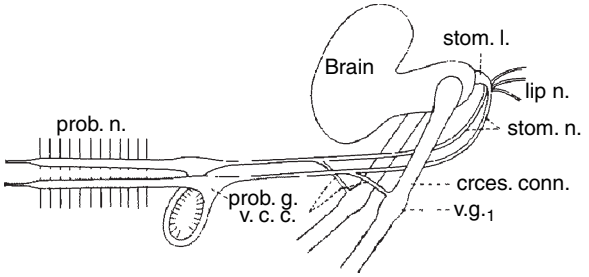


Figure 6 Diagram of stomodeal system of *Amphinomidae* (polychaete). Note the increase in the development of ganglia and connective fibers linking the stomodeum to the brain (Gustafson, 1930). crces. conn., circumesophageal connectives; prob. n., proboscis nerves; prob. g., proboscis ganglion; stom. l., stomodeal lobe; stom. n., stomodeal nerve; v.g.$_1$, first ventral cord ganglion; lip n., lip nerve; v.c.c., ventral cord commissure.

the arrangements of commissures, tracts, and neuropile and would have positive effects on reduced conduction times, elimination of relay interneurons and the increased availability of sensory input to individual neurons and networks (Altman and Kien, 1987).

However, it was quite common for abdominal visceral ganglia to remain independent of the condensation of the rest of the nervous system but nevertheless incorporating neurons for the control of more than one visceral organ. Why this distributed processing remained a viable method for organizing the abdominal nervous system probably had more to do with achieving redundancy and therefore reliability than integrating sensory and motor functions. Interestingly, even in mammals, the autononomic nervous system, a system largely devoted to the control of the viscera, evolved as individual discreet ganglia.

1.25.3 Examples of Visceral Control Systems

1.25.3.1 Crustacean Cardiac Ganglion

One of the simplest invertebrate systems for the control of a particular visceral organ is the nine-cell lobster cardiac ganglion. A muscular heart aspirates blood from the thoracic cavity using passive elastic forces and pumps it out via blood vessels to various parts of the body with neurogenic heart contractions. The heart muscle itself has lost all intrinsic burst activity and therefore requires synchronized bursts of neural input in order to produce its synchronized rhythmic contractions.

Because the cardiac ganglion has proved to be a robust preparation for the study of small neural networks, particularly how endogenous bursting can be produced in nerve cells, it has been examined in many species. Anatomically, the nine neurons that make up the lobster cardiac ganglion contain five large neurons (54–84 μm) located anteriorly and a (22–43 μm) group of four smaller posteriorly located neurons (Figure 8). In the spiny lobster *Panulirus*, all of the neurons are arranged linearly, while in the clawed lobster *Homarus*, the posterior part of the ganglion bifurcates and two small neurons are found in each branch (Hartline, 1967). In crabs, two of the large cells are clustered with the small cells at the posterior end of the ganglion. The meaning of the different clustering is unknown and why this particular cell arrangement has evolved differently is not known. More interesting from an evolutionary perspective is

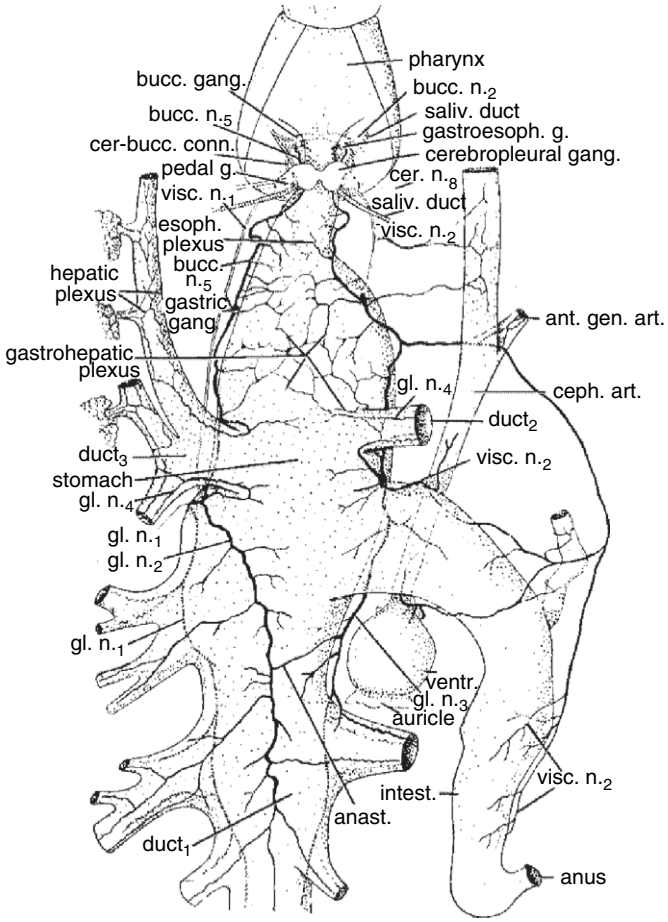


Figure 7 Stomatogastric nervous system of *Acolidia papillosa* (Opisthobranchia, Nudibranchia). Important features are: anast., anastomasis; ant. gen. art., anterior genital artery; bucc. n., buccal nerve; bucc. gang., buccal ganglion; ceph. art., cephalic artery; cer. n., cerebral nerve; cer-bucc. conn., cerebro-buccal connective; gastroesoph. g., gastroesophageal ganglion; gl. n., gland nerve; intest., intestine; saliv. duct., salivary duct; ventr. gl. n., ventral gland nerve; visc. n., visceral nerve. Russell, L. 1929. The comparative morphology of the elysioid and acolidioid types of the molluscan nervous system and its bearing on the relationships of the asoglossan nudibranchs. *Proc. Zool. Soc. Lond.* 1, 197–233.

why all species have nine cells and why some are larger than others.

The burst-generating mechanism appears to reside within the small cells since cutting or blocking results in continued small cell bursting but termination of bursts in the large cells (Mayeri, 1973; Cooke and Tazaki, 1979). Trying to unravel the synaptic connectivity of this small system was hampered for many years by the size of the small cells. Early recordings from large cells suggested that their bursts were triggered by the small cells. It is now known that the small cells produce a slow driver potential endogenously that leads to a burst of spikes. These spikes then activate the large cells synaptically to fire a burst. In Panulirus, it has been found that all small cells synaptically drive all large cells (Friesen, 1975). Because all of the cells are connected to each other electrically, all of the cells, small and large, fire synchronously (Figure 9). Cell 6 elicits a particularly powerful and rapidly antifacilitating EPSP onto cells 1–4, accounting for the abrupt onset of their burst depolarization. The EPSPs from cells 7–9 are smaller but antifacilitate less and often commence prior to the motor burst onset (Friesen, 1975). The synaptic connections between the small cells has been very hard to measure because of their small size, but it has been shown that cell 6 could be correlated with firing of cells 7–9 and of excitation of cell 7 by cell 8 and of cell 8 by cell 9 in a completely feed-forward manner (Friesen, 1975). The key feature in this system is the synchronization of the intrinsically bursting small cells by their electrical and chemical synapses to produce periodic bursts of activity to drive the heart muscle. Such circuits

are extremely robust to perturbations and reliable for the life of the animal. This form of pacemaker evolved to specialized tissue in the hearts of higher animals as exemplified by the sino-atrial node of the mammalian heart. Similarly, other rhythms that consist of periodic discharges with only a single phase per cycle can be generated by neural connectivity with this degree connectivity. For example, each hemisegment of the lamprey is thought to contain a central pattern generator (CPG) in which an indeterminate number of interneurons are synaptically connected with fast and

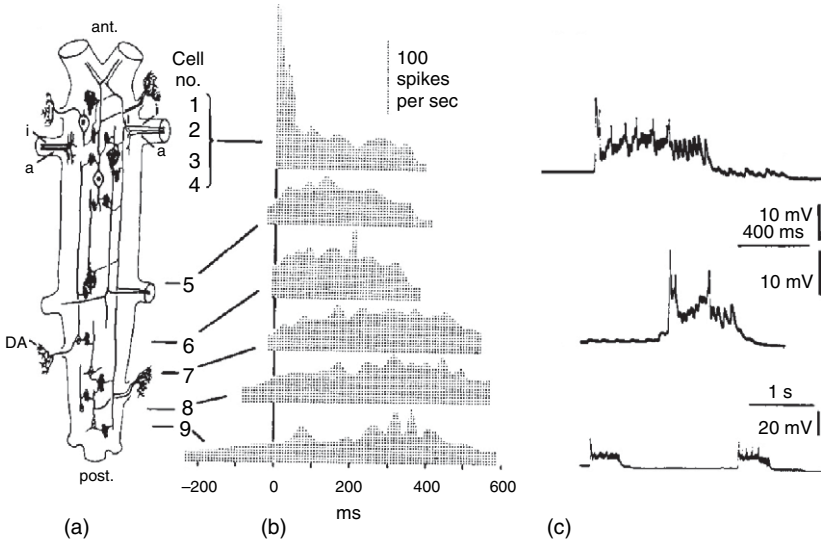


Figure 8 A schematic diagram of the structure of the cardiac ganglion and it's bursting activity in *Panulirus*. a, Location and branching pattern of large (1–5) and small (6–9) cells. Also shown are the entry of the accelerator (a) nerves and inhibitor nerves (also see Figure 10). DA represents a dendritic arborization inside and outside the ganglionic trunk. b, Activation sequence and spike frequency progression of the intrinsic neurons during their burst. c, Intracellular recordings from large cell 1 or 2 at different timescales. a, Modified from Maynard, D. M. 1961. Cardiac inhibition in decapod crustacea. In: Nervous Inhibition (*ed.* E. Florey), pp. 144–178. Pergamon. b, Data from Friesen, W. O. 1975. Synaptic interactions in the cardiac ganglion of the spiny lobster *Panulirus interruptus. J. Comp. Physiol.* 101, 191–205, as cited in Wiens, T. J. 1982. Small systems of neurons: Control of rhythmic and reflex activities. In: The Biology of Crustacea (*eds.* H. Atwood and D. Sandeman), Vol. 4, pp. 193–240. Academic Press. c, Adapted from Bullock, T. H. and Terzuolo, C. A. 1957. Diverse forms of activity in the somata of spontaneous and integrating ganglion cells. *J. Physiol.* (*London*) 138, 341–364. Adapted from Wiens, T. J. 1982. Small systems of neurons: Control of rhythmic and reflex activities. In: The Biology of Crustacea (*eds.* H. Atwood and D. Sandeman), Vol. 4, pp. 193–240. Academic Press.

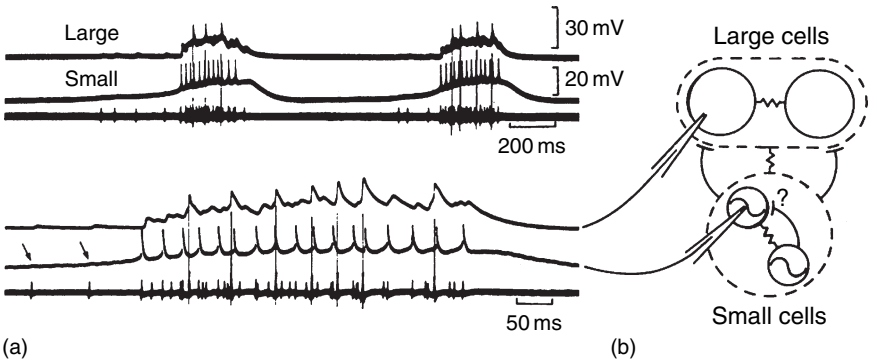


Figure 9 Relation of firing patterns between large and small cells in *Portunus sanguinolentus.* a, Intracellular recordings and extracellular activity, bottom trace at two timescales. b, Possible synaptic organization of the cardiac ganglion in lobsters and crabs. a, Adapted from Tazaki, K. and Cooke, I. 1979. Spontaneous electrical activity and interaction of large and small cells in cardiac ganglion of the crab *Portunus sanguinolentus. J. Neurophysiol.* 42, 1000–1021b, Adapted from Wiens, T. J. 1982. Small systems of neurons: Control of rhythmic and reflex activities. In: The Biology of Crustacea (*eds.* H. Atwood and D. Sandeman), Vol. 4, pp. 193–240. Academic Press.

slow excitatory synapses that synchronize the bursting for each hemisegment (Cangiano and Grillner, 2005).

When the large cells and small cells are considered together, the data suggest the entire network is more that feed-forward. When the large cells are depolarized by injected current, the succeeding small cell burst is accelerated and hyperpolarization has the opposite effect (Watanabe and Bullock, 1960; Tazaki, 1971). This suggests that electrotonic connections between the small and large cells, usually thought of as a way to provide synchronization between neurons, have a more complicated function. Similarly, antidromic spike trains generated in the large cells can elicit spikes or hasten bursts in the small cells (Mayeri, 1973) proving that the large cells also have a feedback onto the small cells.

The mechanism for producing rhythmicity in the cardiac ganglion system illustrates one of the several ways in which bursting behavior has evolved in neural networks that control the viscera. In most rhythm-generating networks, there is almost always one or more neurons capable of generating bursting pacemaker potentials. This type of neuron incorporates a panel of conductances that can generate slow oscillatory potentials entirely autonomously. That such neurons exist was demonstrated conclusively in molluskan neurons by physically isolating them from an *Aplysia* ganglion (Alving, 1968). While these neurons burst in isolation in a saline-containing Petridish, similarly isolated neurons do not burst unless exposed to some modulatory substance such as an amine or peptide. Neurons in this category are termed conditional bursters, that is, conditional upon the presence of a pharmacological agent. This type of bursting cell is common to many phyla and has been studied rigorously in the STG, where both the cellular and functional aspects of the phenomena have been described in detail (Harris-Warrick *et al.*, 1992).

1.25.3.1.1 Bursting pacemaker potentials The ion channels responsible for generating bursting pacemaker potentials had probably already evolved by the time metazoa required neuronally generated rhythmic activity and it was likely that different combinations of channels were employed initially to achieve this goal. Basically there had to be a self-generating mechanism for depolarizing the neuron, a mechanism for the generation of spikes at maximal levels of depolarization although in some cases the neurons could act without spiking, and a third mechanism for terminating the depolarization. These currents have been studied successfully in mollusca using the voltage clamp method due to the fact that the pacemaker potentials are generated near the soma which is itself electrogenic. Between bursts, there is a high resting electrical conductivity corresponding to Na ions, (G_{Na}), which is persistent and voltage independent (Smith *et al.*, 1975; Carpenter and Gunn, 1970). This current gradually depolarizes the membrane as K currents from the preceding burst recede. This slow depolarization activates a persistent voltage-dependent slow G_{Na} and in some cases a parallel Ca current that initiates the burst. The burst is terminated and the membrane is repolarized by activation of a slow G_K and a G_{K-Ca} (Thompson, 1977). During the depolarizing phase, spikes are produced by fast inward Na/Ca currents and delayed rectifier K currents. A third K current was also described that was activated by hyperpolarization and helps control the duration of the interburst interval (Neher, 1971).

Similar mechanisms for bursting in lobster pyloric neurons have also been described. Unlike some of the truly endogenous molluskan bursting pacemaker neurons, the pyloric neurons are conditionally bursting neurons, which must be activated by neuromodulators from 'higher' ganglia. The ionic mechanisms seem to be approximately the same, except that the initial slow pacemaker potential is the result of a decrease in the resting leak G_K (Gola and Selverston, 1981). Pyloric neurons also have a pronounced plateau potential when given a short depolarizing pulse and this potential appears to determine the overall burst length. Finally, bursting pyloric neurons have varying amounts of an I_H that helps control the speed with which the bursting neuron repolarizes and can therefore determine at what phase in the cycle the neuron will fire its burst.

1.25.3.1.2 Modulation of heart rhythmicity There are several mechanisms used to modulate the heart's rhythmicity. Intrinsic reflexes are present that can accelerate and strengthen the heartbeat when the cardiac ganglion is stretched (Maynard, 1961). There also appear to be dendritic processes of some neurons embedded directly in the heart muscle that likely serve a sensory function (Alexandrowitz, 1932). Neuromodulators from the pericardial organ, a neurosecretory structure near the heart, were among the first chemical control substances studied that had distinct effects on the viscera. These modulators, thought to be mainly peptides and amines (Cooke, 1966), when extracted from the pericardial organ and applied topically to the proximal axonal regions of the small cells generally increased burst frequency (Cooke and Hartline, 1975). In the blue

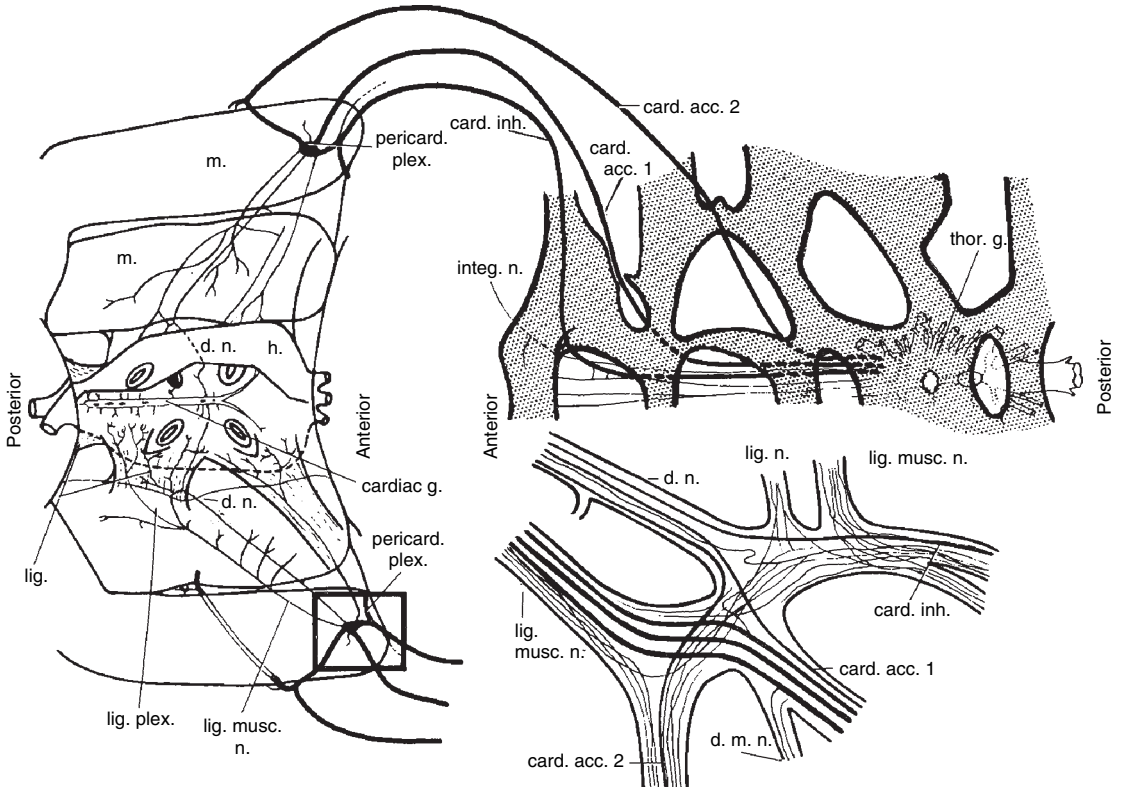


Figure 10 Cardiac nerves of the lobster *Panulirus argus*. Important nerves are card. acc., cardiac accelerator; and card. inh., cardiac inhibitor. Maynard, D. M. 1953. Activity in a crustacean ganglion. I: Cardioinhibition and acceleration in *Panulirus argus*. *Biol. Bull.* (*Woods Hole*) 104, 156–170.

crab *Callinectes sapidus*, a neuron in the commissural ganglion (CG; L-neuron) that was shown to contain dopamine by immunohistochemical staining for its synthetic enzyme tyrosine hydroxolase, innervates the pericardial organ and the cardiac ganglion (Fort *et al.*, 2004). The cardiac ganglion also receives direct innervation from the CNS (Figure 10) that controls its activity in tandem with the neurohormonal innervation (Delgado *et al.*, 2000; Field and Larimer, 1975; Maynard, 1960.)

1.25.3.2 Leech Heart

A key characteristic of annelids is their segmental nature, which in some cases is reflected in the morphology of their viscera and the innervation of these structures. In the gut, neurons innervating the musculature are embedded in the gut wall (Figures 11 and 12). However, the leech heart is also controlled by patterned neural input from CPGs in the segmental ganglia. One of the most well-examined annelid neural systems is the heart CPG network in the leech (Calabrese and Peterson, 1983). The hearts consist of long laterally placed muscular tubes extending the length of the animal. The contractions are in

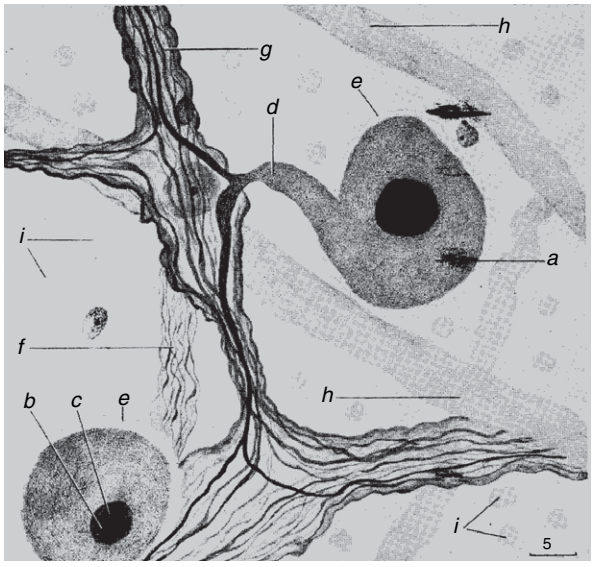


Figure 11 Neurons in the wall of the gut in the leech. a, soma; b, golgi; c, nucleus; d, neck; e, cap; f, neurites; g, axons; h, stroma; i, blood cells. Reproduced from *Z. Zellforsch.*, Vol. 47, 1958, pp. 367–391, Uber die innervation des darmkannales des medizinischen blutgels (*Hirudo medicinalis*), Abraham, A. and Minker, E. With kind permission of Springer Science and Business Media.

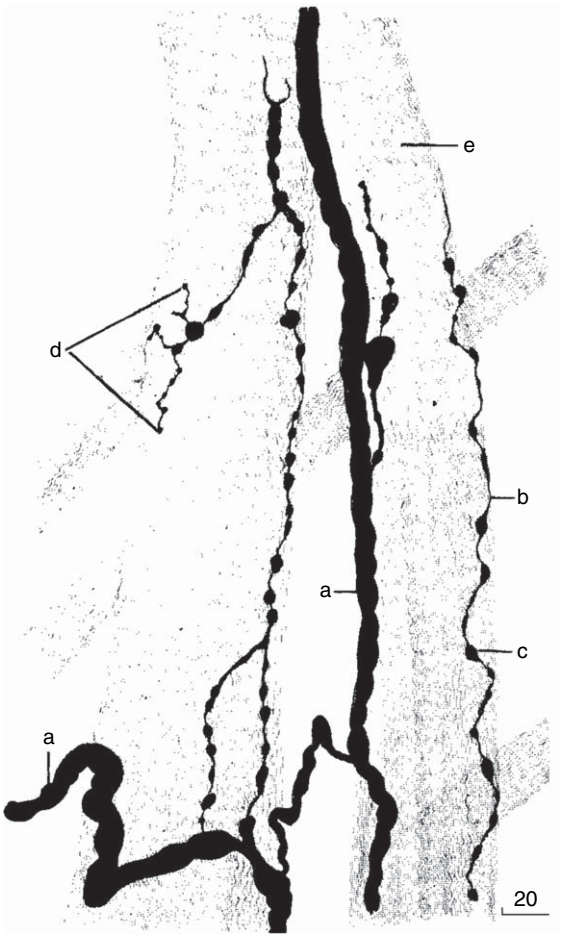


Figure 12 Free nerve endings on midgut muscle of leech *Hirudo* showing innervation of muscle fibers (Abraham and Minker, 1958). a, axons; b, plexus; c, ganglion; d, terminals; e, wall. Reproduced from Abraham, A. and Minker, E. 1958. Uber die innervation des darmkannales des medizinischen blutgels (Hirudo medicinalis). *Z. Zellforsch.* 47, 367–391.

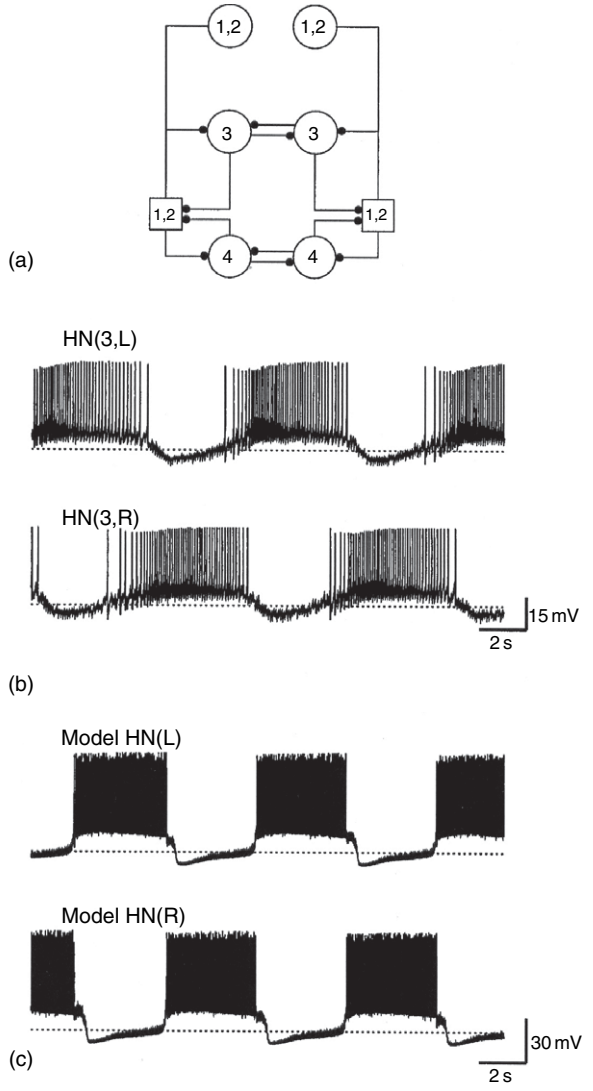


Figure 13 a, Circuit diagram showing inhibitory connections among heart (HN) interneurons that produce pacemaker activity during leech fictive heartbeat activity. Cells are indexed by ganglion and body side. The HN cells of the third and fourth segmental ganglia make reciprocal inhibitory connections across the ganglion midline with each pair constituting an independent neural oscillator. The oscillations are coordinated through their connections with the HN(1) and HN(2) interneurons, which are lumped together here because they are functionally equivalent. b, Typical alternate bursting activity of a pair of oscillator interneurons in an isolated ganglion. c, A model of the same two neurons displaying similar activity. Modified from Calabrese, R. L. and Feldman, J. L. 1997. Intrinsic membrane properties and synaptic mechanisms in motor rhythm generators. In: Neurons, Networks and Motor Behavior (*eds.* P. S. G. Stein, S. Grillner, A. Selverston and D. Stwart), pp. 119–130. MIT Press.

part myogenic, but the rhythm is entrained and controlled by motor neurons in segmental ganglia. These neurons fire rhythmically due to cyclic periodic inhibition from a CPG comprised of seven bilateral pairs of neurons (HN neurons) that occur in the first seven segmental ganglia (Figure 13). The rhythmicity is generated by the first four pairs of interneurons (Peterson and Calabrese, 1982) and is the result of synaptic interactions (reciprocal inhibition) and intrinsic membrane properties. The spiral muscles that make up the heart wall also have intrinsic rhythmicity and continue to contract when the nervous system is removed, although at a much slower rate. The ability for gut or vascular tubes to generate intrinsic rhythms probably evolved early with superimposed neural control coming only in later stages of animal complexity. The normal behavior of the leech heart tubes is rather complicated and represents a condition where the organizing influence of a nervous system is required. Each tube constricts in a coordinated longitudinal pattern, one in a rear-to-front peristaltic wave and the other approximately synchronously (Thompson and Stent, 1976). About every 20 heartbeats, the two tubes trade roles and these changes in

coordination are always reciprocal and occur with remarkable regularity. The heartbeat pattern generator comprises two different oscillators: a timing oscillator composed of the HN(1)–HN(4) interneurons (Figure 13) responsible for the beat timing; and the switching oscillator which is responsible for the coordination state. Each of these two oscillators is autonomous but they connect to two HN(5) nonautonomous neurons. These two neurons are able to blend the periods of the two oscillators and connect them to the variable phase neurons HN(6) and HN(7). The pattern generator produces the correct phases of activity in the HE motorneurons through direct inhibitory connections from a subset of the HN interneurons that includes members of both the timing oscillator cells and the variable phase interneurons. The HE motor neurons therefore entrain the intrinsic rhythm of the heart muscle very effectively.

Initially, the explanation for the operation of the CPG relied on inhibitory synapses between the reciprocally connected pairs of HN neurons in the third and fourth segmental ganglia. Subsequent studies, which successfully blocked the inhibitory synapses without interfering with currents in the HN cells, demonstrated that these neurons were capable of intrinsic bursting (Cymbalyuk *et al.*, 2002). Both mechanisms, endogenous bursting and synaptic interactions, work synergistically to produce a reliable yet flexible rhythm. The coexistence of bursting using pacemaker potentials and bursting being generated by network properties are found throughout invertebrate CPGs.

The period of the heartbeat is controlled by neuromodulators from a variety of sensory neurosecretory and motor pathways (Arbas and Calabrese, 1990). A family of endogenous RFamide peptides has been shown to modulate the rhythm and these substances have been localized to particular neurons. The target for this central modulation was shown to be the oscillator neurons in the third and fourth ganglion.

The peptide FMRFamide in particular is effective in slowing the heart rate by acting on I_K channels and by decreasing the amplitude of inhibitory synaptic transmission (Simon *et al.*, 1992). As already noted, the use of neuromodulators to control visceral networks is ubiquitous and a general solution used by all animals.

1.25.3.3 Snail Feeding CPG

Considerably more complicated than either the single-phase crustacean heart rhythm or the alternating peristaltic and synchronous leech heart rhythm is the molluskan feeding rhythm. We can consider two different feeding rhythms that have evolved parallel mechanisms for operation of the feeding apparatus. Motoneurons controlling the feeding rhythm of the snail *Lymnaea stagnalis* receive synaptic inputs from a CPG located in the cerebrobuccal ganglion. The CPG controls a structure called the radula, which undergoes protraction and rasping movements when the animal feeds. The CPG neurons (Figure 14a) are activated by chemosensory inputs from the lips that strongly depolarize a set of protraction phase interneurons called N1Ms, and trigger the production of endogenous plateau potentials that maintain protraction phase activity (Kemenes *et al.*, 2001). This activation is further supported by synaptic excitation from N1L and the SO. The combined N1M and N1L activity has a biphasic effect on the rasp phase interneurons that consists of an initial hyperpolarization followed by a slow depolarization that activates and triggers plateau potentials in the N2v and by electrical connections to N2d rasp phase interneurons. This activity terminates the plateau potentials in N1M and ends the protraction phase by increased inhibition from N2v and N2d on all of the protraction phase interneurons and the SO neuron. The rasp phase ends when the plateaus in N2v and N2d terminate spontaneously. This releases another set of interneurons, N3t and N3p, which generate swallowing, from inhibition by both protraction and rasp phase interneurons. Because N3t has a strong postinhibitory rebound (PIR), it rebounds from its inhibition and fires strongly, pulling the N3p interneurons along with them because of electrical connections. Once the swallowing phase stops, continued sensory stimulation starts a new cycle (Straub *et al.*, 2002).

The three consecutive phases that are produced by the CPG control the firing sequences of the motoneurons and subsequent muscle contractions (Figure 14b). The sequence of firing in this system derives from the synaptic interactions and the intrinsic properties of the interneurons in the CPG. This pattern can be demonstrated *in vitro* by stimulating the SO neuron which starts a sequence of bursts from N1 to N2 to N3 in that order. By stimulating SO, EPSPs are produced in the approximately 10 N1 neurons which then burst together and in so doing inhibit the SO, N2, and N3 neurons simultaneously. When the N1 burst terminates, N2 fires and continues to inhibit N3 and when N2 stops firing, N3 is free to fire. When the three N3 neurons fire, they turn off the SO and the N1 neurons. As with many mollusks, there is some degree of myogenic activity in the feeding muscles

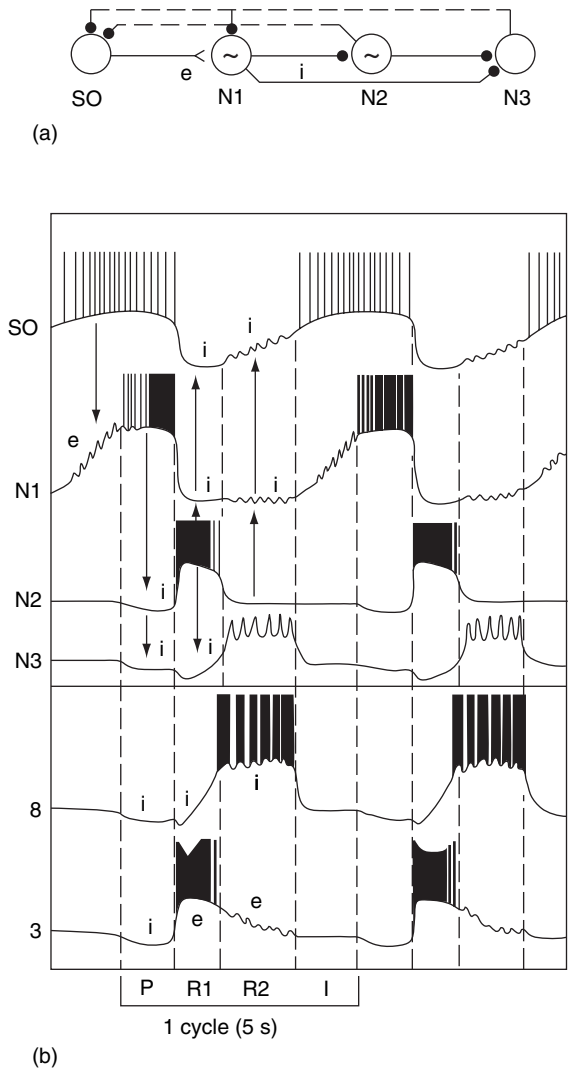


Figure 14 Summary of the CPG in the *Lymnaea* feeding system. a, The hypothetical wiring diagram showing the minimum number of connections required to produce the activity pattern shown in (b). b, Output pattern of the four interneuronal types and two examples (8 and 3) of motoneuron output when the SO is on either spontaneously or as a result of steady depolarization. Arrows indicate synaptic connections, e for excitatory and i for inhibitory. Reproduced from Rose, R. M. and Benjamin, P.R. 1981. Interneuronal control of feeding in the pond snail *Lymnaea stagnalis*. ii: The interneuronal generating feeding cycles. *J. Exp. Biol.* 92, 203–228, with permission from The Company of Bilogists Ltd.

but not enough to insure the coordination of the muscles for different behaviors.

Isolation of the neurons making up the CPG by placing them in tissue culture has revealed the intrinsic cellular properties of the constituent neurons. The N1 interneurons were able to generate intrinsic plateau properties when properly stimulated while the N2 interneurons could only generate plateau potentials in the presence of acetylcholine. Other isolated neurons in the feeding CPG circuit had no significant intrinsic properties relative to the feeding rhythm. The lateral N1, the dorsal N2, the phasic N3, and the slow oscillator neuron had their firing patterns determined by cholinergic and glutamatergic synaptic inputs from other CPG interneurons, which could be mimicked in culture by pharmacological agonists. In addition to the SO neuron, stimulation of the cerebral ventral 1a neuron (CV1a) was shown to produce a fictive feeding pattern in isolated ganglia.

The control over the feeding rhythm, as in the cardiac and leech heart, consists of both synaptic and neuromodulatory inputs to the feeding CPG. Direct stimulation of the cerebral ventral neuron (CV1a) and SO leads to a strong CPG-driven fictive feeding pattern (Kemenes *et al.*, 2001). However, when the lips are stimulated with a natural stimulus such as sugar, neither CV1a nor SO1 is involved in the initial activation of the rhythm. Once the behavior begins, CV1a has effects on motor neuron burst duration and SO in setting the overall frequency. Cerebral to buccal interneurons that may be homologous to CV1 in *Lymnaea* have been identified in many other molluskan species, including *Aplysia* (CB12; Rosen *et al.*, 1991), *Limax* (CB1; Delaney and Gelperin, 1990), *Pleurobranchia* (phasic paracerebral neurons; Gillette *et al.*, 1982), and *Achatina* (C1; Yoshida and Kobayashi, 1992). As with *Lymnaea*, all of these can drive feeding rhythms when stimulated intracellularly, can respond to food stimuli but are not necessary to activate the rhythym when chemosensory fibers are stimulated. Similar homologies exist between the SO and other buccal neurons that can drive feeding rhythms. In *Aplysia*, which we will consider separately, several buccal neurons can drive the rhythm (Susswein and Byrne, 1988; Kabotyanski *et al.*, 1998). Similar but not homologous neurons have also been found in *Helisoma* (Quinlan *et al.*, 1997), *Clione* (Arshavsky *et al.*, 1989), and *Planorbis* (Arshavsky *et al.*, 1988).

The amine 5-HT contained in the cerebral giant cells acts on motor neurons that are part of feeding CPG including B4, B8, and B4CL (Straub and Benjamin, 2001). All of these neurons were depolarized past firing threshold for prolonged periods of time by 5-HT. Conditional bursting occurred in the B4 and B8 cells but not in the B4CL neurons and the bursting frequency was increased by CCG tonic firing. 5-HT also increased PIR in all three neuron types. As will be the case in all of the systems discussed however, how these modulators are used during the actual behavior of the animal is difficult to ascertain.

1.25.3.4 *Aplysia* Feeding

A similar mechanism for the control of the feeding apparatus occurs in the marine mollusk *Aplysia californica*. Despite the fact that the muscles themselves still have some intrinsic rhythmicity, the evolution of multifunctional circuits operating the radula and the other mouth parts led to a new level of complexity including a heavy reliance on neuromodulation. As in other circuits, the predominant role of reciprocal inhibitory synapses has evolved to insure that antagonists do not fire at the same time, no matter what specific behavior occurs, and to be the sole or supportive mechanism for generating alternate bursts. Similarly, many of the neurons in the circuit are intrinsic or conditional bursters – conditional on the presence of neuromodulatory input. The particular circuitry shown here has been useful in illustrating how a small ensemble of neurons can perform several different functions by making only small changes to the circuit (Jing *et al.*, 2004). The basic kernel of the motor circuit is comprised of two reciprocally inhibitory neurons B63 and B64 (Figure 15). Depending on which higher-order neurons are activated, the kernel circuit along with other neurons will form both behavior-specific (used only for one behavior) and behavior-independent (that is capable of multiple behaviors) modules. Behavior-specific modules such as biting, swallowing, and rejection are encoded by combinations of output from CBI-2 and CBI-4 which are higher-order interneurons in the cerebral ganglion (Figure 15a). Encoding of behavior-independent modules for swallowing or rejection is achieved by adding neuron B51 to the circuit (Figure 15b).

The radula has also been used as a model system with which to study the co-evolution of the nervous system and the biomechanical plant it controls (Sutton *et al.*, 2004). Sutton *et al.* make the assumption that evolutionary adaption acts on behavior and that the most intimate causal factor is the biomechanical plant, the muscles, and parts of the body moved by the muscles. The evolution of the nervous system must therefore be seen in the context of the unity between all three elements – behavior, musculosketal plant, and nervous system (see also Dumont and Robertson, 1986).

1.25.3.5 The Crustacean Stomatogastric System

The crustacean stomatogastric system is one of the best characterized invertebrate visceral nervous systems but very little is known about it in terms of evolutionary development. Like lobsters and crabs, insects also have a stomatogastric nervous system that has been described as an invertebrate homologue of the vertebrate visceral autonomic nervous system (Hartenstein, 1997). In crustaceans, nerve cells are located on the fore and hindgut which supply motor nerves to the stomach (Figure 16). The neurons are connected to a large ganglion in the head (supra-esophageal) and to commisures which circle the esophagus and supply the stomatogastric ganglion (STG) with various kinds of descending input.

It is not known when the crustacean digestive tract evolved from producing simple peristaltic movements to become capable of complicated food maceration and filtering behaviors. One can speculate that the ability to reduce feeding time in the open, where animals could easily be preyed upon, conferred some selective advantage to storing food in a large cardiac sac for later maceration and digestion. This would require the stomach to have not

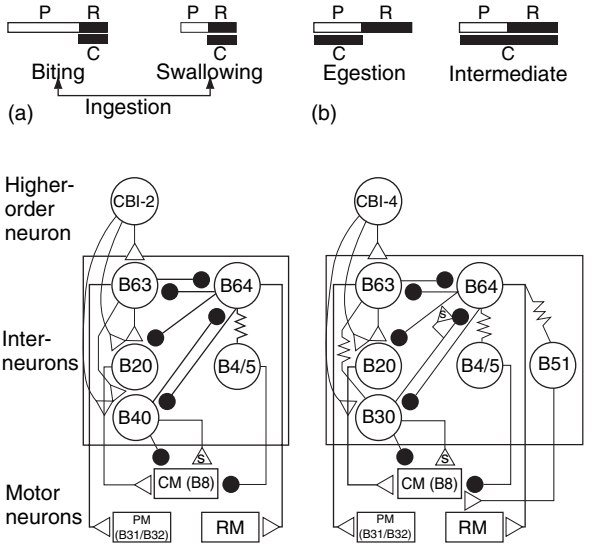


Figure 15 Fictive motor programs elicited in the isolated CNS by stimulation of the CBIs (Rosen *et al.*, 1991). The multiple motor patterns and the circuits that underlie them are shown here diagrammatically. a, Classification of the motor programs and their relation to behavior. Each cycle of the program comprises a protraction (P) phase that is followed by a retraction (R) phase. Motor programs can be classified into four types depending on the phasing of the radula closer (C) motor neuron. b, Schematic diagrams of the feeding circuits that produce the multiple motor outputs. On the left, the circuit that is activated by CBI-2 mediates ingestive-biting-like, egestive, and intermediate programs. On the right, the circuit that is mediated by CBI-4 and mediates ingestive-swallowing-like, egestive, and intermediate programs is shown for comparison. Adapted from Jing, J., Cropper, E. C., Hurwitz, I., and Weiss, K. R. 2004. The construction of movement with behavior-specific and behavior-independent modules. *J. Neurosci.* 24, 6315–6325, with permission from Society of Neuroscience.

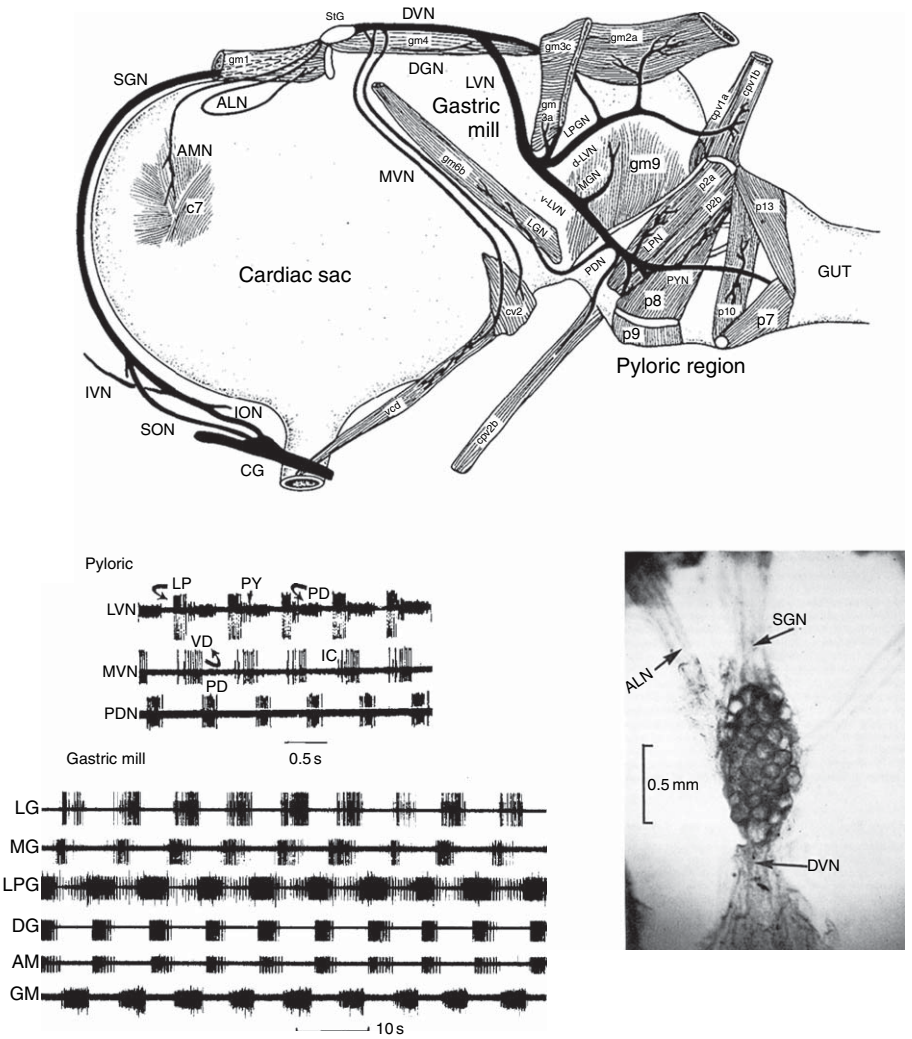


Figure 16 Diagram of the lobster stomach illustrating the four basic divisions: esophagus, cardiac sac, gastric mill, and pylorus. The STG lying on top of the stomach contains about 30 neurons. The axons of these neurons pass out the ganglion via the dorsal ventricular nerve (DVN) to innervate the striated muscles controlling constriction and dilation of the pyloric filter and gastric mill. Beneath the diagram of the stomach are extracellular recordings of the pyloric and gastric rhythms as recorded from the different motor nerves shown in the diagram in a preparation with commissural ganglia attached. To the right of the recordings is a picture of the STG as it appears in the dissecting microscope.

only a simple storage and mixing function but also the ability to macerate and filter the food. Neural control over these processes would lead to the organization of local CPGs in the stomatogastric system that in turn would be under the influence of higher centers. In addition, this system evolved the membrane receptor proteins and second messenger systems necessary to enable the stomach to respond to neuromodulatory substances in complex ways.

1.25.3.5.1 The stomatogastric CPGs The STG, located in the ophthalmic artery just above the stomach, contains the complete neuronal infrastructure for the generation of two visceral behaviors – the gastric mill and the pyloric. The isolated STG can be observed in Figure 16. The gastric rhythm, produced by 11 neurons, is for 'chewing' the food that has been swallowed and stored in the cardiac sac division of the stomach (Figure 16). Contractions of the cardiac sac force coarsely shredded food into the gastric mill. Here the three teeth of the gastric mill move in a more or less periodic pattern which grinds the food but are heavily modulated by sensory feedback which adjusts the pattern to the consistency of the material in the stomach. The pyloric rhythm controls a pump – filter apparatus at the caudal end of the stomach (Figure 16) – and while it can adjust its speed, appears to work almost continuously (Rezer and Moulins, 1983). The neurons in

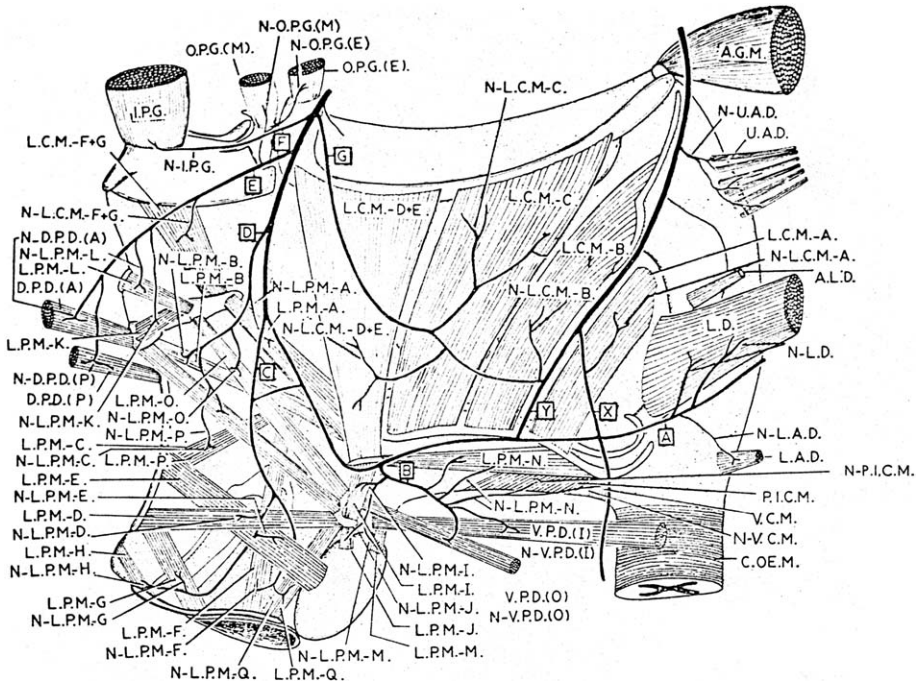


Figure 17 Diagramatic lateral view of the foregut of the crab *Paratelphusa guerinii* (Milne-Edwards) showing the muscles and their innervation.

both CPGs have dual functions. With the exception of two that are interneurons, the remainder have evolved specific biophysical and synaptic properties to not only produce coherent synchronized patterns as a result of their cellular properties and synaptic interconnectivity, but to also act as motor neurons transmitting spike potentials to the appropriate neuromuscular junctions. The distribution of the motor nerves supplying muscles in the lobster and crab are illustrated diagrammatically in Figures 16 and 17. Unlike the molluskan examples that have been discussed, the crustacean stomach muscles are striated and are morphologically and functionally identical to all other 'voluntary' muscles such as those in the limbs and therefore require neural input for contraction. The neurons therefore have dual functions: to generate the motor pattern within the ganglion and to signal the muscles outside the ganglion (shown for both rhythms in Figure 16). The synapses use both acetylcholine and glutamate but interestingly produce mainly inhibitory connections within the ganglion and exclusively excitatory connections at the neuromuscular junction. In addition, like some synapses in vertebrates, for example, in the retina, the stomatogastric neurons can release their neurotransmitters without a spike invading the presynaptic terminal, that is, as a graded function of membrane voltage at the synapse. At the neuromuscular junction, however, the release is pulsatile.

1.25.3.5.2 Inputs from higher centers The STG is connected to other parts of the CNS by a single input nerve, the stomatogastric nerve (SGN). This nerve is made up of four separate nerves that emerge from the paired CGs, the superior and inferior esophageal nerves (SONs and IONs). In addition, an unpaired nerve, the inferior ventricular nerve (IVN in Figure 16), emerges from the base of the supraesophageal ganglion and enters the esophageal ganglion at the point where the SONs and IONs come together to form the SGN. Two histaminergic fibers in the IONs synapse directly onto specific neurons in the STG. Other neurons with cell bodies in the esophageal ganglion turn on the gastric mill rhythm and strongly affects the pyloric rhythm by binding to muscarinic receptors (Nagy and Dickinson, 1983). Another neuron, the pyloric suppressor (PS) terminates the pyloric rhythm (Cazalets *et al.*, 1990). The types of descending neurons appear to differ among species and has by no means been worked on in comparative terms. For example, the European lobster *Hommarus gammarus* has two pairs of neurons, CGs and GIs, which act as command fibers for the gastric

mill (Combes *et al.*, 1999). The CG neurons excite the LPGs and GMs, while the GI neurons inhibit the LG, MG, and DG neurons. Each pair of neurons produces a different pattern and the combined action of the two determine the actual output.

1.25.3.5.3 The canonical STG circuit Although the stomatogastric system had been described anatomically in the 1800s (Mocquard, 1883), it was not until the advent of intracellular recording that detailed functional circuitry within the STG was described. There are four separate CPGs in the stomatogastric system – the gastric and pyloric CPGs are entirely contained in the STG, while the cardiac sac and esophageal CPGs have some neurons in the STG but the location of the other neurons has not been localized. The connections for some of the cardiac sac and esophageal neurons have been described but the complete CPG circuits are not known. However, the pyloric and gastric CPG circuitry has been fully described from work on many different species and although there is some variation in cell numbers (Tazaki and Tazaki, 1997), the overall 'canonical' circuitry (Figure 18) has been known for many years.

The pyloric and gastric mill neural circuits are shown in Figure 18. The pyloric circuit is comprised of two PD cells, eight PY cells, and one each of VD, LP, IC, and AB cells. The circuit was originally described by Maynard (1972) and completed in 1975 (Maynard and Selverston, 1975). In a combined preparation with the two CGs and the single esophageal ganglion intact, all of the neurons in the pyloric network burst intrinsically. Because they require neuromodulatory inputs from the commissurals, however, they are sometimes referred to as 'conditional' bursters. Without this modulatory input, the intrinsic conductances underlying bursting are not sufficiently activated and the cells are

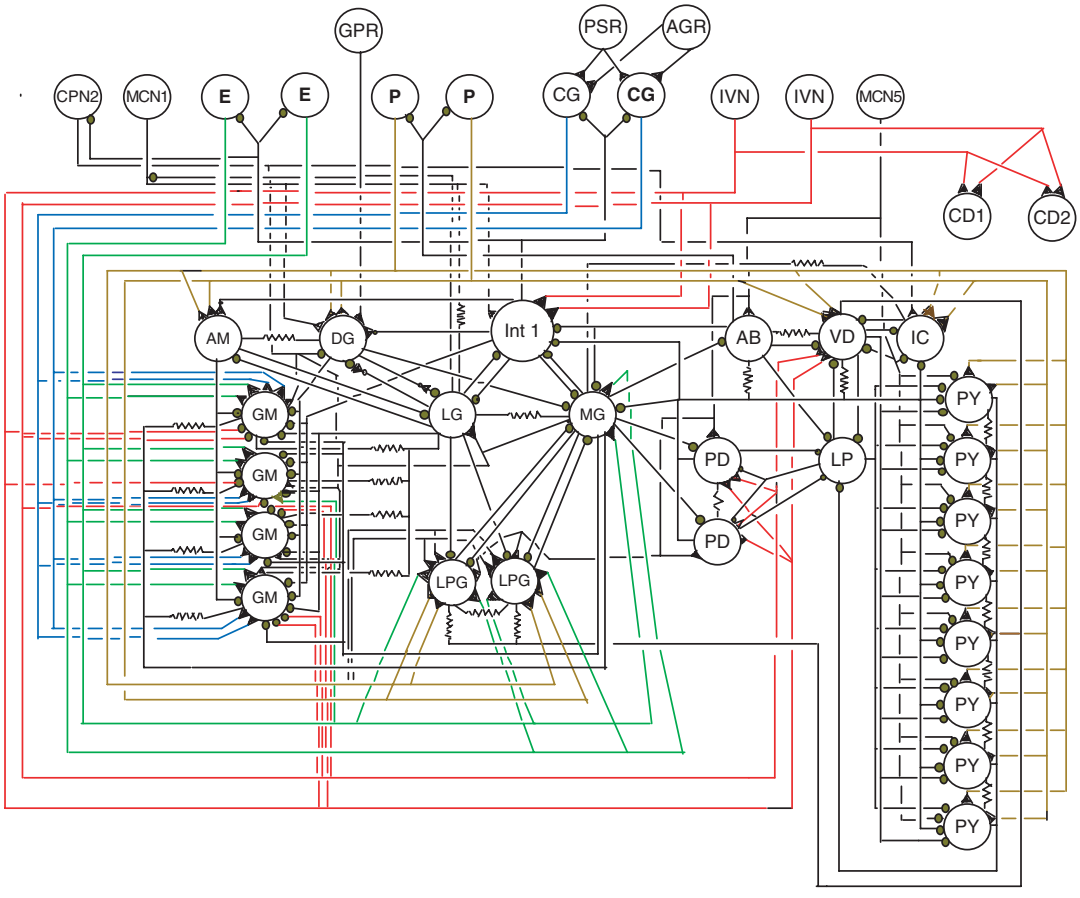


Figure 18 Canonical circuit for the lobster stomatogastric system. The left cluster containing the LG, MG, int 1, LPGs, GMs, and AM (11 neurons) make up the gastric mill CPG. The right cluster, PDs, AB, VD, IC, LP, and PYs (14 neurons), comprise the neurons of the pyloric CPG. Both pyloric and gastric CPG neurons are located in the STG. The E and P neurons are located in the CGs. Other neurons shown represent various sensory and neuromodulatory inputs to the STG and are discussed in the text.

either silent or fire slowly but not in bursts. The AB neuron occasionally bursts when isolated from neuromodulatory input and when inputs are present it has the fastest rhythm, thus acting as a pacemaker for the other pyloric cells. Simultaneous intracellular recordings can be obtained from all of the pyloric neurons. By deleting single neurons from the circuit sequentially, it has been shown how the synaptic connectivity and the intrinsic ability to burst combine to produce a robust three-phase burst pattern (Miller and Selverston, 1982). The ionic mechanism for bursting is different from that described for molluskan neurons. Basically a decrease in the K leak current depolarizes the neuron until a voltage-gated Ca and Na current is activated (Gola and Selverston, 1981). This increases the rate of the depolarization and activates the fast spiking and plateau currents. Inactivation of the Na and Ca currents combined with Ca-activated K current brings the cell back to its resting potential. Other currents, particularly I_A and I_H play an important role in burst timing and will be considered later.

The circuitry of the gastric mill CPG was established in 1974 (Mulloney and Selverston, 1974a, 1974b; Selverston and Mulloney, 1974) and has undergone only minor revision since then. The gastric mill CPG appears to work entirely as a result of the synaptic interactions with no single neuron or group of neurons acting as the pacemaker for the rhythm. The idea that within one ganglion there coexists two fundamentally different mechanisms (the other being intrinsically bursting cells) for generating motor patterns is unique but is logical if one considers the behaviors involved, the constant pumping of the pylorus and the intermittent grinding of the gastric mill.

The inhibitory synapses in the gastric mill play the determining role in pattern formation. The key LG and DG neurons evoke two types of IPSPs in follower neurons (Elson and Selverston, 1995). The fast type rises rapidly (100–300 ms), is mediated by an increased chloride and K conductance and resembles the glutamatergic IPSPs described for the pyloric CPG. The second, slow type of IPSP, has a long rise time (1–2 s) and is mediated by an increased conductance to K. The fast inhibition occurs alone at connections from DG and LG to power stroke motor neurons (MG and GM). Slow inhibition occurs in parallel with fast inhibition (producing dual component responses) at LG to LPG and Int 1. DG evokes a pure slow IPSP onto the LPG neurons.

While the two CPGs control separate stomach musculature, there are many direct synaptic and electrotonic connections between neurons belonging to each circuit (Figure 18). In addition, several neurons that are located in the CGs receive synaptic inputs from the neurons in each CPG and feedback timed inputs to neurons in both CPGs. This is most apparent in intracellular recordings from the DG and AM neurons, which show bursts of EPSPs that originate from P cells which are modulated by the AB neuron. Since each CPG operates at different frequencies, the possible functions of these connections has been investigated (Bartos *et al.*, 1999).

1.25.3.5.4 Ion channels in STG neurons As already mentioned, several voltage-gated channels are responsible for the intrinsic burst-generating mechanisms found in STG neurons. Several other channels are particularly important in controlling various aspects of the bursting behavior and therefore properties of the behavior itself. The somata of STG neurons contain only outward ion channels and are inexcitable (Graubard and Hartline, 1991; Golowasch and Marder, 1992). Two of the other important currents located on the processes in the neuropile are I_H and I_A – both found to a greater or lesser extent in all STG neurons. I_H is activated by hyperpolarized voltages and plays an important role in determining the extent of PIR which occurs after inhibition. I_A is a transient depolarization-activated K current which delays the onset of bursting. Voltage clamp studies on individual pyloric neurons have shown that the distribution of I_A channels differs between cells. It is largest in PD and PY cells, smaller in AB, LP, and IC cells and not detectable in the VD cell (Tierney and Harris-Warrick, 1992). When I_A currents are blocked with 4-AP, the cycle period is decreased by ~20%; there is a general enhancement of activity in all of the cells and there is a change in phasing of follower cells with respect to the PD/AB pacemaker group.

1.25.3.5.5 Chemical modulation The cells of the STG require modulatory input from the CGs in order for rhythmic activity to take place. The CGs release an as yet unknown mixture of neuromodulatory substances into the neuropile of the STG thus enabling the gastric and pyloric CPGs to operate. The modifications to the cellular conductances are due to phosphorylation mechanisms following the activation of kinases by second messenger systems, the concentrations of which have been raised by G-protein/membrane receptor interaction with the neuromodulators. The ionic conductances have kinetic profiles which are directly responsible for establishing burst periods in isolated cells by acting on slow conductances and this along with the

synaptic strengths of the faster ionotropic synapses determine the temporal and spatial characteristics of the motor pattern. So if we consider the isolated STG, we see that there have evolved a large number of overlapping mechanisms that are present which control the output patterns driving the gastric and pyloric regions of the stomach. The CPGs, with neuromodulatory input from the two higher ganglia generate the basic autonomous pattern. Various combinations of other modulatory substances, mostly amines and peptides, can also be released into the ganglion to produce many different patterns by:

- reconfiguring the gastric or pyloric circuit to generate different variations of the main pattern;
- switching one or more neurons from one pattern to another;
- combining a subset of neurons from several CPGs to form an entirely new circuit; or
- fusing two complete CPGs to form a new combined circuit generating a new and novel pattern.

The modulators can be released from the terminals of neurons or be carried to the neuropil in the blood after being released from neurohemal structures such as the pericardial organ. Together, these mechanisms allow the neural circuits controlling the viscera to produce many more motor patterns than are required to process food. Since it has not been experimentally tractable to record both rhythms while at the same time monitoring the levels and types of neuromodulators present in the STG neuropil, the reason for all this flexibility is not known. Nevertheless, it is unlikely that all of these mechanisms were selected for without their having some function, at least some of the time, but at present one can only speculate whether or not they are historical leftovers or really have a clear purpose.

1.25.3.5.6 Sensory control The stomach wall and the striated muscles that operate the gastric mill and pyloric pump contain sensory receptor cells sensitive to stretch (mechanoreceptors) and others inside the gut that respond to chemical stimuli. Not all of these have been investigated in the stomatogastric system but single sensory fibers have been shown to be able to affect the pattern on a cycle-by-cycle basis. Receptors in the posterior part of the stomach (PSRs) are stimulated by rhythmic movements and their action, via the CGs, is to activate both the gastric and pyloric rhythms (Nagy and Moulins, 1981). One feedback mechanism that has been described is a sensory neuron (AGR) close to the STG that receives stretch receptor innervation from the powerful GM muscles. The AGR can either enhance or restrain the output to these muscles depending upon the amount of force being sensed (Simmers and Moulins, 1988). In this sense, the AGRs act like both the Golgi tendon organs and muscle spindles of vertebrates which evolved quite separately to perform similar functions. The AGRs in the European rock lobster *Hommarus gammarus* synapse onto two pairs of command neurons called CG and GI which are located in the CG. When the gastric mill is active *in vitro* weak firing of the AGR occurs in time with the gastric mill rhythm, when all the power stroke neurons are synchronously active. However, with strong AGR firing, the phase relationships switch to a different pattern in which lateral and medial teeth motor neurons fire in antiphase (Combes *et al.*, 1999). In this case, the feedback from a single mechanosensory neuron is able to specify two different motor pathways in an activity-dependent manner.

Another set of stretch receptors call the gastropyloric receptors (GPRs) do not act via the CGs but instead form a closed loop system in which the GPRs display endogenous rhythmicity (Katz and Harris-Warrick, 1990a). The GPRs found in crabs activate the pyloric rhythm via cholinergic mechanisms and cause some of the gastric mill neurons to fire in pyloric time. The later result appears to be due to the co-release of serotonin (Katz and Harris-Warrick, 1990a, 1990b). Finally, bilateral sensory neurons in the cardiac gutter of the crab stomach project to both the gastric and pyloric feeding circuits (Beenhakker *et al.*, 2004). When stretched artificially, these neurons initiate chewing movements and modify the pyloric filtering movements via projection neurons in the CGs.

1.25.3.5.7 Evolution of the stomatogastric system The question of how visceral nervous systems evolved is complicated by the fact that a comparative study of the systems described in this chapter (leech heart, snail, and aplysia feeding, etc.) has not been undertaken. That is understandable since most of them were investigated with other goals in mind – generally a mechanistic description of how rhythmic patterns are generated in animal models. However, in order to understand how these systems evolved, we have to look at the same system in a variety of different species that have evolved at different times based on phylogenic descriptions. Commonalities and differences between species can suggest hypotheses for how the system evolved to its present form. The stomatogastric system is probably the only one of these systems that has been studied in sufficient detail and at enough different levels to attempt this comparison. An excellent description of the evolution of the stomatogastric system has been put forward by Katz

(1991) and by Katz and Tazaki (1992). One principal outcome of these studies has been the suggestion that while the neural circuits have remained stable over time, enormous variations in neuromodulators and external structures have evolved to meet the needs of animals living in different environments and utilizing different food sources.

The phylogenetic tree for the decapod crustaceans shown in Figure 19 is a compilation of various phylogenetic classification schemes (Burkenroad, 1983; Bowman and Abele, 1982; Schram, 1986; Kim and Abele, 1990) that have been suggested by Katz and Tazaki (1992). Such an analysis is important in tracing the evolution of the nervous system since there is no fossil record and the only possible approach is a phylogenic tree.

The Katz–Tazaki formulation makes it quite clear that the changes which have occurred in the stomatogastric system over evolutionary time can be studied by examining various decapod classes and though incomplete, is probably better than any other analysis of neural systems. Figure 20 shows three stomatogastric systems from the clawed lobster *Homarus*. The Caribbean spiny lobster *Panulirus argus* and the crab *Callinectes* are illustrated diagrammatically. The organization at the level of the major ganglia is similar, with the subesophageal ganglion connecting to the paired CGs which in turn connects to the esophageal ganglion (og) via the paired SONs and IONs (son and ion). These four nerves coalesce to form the SGN, the principal input to the pattern generators in the STG. The principal variation between these three species appears in the arrangement of motor nerves leaving the ganglion. This is due to the different size and position of ossicles within the gastric mill (Figure 21) and the different arrangement of muscles in both the gastric mill and pyloric regions of the stomach (see also Figure 17).

The anatomical differences that have evolved extend to the arrangement of neurons in the CPGs as can be observed in Table 1, which is a

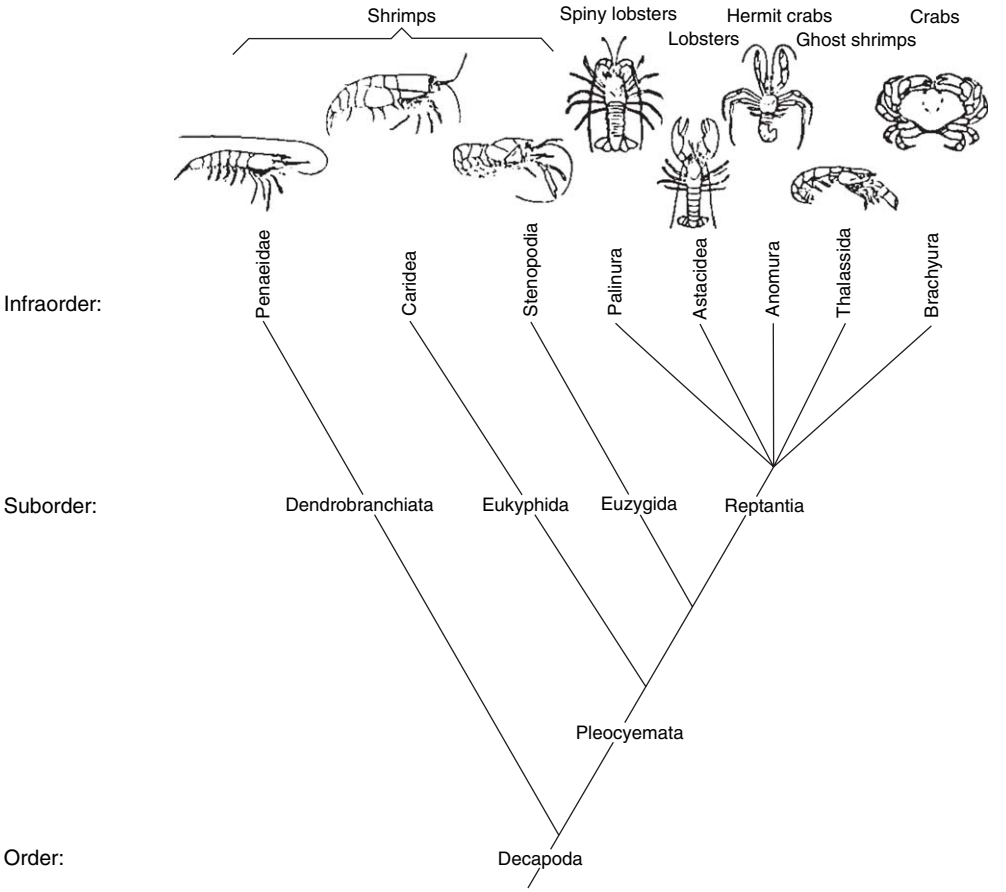
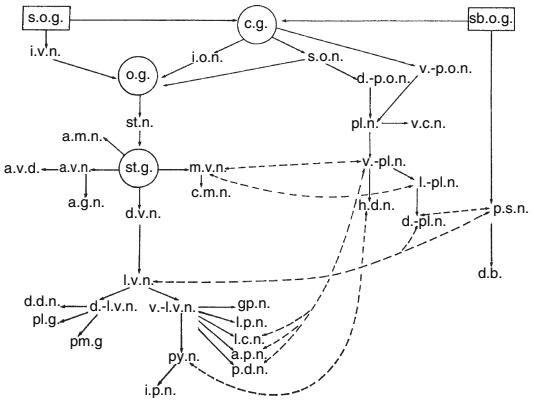
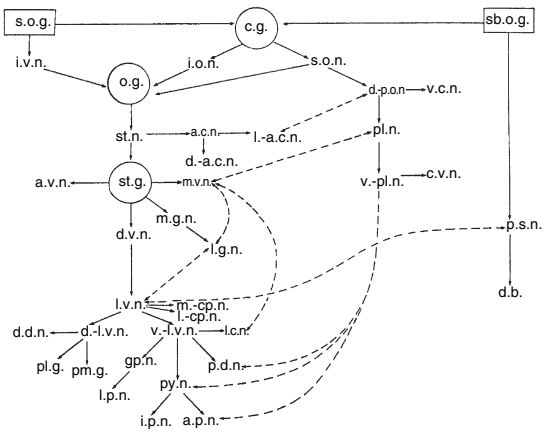


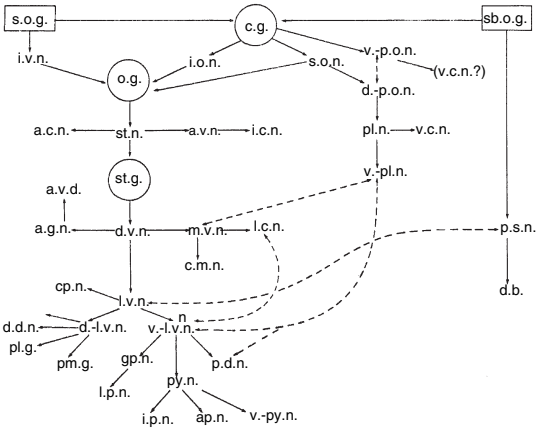
Figure 19 Diagram of the phylogenetic relationships between decapod infraorders. Relationships between reptantian infraorders have not been determined. Modified from Katz, P. S. and Tazaki, K. 1992. Comparative and evolutionary aspects of the crustacean stomatogastric system. In: Dynamic Biological Networks: The Stomatogastric Nervous System (*eds.* R. M. Harris-Warrick, E. Marder, and A. I. Selverston), pp. 221–261. MIT Press.



Panulirus argus



Callinectes sapidus



Homarus americanus

Figure 20 Diagrams showing the layout of the stomatogastric nervous systems of *Panulirus*, *Homarus*, and *Callinectes*. Modified from Maynard, D. M. and Dando, M. R. 1974. The structure of the stomatogastric neuromuscular system in *Callinectes sappidus, Homarus americanus* and *Panulirus argus* (Decapoda Crustacea). *Philos. Trans. R. Soc. Lond.* 268, 161–220.

Table 1 Invention of the pyloric region and number of cell types in different species as summarized in Tazaki and Tazaki (1997)

	Motor neuron				
Muscle	*C. borealis*	*P. interruptus*	*P. serratus*	*P. japonicus*	*S. oratoria*
cpv1, 2	PD (2)	PD (2)	PD (2)	PD (2)	PD (2)
cpv3, 4	LP	LP			
cpv5		LP			
cpv6	LP			LP	
cpv7	IC	LP			
cpv8	LP				
cpv11	PY				
p1	LP (1)	LP (1)	LP (1)	LP (1)	PY1 (1)
p2	PY (5)	PY (8)	PY (2)	PY (2)	PY2 (2)
p8, 11	PY	PY		PY	
p12–14	PY	PY			
p3, 7, 10	LPG (2)	PY	?	LPG (2)	?
p15	LPG				
cv1	VD (1)	VD (1)	?	VD (1)	VD (1)
cv2	IC (1)	IC (1)	?	IC (1)	PCP (1)
cv3	IC	IC	?		
Interneuron	AB (1)	AB (1)	AB (1)	AB (1)	AB (1)

Reproduced from *J. Comp. Physiol. A*, Vol. 181, 1997, pp. 367–382, Neural control of the pyloric region in the foregut of the shrimp *Peneaus* (Decapoda Penaeidae), Takazi, K. and Takazi, Y. With kind permission from Springer Science and Business Media.

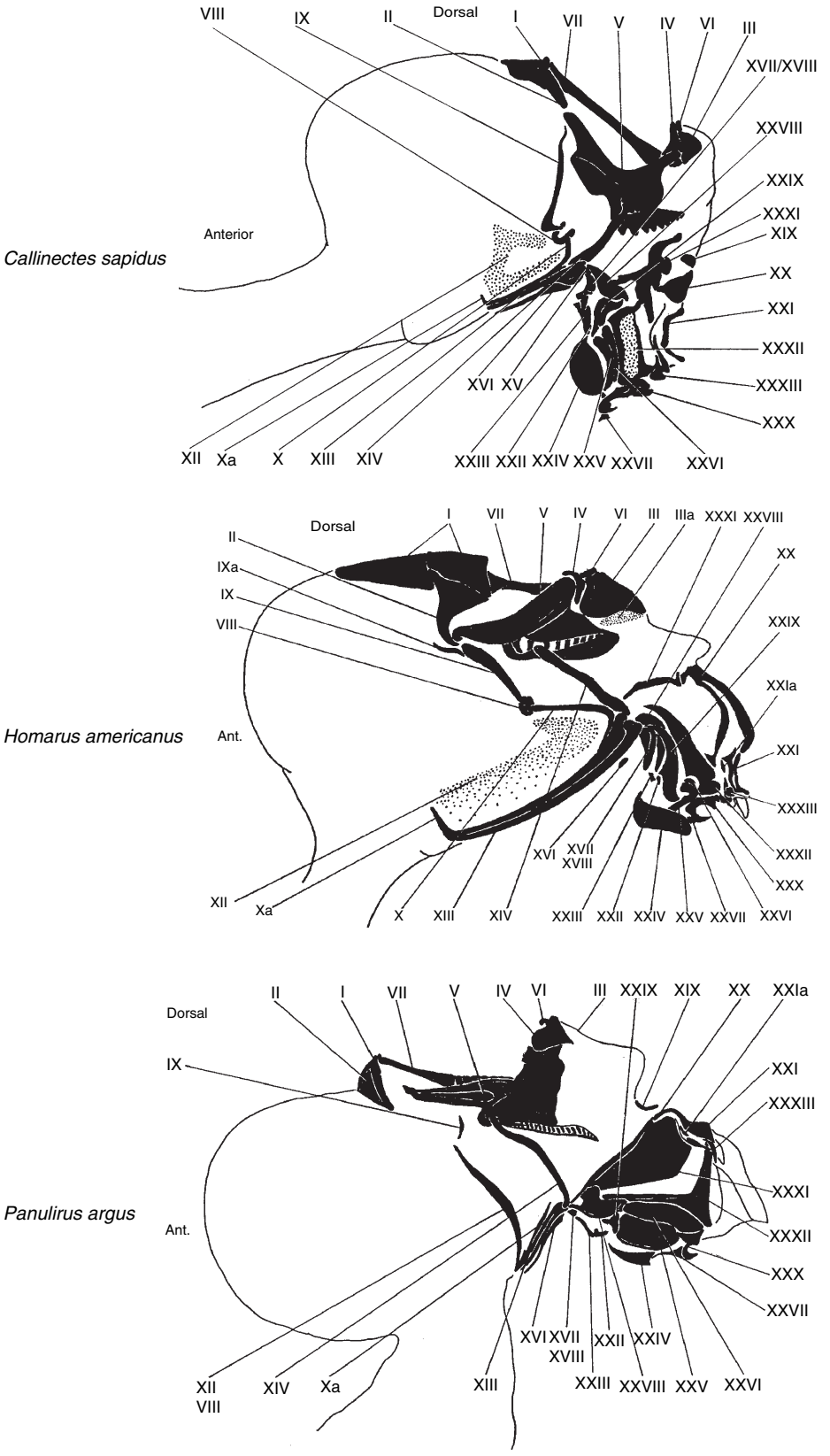


Figure 21 Diagrams of lateral views of stomach ossicles of the same three species as shown in Figure 20. Modified from Maynard, D. M. and Dando, M. R. 1974. The structure of the stomatogastric neuromuscular system in *Callinectes sappidus, Homarus americanus* and *Panulirus argus* (Decapoda Crustacea). *Philos. Trans. R. Soc. Lond.* 268, 161–220 (figures 2, 3, and 4), with permission from The Royal Society.

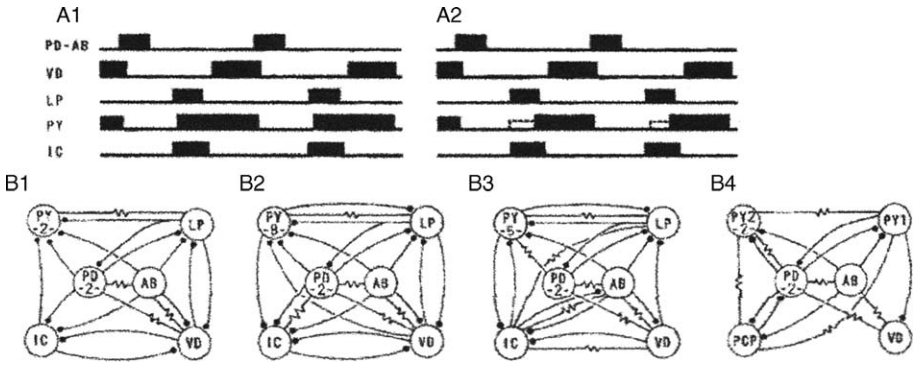


Figure 22 Diagram of pyloric cycles and differences in connectivity among four species. A1, *Penaeus*; A2, *Panulirus*; B1, *Penaeus*; B2, *Panulirus*; B3, *Cancer*; B4, *Squilla*.

compilation of elements making up five different crustacean pyloric systems (Tazaki and Tazaki, 1997). As a result, there are also differences in the motor output patterns and synaptic connectivity between pyloric neurons (Figure 22).

The constancy in structure and CNS topology lends some credence to the idea that overall the principal features of decapod visceral control evolved in a similar way and that fine-tuning of these circuits may be more the result of neuromodulator variation than the rearrangement of neurons and synapses.

1.25.4 Some General Conclusions Regarding the Evolution of Visceral Nervous Systems

- Using the same components – neurons, synapses ion channels, etc. – all visceral nervous systems have evolved rhythmic central pattern-generating networks with which to control automatic functions such as digestion, circulation, and respiration.
- These CPGs are robust and sensitive due to their intrinsic dynamics.
- Cellular and synaptic properties are stable despite channel turnover and plastic changes due to homeostatic mechanisms.
- Neuromodulators provide multifunctionality to circuits by altering their cellular and synaptic properties via second messenger-induced phosphorylation mechanisms.
- Neurons forming visceral control mechanisms are generally located in ganglia near the structures being controlled. Within the ganglia, neurons can be identified based on location, physiological properties, connections with other neurons and effector organs, molecular properties, and many other criteria.

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Relevant Website

http://tolweb.org – The Tree of Life Web Project (ToL) is a collaborative effort of biologists from around the world.

1.26 Cognition in Invertebrates

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Glossary

classical conditioning Learning about relationships in the environment.

cognition The use and handling of knowledge.

episodic memory In humans the consciously recollected memory for facts and events as they are characterized by their contents (what), their time of occurrence (when), and their locations (where). Episodic-like memory in animals stores the what, when, and where for important events (food). (See Section 1.26.6.)

explicit knowledge Knowledge encoded as representations, such as places, facts, and events.

habituation Reduction of responsiveness after multiple application of the same stimulus. Habituation is discriminated from sensory adaptation and motor fatigue by its stimulus specificity, spontaneous recovery, dishabituation by a strong stimulus, and by a saving effect (stronger response decrement after multiple sessions of habituation).

implicit knowledge Knowledge encoded in actions and associations, for example, acquired through predictive learning.

innate preferences, innate stereotypy Simple forms of behavior based on phylogenetic memory.

nonelemental learning Associative forms of learning in which individual events are ambiguous and only logical combinations of them can be used to solve the problem. Examples are described in Section 1.26.4.

occasion setting A learning situation in which a stimulus, the occasion setter, sets the occasion for when a predictive

	relationship applies. Contextual learning is closely related to occasion setting (see Section 1.26.5.3).
operant conditioning	Learning about the consequences of one's own behavior.
phylogenetic memory	The species' memory. The information stored in the genome as acquired by mutation and selection during evolution.
predictive learning	Using operant and classical conditioning to predict future events.
rule learning	The ability of an animal to infer rule information from a number of different examples connected by a common feature (see Section 1.26.5).
search image	Animals searching for a target the knowledge about which has either been learned or is known on the basis of innate information (phylogenetic memory).
sensitization	Increase of responsiveness after the experience of a strong stimulus.
working memory	A transient form of active memory that combines both information retrieved from a permanent store and actual incoming information (see Sections 1.26.6.1 and 1.26.6.2)

1.26.1 Introduction

Animals are characterized at all levels of neural complexity by a continuous flow of energy, material, and information. Behavior is an animal's means for surviving the destructive forces of entropy on both long and short timescales. Evolution maintains and in most cases improves complexity over multiple reproductive life cycles. To achieve this goal the environment evaluates stochastic changes in the material inheriting the organism's organizational information, and the information thus selected is transmitted from one generation to the next. Thus, evolution is in essence an information-creating (in some cases information-destructing) process, and the inherited information characterizing each organism may be called the species-specific phylogenetic memory.

Animals also collect and store individual memories on a short timescale through learning by experience. Information based on repeated experience gathered by an individual organism in attempts to cope with the immediate demands of the environment is evaluated through environmental feedback, either reinforcing or reducing the impact of the underlying cellular mechanisms on the organism's future responses. Individual memory is therefore based on learning, which is the capacity to modify the individual's behavior on a relatively permanent basis through the acquisition of new information based on individual experience. Both kinds of memory, phylogenetic and individual memory, can be understood as organismic devices to predict the future and to reduce the environment's uncertainty. They are thus instantiations that link the past with the future, creating a present that is better adapted to the organism's requirements. Individual memory is incorporated into the framework provided by the phylogenetic memory, facilitating and shaping individual learning processes. Thus, the framework within which experience-based learning occurs is tuned to the demands expected during the individual organism's lifetime.

Cognition can be viewed as the integrating process that utilizes both forms of memory, creates an internal representation of the world and a basis for expecting the future of the animal's own actions within the experienced environment. It thus allows the animal to decide between different options in reference to the expected outcome of its potential actions. All these processes occur as intrinsic properties of the nervous system and provide an implicit form of knowledge for controlling behavior. None of these processes need to – and certainly will not – become explicit within the nervous systems of most invertebrates, but, as this review will show, such processes must be assumed to also exist in invertebrates with proper nervous systems (albeit over a large range of levels of complexity) to account for their behavior.

The results of these integrating cognitive processes are manifold and can span a gradient of possibilities. In such a gradient, at one extreme, one can find organisms dominated by their inherited information and having minimal experience-based adaptation; at the other extreme, phylogenetic memory may merely provide a broad framework, and experience-based memory will dominate. The factors determining the specific combination and weights of inherited and experience-dependent memories in an individual are not yet well understood. A short individual lifetime, few environmental changes during a lifetime, and highly specialized living conditions will favor the dominance of inherited information; a longer individual lifetime, less adaptation to particular environmental niches, and rapid environmental changes relative to the life span reduce the impact of phylogenetic memory and increase the role of individual learning. Social living style also seems to be a defining factor for the balance between these different forms of memory. In social animals, learning has to play an important role, because the species' genome must equip the individuals for acting under much

more variable environmental conditions because of the society's longer lifetime, and because the communicative processes within the society demand a larger range of cognitive processes.

It has also been suspected that the complexity and size of the nervous system may be related to the dominance of inherited or experience-dependent memories, in the sense that individual learning demands a larger nervous system having greater complexity. However, the primary parameter determining the size of the nervous system is body size, and secondary parameters such as richness of the sensory world, cognitive capacities, and abundance of motor patterns are difficult to relate to brain size, because such parameters cannot be adequately measured, and thus a comparison based on them is practically impossible between animals adapted to different environments. Although the relationship between brain size and cognitive capacity is still unclear, it is obvious that animals differ with respect to their sensory, motor, and cognitive capacities (see Evolution of the Elephant Brain: A Paradox between Brain Size and Cognitive Behavior). Individual learning within the species-specific sensory and motor domains will lead to more flexible behavior, and thus to more advanced cognitive functions. Predicting the future will therefore be less constrained, and more options will enrich the animal's present state.

Although we do not attempt to strictly define the cognitive components of behavior, the essential characteristics of cognition are: (1) rich and cross-linked forms of sensory and motor processing; (2) flexibility and experience-dependent plasticity in choice performance; and (3) long-term (on the timescale of the respective animal's life span) adaptation of behavioral routines. These three features allow the creation of novel behavior through different forms of learning and memory processing. Among them, we can cite (1) rule learning, (2) observatory learning during navigation and imitation, and (3) recognition of group members and individuals in a society. All these characteristics are based on implicit forms of knowledge and do not require any explicit (or conscious) processing. However, internal processing at the level of working memory (or representation) as an indication of rudimentary forms of explicit processing may exist in invertebrates within the context of observatory learning and social communication, and will be discussed.

We shall first consider forms of behavior that are dominated by innate components, and raise the questions of how innate and learned behavior are related and whether innate behavior involves cognitive components. The following sections focus on experience-dependent forms of behavior and follow in the order of ascending complexity: from elemental forms of associative learning to rule learning and observatory learning in navigation and communication. A key component in cognition is memory, not only as a storing device, but also in the form of working memory, the implicit form of representation which may provide the substrate for neural operations underlying decision making in relation to the expected outcome of the animal's actions. Both aspects of memory will be discussed.

1.26.2 Dominance of Innate Behavior

Invertebrates are dominated to a great extent by innate behavior, and some researchers have gone so far as to characterize insects and crustacea as "mindless machines" (Gould and Gould, 1982). Historically, innate preferences for biologically relevant sensory cues were used to characterize innate behavior. This view, which is still represented in ecological approaches to invertebrate behavior, assumes that invertebrates are irresistibly attracted to some sensory cues, which act as magnets on different phases of invertebrate behavior. At the origin of this notion, one can certainly find the preconception that invertebrates are essentially reflex machines with limited forms of experience-dependent plasticity. Although we shall focus on learning-related flexibility as a characteristic of invertebrate cognition, it is worthwhile to first recognize the complexity and richness of innate sensory–motor routines and their relationship to learned behavior.

1.26.2.1 Selection of Habitat, Feeding, and Foraging

Earthworms not only eat their way through the soil, swallowing the dirt they encounter, but feed selectively in many ways, as described by Darwin (1882). They leave unsuitable ground and select more productive soil, and they crawl out of their burrows at night to feed on the leaves of certain plant species. The earthworm grasps the tapered tip opposite the petiole, and then pulls the leaf back into the burrow to be eaten underground (Edwards and Lofty, 1972). Selection of habitat and food sources is commonly based on 'search images', innate sensory–neural filters that inform the animal about the desired food. Innate preferences are difficult to demonstrate because they require an absolute control of the animal's experience prior to the experiments in which it is confronted with the stimuli to be tested. If such control is absent, it is practically impossible to conclude anything about potential innate preferences, because the animal's

choice may simply reflect its previous experience with natural stimuli in its environment. Despite the difficulty of achieving such a control, some studies have appropriately addressed the question of innate preferences and showed that such preferences do indeed exist. However, they have also pointed out that choice behavior driven by this programmed signal responding is subjected to dramatic modifications based on first experiences. Thus, although such preferences may exist, they seem to be essentially useful in guiding the animals' behavior in their first confrontations with the external world.

A clear example of changes in innate preferences due to previous experiences is provided by the cuttlefish *Sepia officinalis*. In this animal, Darmaillacq *et al.* (2004a) recently showed that on the third day post-eclosion, when the young cuttlefish have consumed their vitellogenic resources, they exhibit clear preference for shrimps over crabs and young fish of comparable sizes. However, by making the preferred prey distasteful with quinine, it is possible to induce a preference for an originally nonpreferred prey item in 3-day-old and naïve cuttlefish, demonstrating the flexibility of this initial behavioral preference in response to previous individual experience. Such a learning is extremely fast (Darmaillacq *et al.*, 2004b), a fact that indicates to what extent innate preferences may be easily modified.

In insects, several studies have addressed the question of appetitive choice behavior as driven by innate preferences. In the moth *Helicoverpa armigera*, for instance, Cunningham *et al.* (2004) have shown that experience-deprived adult moths raised in isolation exhibit innate preferences for phenylacetaldehyde when tested in dual-choice wind tunnel tests. Again, this preference changes rapidly if a nonpreferred volatile substance is paired with a feeding stimulus (sucrose solution). In honeybees, a similar result was found with respect to color preferences. Honeybees whose visual experience was strictly controlled in a flight cage in which no rewards were provided on colored targets showed consistent preferences for some dominant wavelengths when presented with such targets in their first active foraging flight (Giurfa *et al.*, 1995). Colors preferred by naïve bees were human-blue and human-yellow, which correspond to those hues that experienced bees learn faster and better when trained with a single color/sucrose reward association (Menzel, 1967). However, all other hues which are originally not chosen rapidly become preferred if they are explicitly paired with sucrose reward (Giurfa *et al.*, 1995).

The intimate connection between innate and learned behaviors and their mutual replacement for behavioral control also becomes obvious in host selection by the two species of the stem-borer parasitoids *Cotesia glomerata* and *Cotesia flavipes* (Hymenoptera, Braconidae) (Geervliet *et al.*, 1998; Potting *et al.*, 1997; Vet, 1996; Vet *et al.*, 1995). While *C. flavipes* exhibits innate preference for its host's odors, the larvae of *Pieris brassica* (Lepidoptera), the closely related *C. glomerata*, learns the varying odor profiles of its Pieris host larvae, which depend on the plants it feeds on. No other differences in behavior between these two species were found, indicating that experience-dependent adjustment and innate stereotypy are two close strategies and are not related to any great differences between the neural systems involved.

The interesting question of whether innate preferences are deleted or inhibited by experience (and thus available at different moments of an invertebrate's life) has been addressed in bumblebees (Gumbert, 2000). Naïve bumblebees show color preferences that are similar to those exhibited by honeybees. After being trained to a given colored target by pairing it with sucrose reward, the bees chose novel test colors according to their similarity to the trained color. Thus, bees generalized their choice from the trained to novel, similar colors; however, if the trained and the test colors were perceptually dissimilar to the bees such that generalization did not occur, the original innate color preferences reappeared and guided the bees' choices. Thus, bumblebees show innate preferences for certain colors not only prior to color learning but also after intensive learning when choosing among very different novel colors (Gumbert, 2000).

These chosen examples among many similar ones from other invertebrates allow us to conclude that (1) invertebrates exhibit innate preferences for signals allowing to rapidly and efficiently detect biologically relevant stimuli in their first encounters with them; (2) such preferences can be drastically modified by the animals' experience such that plasticity and not rigidity of behavior should be underlined in the case of innate preferences; and (3) such preferences can be available in experienced adults such that they can be retrieved under particular circumstances, when the learned information is no longer useful. It is still unknown how these preferences can be hard-wired in the naïve invertebrate nervous system, but they have been selected through the species' evolutionary history and thus belong to its phylogenetic memory.

1.26.2.2 Early Programmed Learning

Early programmed learning occurs in young animals exposed to particular stimuli during a critical period. As a consequence of this process, also called imprinting, the animal later prefers the 'imprinted' stimuli. This kind of programmed learning has also been documented in invertebrates, particularly in insects. For instance, mosquitoes *Culex quinquefasciatus* use chemical cues to locate suitable water pools for oviposition. An individual mosquito's preferences for these odors can be altered greatly by prior experience (McCall and Eaton, 2001). In particular, change in odor preferences could be demonstrated following exposure to the odor during development or pupal eclosion, suggesting that some form of larval or early adult imprinting occurred.

In social Hymenoptera, parental relationships involve the use of specific signals, some emitted by the young animals, and others by the adults. Some of the signals are learned during an early experience (Dobson, 1989). The behavioral responses of parasitic wasps to chemical cues from their hosts and host plants are known to be affected by genetic and environmental components. Gandolfi *et al.* (2003) have shown that true pre-imaginal learning of olfactory cues occurs in the parasitic wasp *Hyssopus pallidus*. While parasitoids are not able to learn the fruit cues in the adult stage, exposure to fruit odor at early pre-imaginal stages significantly increases the adult response to frass from fruit-fed caterpillars (Gandolfi *et al.*, 2003). The olfactory memory persisted through metamorphosis, with a retention time of 14 days. Pre-imaginal learning was not confined to fruit cues, but was also demonstrated for menthol, an olfactory host- and fruit-independent cue.

A fascinating case of imprinting in insects is provided by studies on slave-making ants, which are characterized by socially parasitic founding of colonies (i.e., colonies in which two species of social insects coexist, one of which is parasitically dependent on the other) and the pillage of broods from neighboring host colonies during slave raids. Slave-making ants invade colonies of other ant species and transport the pupae back to their own nest. Adults emerging from these pupae react and work for the slave-making species as if it were its own species (Isingrini *et al.*, 1985; Carlin and Schwartz, 1989). The basis for such phenomenon may be olfactory imprinting processes by which the slave ants learn to recognize the slave makers as members of their own species. This hypothesis is subject to debate because it has been argued, for instance, that the capacity of larvae to imprint on the odor of their natal nest might make an ant species unsuitable for slave makers. An ant worker pillaged as pupa after pre-imaginal imprinting might identify slave-maker brood as alien and refuse to care for them (D'Ettorre and Heinze, 2001). However, the problem is solved if the hydrocarbon cuticular profiles, the distinctive olfactory mark of each species, of both the slaves and the slave makers are similar. This seems to indeed be the case (Lenoir *et al.*, 2001; D'Ettorre *et al.*, 2002).

The mechanistic basis of early olfactory learning has been studied in some insects. In the fruit fly *Drosophila melanogaster*, Devaud *et al.* (2003) showed that synaptogenesis in the antennal lobe, the primary olfactory neuropile in the insect brain, starts in late pupa and continues during the first days of adult life, at the same time as the behavioral response to odors matures. The antennal lobe is made up of functional units with a globular structure called the glomeruli, which can be recurrently identified due to their position and morphology. Individual olfactory glomeruli (DM6, DM2, and V) in *Drosophila* display specific growth patterns between days 1 and 12 of adult life. Experience can modify the olfactory pathway both structurally and functionally, as shown by adaptation experiments. The modifications associated with this form of learning seem to take place at a critical age. Exposure to benzaldehyde at days 2–5 of adult life, but not at 8–11, causes behavioral adaptation as well as structural changes in DM2 and V glomeruli. Taken together, these data demonstrate an imprinting-like phenomenon in the olfactory pathway of young *Drosophila* adults, and illustrate its glomerulus-specific dynamics.

1.26.3 Elemental Forms of Associative Learning

Rapid changes in environmental contingencies require flexible capacities with which organisms can come to expect biologically significant events and modify their behavior in anticipation of those events if behavior is to remain adaptive; that is, increase the probability of obtaining beneficial consequences and avoiding harmful ones (Reif, 1998; Sutton and Barto, 1998; Dickinson and Balleine, 2002). The acquisition of such anticipatory behavior relies on simple associative principles.

These principles are centered on computing error signals: whenever the current expectation is violated, error signals trigger processes in the brain which serve to both keep the animal's expectation

up-to-date and minimize such errors. The most potent error signals are generated by unexpected situations or events with biological relevance (i.e., an unconditioned stimulus, US): upon first perceiving such an unexpected US, coincidence detectors evaluate any neural activity preceding or overlapping with the error signal generated by the US. *A priori* it is irrelevant if this neural activity stems from the animal's behavior (abbreviated: BH) and/or from any environmental stimulation (i.e., a conditioned stimulus, CS). The preceding events can serve as predictors of the US, if their temporal connection with the US is consistent enough. Depending on the state of the animal and the nature of the environment, several more encounters with the US and its preceding events are required for a reliable memory to form. A reliable memory means that fully expected USs no longer generate any error signals, and hence the animal does not need to update its expectations any further. In such a state, the animal can behave adaptively: it expects the consequences of any behavior or situation.

There are two basic ways in which such memories can be acquired. In operant (or instrumental) conditioning the behavior (BH) of the animal is essential for these memories to form; indeed, it is the predictor by which the animal controls the occurrence of the US. If, on the other hand, the animal's behavior is dispensable for learning to occur, the situation is termed classical (or Pavlovian) conditioning. In freely moving animals, these two systems work together to guide adaptive behavior; Pavlovian learning often provides information to guide the selection of a particular behavioral strategy. But these systems can also work independently and even in opposition to one another (Ellison and Konorski, 1964; Balleine, 2001).

1.26.3.1 Invertebrate Classical Conditioning

The main associative principle for the conceptual understanding of classical conditioning is the notion of stimulus substitution (Pavlov, 1927). In the naïve animal, the CS initially triggers no or little response, that is, there is a transmission block from the sensory pathway to the motor circuits. If this CS is paired consistently enough with an initially unexpected US, the locations in the nervous system where the sensory information about the two events converge are modified such that the CS now comes to elicit a behavioral response, often mimicking the response to the US. The pairing has created new connections or removed transmission blocks so that the sensory circuitry processing the CS can now access some of the response-producing motor circuits that were previously engaged only by the US. By acquiring some of the response-eliciting properties of the US, the CS prepares the animal for the US and minimizes the error signals that future USs generate.

For instance, in *Aplysia* skin, sensory neurons make direct synapses onto motor neurons that control the defensive gill withdrawal reflex. Upon a light touch to the naïve animal's siphon, these sensorimotor synapses fail to conduct and the gills are not withdrawn. However, if the touch is repeatedly paired with a noxious stimulus (such as an electric shock) which does elicit gill withdrawal, the light touch alone eventually comes to elicit a gill withdrawal, whereas before such training it did not (see, e.g., Kandel *et al.*, 1979, 1983; Walters *et al.*, 1979, 1981; Carew *et al.*, 1981, 1983, 1984; Kandel and Schwartz, 1982; Hawkins *et al.*, 1983, 1986, 1989, 1998; Walters and Byrne, 1983a, 1983b; Abrams and Kandel, 1988; Byrne *et al.*, 1990). This increase in synaptic efficacy (facilitation) is brought about by the action of the neuromodulator serotonin released by modulatory interneurons during the electric shock. The sea slug *Aplysia* was most helpful for identifying synaptic facilitation as an instance of stimulus substitution and thus establishing a neurobiological basis for a psychological concept.

The discovery of such simple biological processes was of tremendous importance for the study of classical conditioning in more complex invertebrate brains such as those of insects. This can be exemplified in olfactory learning. The similar structure of the first few processing stages of smell in vertebrates and invertebrates is intriguing. Sensory neurons expressing the same olfactory receptor converge on aggregates of glomerular neuropil structures, where they synapse onto local interneurons and projecting neurons. In both vertebrates and invertebrates the pattern of neural activity in the glomeruli is odor specific. In insects, the projection neurons transmit this pattern to the next synapse in the calyces of the mushroom bodies. Evidence from aversive conditioning in *Drosophila* and from appetitive conditioning in *Apis* points toward an initial CS conduction failure either at the input synapse, or the output synapse of the mushroom body Kenyon cells, or suggests additive effects at both (Heisenberg, 2003). In *Drosophila*, a group of animals is exposed to one of two initially equivalent odors at a time in a vial coated with an electried grid (e.g., Tully and Quinn, 1985; Tully, 1991; Tully *et al.*, 1994). One of the odors is paired with an electric shock. After a few such odor–shock pairings, the animals are exposed to each of the odors

from opposing vials in a decision chamber and have to make a choice between the odors. In a successful conditioning experiment, the large majority of flies will avoid the odor associated with the shock. In the honeybee, individually harnessed hungry animals initially rarely respond to an odor presentation with an extension of their mouthparts (proboscis), whereas they will almost invariably do so if a sugar solution touches their antennas (e.g., Menzel, 1990; Menzel and Müller, 1996). If the odor is paired with the sucrose stimulation even only once, a stable memory of this association is formed and the animal will exhibit the conditioned proboscis extension response (PER) to future presentations of the odor alone. In both insects, the information about the identity of the odors is relayed to the behavior-initiating centers deep in the brain via the glomerular olfactory lobes and the mushroom bodies. Neurons containing the neuromodulators octopamine and dopamine have been found which could modulate any of the synapses in this pathway (Hammer, 1993, 1997; Schwaerzel *et al.*, 2003). Interestingly for the notion of expectation and anticipation, the octopaminergic neuron VUM_{mx1} exhibits activity paralleling the animal's expectation: it responds to unexpected sucrose presentations, but not to expected ones (Hammer, 1993, 1997). While there is evidence in vertebrates of how this reduction in the error signal may be implemented biologically, such evidence is still lacking in invertebrates. Conditioning using the PER has been so successful that it has been established in other insects such as moths and flies (Medioni and Vaysse, 1975; DeJianne *et al.*, 1985; Holliday and Hirsch, 1986; Brigui *et al.*, 1990; Fan *et al.*, 1997; Fresquet, 1999; Hartlieb *et al.*, 1999; Daly and Smith, 2000; Fan and Hansson, 2001; Skiri *et al.*, 2005) and for use with mechanosensory stimuli (Giurfa and Malun, 2004).

There is another advantageous snail model system for predictive learning, the pond snail *Lymnaea*. Its feeding behavior can be classically and its respiratory behavior operantly conditioned. Appetitive classical conditioning of *Lymnaea* feeding behavior involves either a tactile (touch to the lips) or a chemical (amylacetate) CS and a sucrose US (Kemenes *et al.*, 2002; Jones *et al.*, 2003; Staras *et al.*, 2003; Straub *et al.*, 2004; Fulton *et al.*, 2005; Korneev *et al.*, 2005). A single presentation of either CS with a sucrose solution will enhance *Lymnaea*'s bititing frequency in the presence of future CSs presented without the sucrose. Changes in neuronal activity and cellular properties that were recorded following tactile conditioning occur at all levels of the system, including central sensory pathways, modulatory interneurons, central pattern generator (CPG) interneurons, and motoneurons involving several possible plasticity sites. *Aplysia* biting behavior can be conditioned similarly (Lechner *et al.*, 2000a, 2000b). A brief touch to the lips with a small brush (CS) does not usually elicit a bite. But when this touch is consistently paired with a food reward (US), the touch alone suffices to elicit the bite. This procedure has been transferred to a reduced preparation, where peripheral nerve stimulation replaces the CS and US and motor nerve recordings monitor the behavior (Mozzachiodi *et al.*, 2003).

A third molluskan model system, the predatory snail *Hermissenda*, has revealed principal aspects of classical conditioning. The conditioning procedure consists of pairing light CS with high-speed rotation, or orbital shaking (US) which are aversive stimuli for the snail (Crow, 2004). Two responses are elicited by rotation, a reduced rate of forward locomotion and foot shortening. The two sensory structures mediating the CS and US are central, and thus their synaptic projections remain intact after isolation of the nervous system. Because the neurons that contribute to the neural circuitry controlling the unconditioned responses (URs) and conditioned responses (CRs) are identified, and can be studied in semi-intact nervous systems, an explanation is within reach of how conditioning is expressed in behavior generation.

Recently, earthworms have also been classically conditioned to anticipate potentially dangerous illumination after a brief vibration by longitudinally contracting their body (Watanabe *et al.*, 2005). Other invertebrate model systems for classical conditioning include cockroaches and crickets (Matsumoto and Mizunami, 2002, 2004; Matsumoto *et al.*, 2003; Watanabe *et al.*, 2003; Kwon *et al.*, 2004; Lent and Kwon, 2004; Pinter *et al.*, 2005).

The notion of predictability and error signal minimization developed mainly from classical conditioning experiments. In the course of these experiments, a number of paradigms were developed which now can be used to probe invertebrate preparations for the biological substrate underlying these learning phenomena. The first hint at predictability at the chore of classical conditioning came from its strict dependence on the relative timing of CS and US. In order for classical conditioning to occur, the onset of the CS has to occur before the onset of the US. Simultaneous or reverse (backward) pairings yield very little to no learning (Hellstern *et al.*, 1998). This result also contradicts the notion that simple pairings were sufficient for conditioning to occur. The next challenge to the simple pairing

hypothesis was to randomly add US-alone presentations, so as to render the CS nonpredictive, but to preserve the total number of paired CS–US presentations from successful experiments. Strengthening the predictability hypothesis, a US that is presented equally often with or without the CS prevents any CS–US conditioning that would normally have occurred (Rescorla, 1968). More sophisticated experiments included a second CS. In sensory preconditioning (Brogden, 1939; Kimmel, 1977), CS1 and CS2 are first presented together, without any US. In a second training phase, one of them (CS1) is paired with the US until the CS1–US association is formed. In the test phase, CS2 is tested alone. Successful sensory preconditioning experiments also contradict the simple pairing hypothesis, since CS2 was never paired with the US (Brembs and Heisenberg, 2001; Guo and Guo, 2005; for the honeybee, Müller *et al.*, 2000). In blocking experiments (Kamin, 1968), a first training phase consists of CS1–US pairings. In the second training phase, CS2 is added, so that the compound CS1–CS2 is paired with the US. The idea behind this experiment is that the prediction error signal for the US is zero after the first training phase (CS1 fully predicts every US occurrence) and as such nothing will be learned about the 'redundant' CS2. Hence the term 'blocking': the learning about CS1 blocks any learning about CS2, even though it was paired with the US sufficiently to produce classical conditioning had the first training not occurred (see Brembs and Heisenberg, 2001 for invertebrate literature on blocking). In the honeybee, the data on blocking are controversial (Smith and Cobey, 1994; Gerber and Ullrich, 1999). It is yet to be resolved whether the kind of odor stimulus (Linster and Smith, 1997) or the adequate control group will help to determine that blocking is a reliable phenomenon.

Perhaps not very surprisingly, all mechanisms and changes found to subserve classical conditioning in any of the above-mentioned model systems affect the CS pathway, emphasizing the universal role stimulus substitution plays in classical conditioning.

The relationship between classical conditioning procedures and predictive learning has often provided the justification for using this paradigm to study the neural bases of animals' basic cognitive or representational capacities. Nevertheless, it is rarely recognized that, at an adaptive level, cognitive capacities, such as those involved in encoding the predictive relations between stimuli, can be of little functional value to the purely Pavlovian organism. The sensitivity of cognitive systems to sources of information would seem more likely to provide the basis for modifying, even withholding or reversing, the direction of behavior in response to that information, something that demands greater behavioral flexibility than the system mediating classical conditioning provides. Hence, through developing an understanding of operant conditioning, one may actually come closer to establishing the functional role of cognitive processes in behavior.

1.26.3.2 Invertebrate Operant Conditioning

Just as in classical conditioning, error signals from unexpected events drive operant conditioning. The main associative principles using these signals are the concepts of initiating and gating behavioral activity. In the naïve animal, a behavior initially without a biologically relevant consequence is suddenly followed by the perception of an unexpected US. In sufficiently ambiguous situations, the animal has to first initiate a number of behavioral programs in order for the coincidence detectors to successfully cross-correlate this behavior with the novel US ('trying out'): any of its behaviors around the given time may have triggered the US. The significant cross-correlation between behavior and US eventually leads to modifications that act as a gate, letting only the behaviors pass that will maximize reward or minimize punishment (operant behavior). It is hypothesized that during operant conditioning separate processes modify the subsequent, more persistent initiation of behavior (operant memory). In contrast to classical conditioning, there is evidence from invertebrates on how the error signals may be generated in the brain. Whenever an action is initiated, a corollary discharge (or efference copy) is fed forward as a control signal to be compared with the sensory input. The difference between this control signal (expectation) and the actual sensory input generates the error signal (for a review, see Webb, 2004).

Isolating a behavior from any consequences other than a single biologically significant one (i.e., a US) is difficult. Hence, it is not surprising that there are only a few operant conditioning experiments which have demonstrated a reduction analogous to classical conditioning (one predictor – one consequence, BH → US).

Drosophila suspended at the torque meter is probably as isolated from operant feedback as technically possible in an intact animal (Heisenberg *et al.*, 2001). Glued at head and thorax to a copper wire hook, it is clamped to a device that measures the fly's yaw torque (yt) (i.e., the force the fly generates when it attempts to turn about its vertical axis). The animal is tethered completely motionless

and its visual environment is entirely homogeneous (since the head is fixed). In such a constant stimulus situation the animal may beat it wings, move its legs, bend the abdomen, or extend the proboscis with no consequences at all. The experimenter may choose to make a biologically relevant stimulus (e.g., an unpleasant heat beam, US) contingent on one of the behaviors the animal generates. For the fly, it means that without any warning from the environment, temperatures suddenly rise to an unpleasant level. The animal has to find out how to switch the heat off. There is no way the animal can know that the experimenter coupled the heat with yt values that correspond roughly to, say, right turns. The animal has to find this correlation by trying out different behaviors until it finds the correct flight control maneuvers that eventually lead to more tolerable temperatures. This is a completely arbitrary situation in which the animal has to spontaneously produce a range of behaviors. This array of different motor outputs must then be cross-correlated with the sensory input. Only behaviors with significant cross-correlations are to be maintained (gating). In principle, such a gating mechanism is neurobiologically straightforward: the neurons generating the relevant behaviors are active when the neuromodulators signaling the heat on- and offset are being released. These neurons will be modified by activity-dependent plasticity to bias any future behavior toward the behaviors controlling the heat. Such a process has been found in a similarly isolated behavioral preparation, that is physiologically much more accessible, the operant conditioning of feeding behavior in *Aplysia* (Nargeot *et al.*, 1997, 1999a, 1999b, 1999c; Brembs *et al.*, 2002, 2004; Brembs, 2003a, 2003b). In this paradigm, spontaneous bites (or the corresponding neural patterns in the reduced preparation) are rewarded by dopamine-releasing nerve stimulation. A series of experiments suggests that dopamine-dependent modifications in the biophysical properties of a neuron that is active late during the rewarded behavior (and is critical for the type of behavior – biting) are partly responsible for the increased probability of emitting biting behavior in contingently reinforced animals as compared to control animals.

Thus, similar to classical conditioning, the psychological concept of behavior selection in operant conditioning could be related to a neurobiological mechanism.

However, it is more difficult to imagine the neurobiological mechanism which modifies behavior initiation during operant conditioning. After all, the initiation of the behavior may take place quite some time before the US is perceived and therefore the neurons initiating behavior may have been silent for any amount of time. At the time of this writing, there is only one hint as to whether behavior-initiating neurons actually are modified, but there is no data about any possible mechanisms by which such modification may occur. One is tempted to draw from the concept of the efference copy to hypothesize about how this gap may be closed. Efference copies are corollary discharges during behavior that allow the animal to discern self-motion from passive motion. The connection of this neural activity with the sensory organs and the motor circuits make efference copies suitable candidates for the memorization of potential consequences of their respective actions and thus the link between behavior selection and initiation (Webb, 2004).

While there is no data confirming or falsifying these speculations to date, there are more operant conditioning paradigms which may be helpful in understanding the biological implementation of cognitive concepts in invertebrates.

The honeybee antennae can be operantly conditioned to assume a certain posture (Kisch and Erber, 1999). In a more reduced preparation, the activity of the fast flagellum flexor muscle is recorded extracellularly from the scapus of the antenna (Erber *et al.*, 2000). Whenever the muscle activity exceeds a defined reward threshold, the animal is rewarded with a drop of sucrose solution. After several US deliveries, the frequency of the muscle potentials increases significantly over the spontaneous frequency. The conditioned changes of frequency can be observed for 30 min after conditioning and do not occur in yoked controls, which were rewarded independent of nerve activity.

Aversive operant conditioning of *Lymnaea*'s aerial respiratory behavior involves stimulating the animal's pneumostome when it surfaces and attempts to breathe (Lukowiak *et al.*, 1996, 1998, 2000, 2003; Lukowiak and Syed, 1999; Spencer *et al.*, 1999; Haney and Lukowiak, 2001; McComb *et al.*, 2002, 2003; Sangha *et al.*, 2002, 2003; Scheibenstock *et al.*, 2002; Spencer *et al.*, 2002). The stimulation is aversive, which leads to the animal closing the pneumostome and learning as training progresses to reduce its attempts to open the pneumostome. Instead of aerial respiration, the animals rely on cutaneous respiration for oxygen and hence it could be shown that the decrease in pneumostome openings was not due to a generally detrimental effect of the procedure. The neural network mediating the behavior is simple and well studied, making *Lymnaea* a very promising model system for the understanding of behavior initiation and selection.

1.26.3.3 Invertebrate Composite Operant Conditioning

Of course, in freely moving animals, a CS rarely occurs independent of all the animal's behaviors, just as a US will very rarely be contingent on a behavior independent of any other stimulus present. Such situations (i.e., with a BH, a CS, and an US present) are called composite operant conditioning. *Drosophila*, *Aplysia*, and the honeybee are also the most widely used model systems for studying composite operant conditioning. Exploiting their unique technical advantages, one paradigm each in both *Drosophila* and *Aplysia* has been explicitly developed to combine operant and classical components in a single preparation. If one adds coloration of the (unpatterned) arena as a CS to the operant yaw torque learning paradigm described above for *Drosophila*, one creates an instance of composite operant conditioning (Heisenberg *et al.*, 2001). Whenever the yaw torque of the fly is in one of the two domains (roughly corresponding to left or right turns, respectively), the arena surrounding the animal is illuminated in one color; if the yaw torque moves into the other domain, the coloration changes as well. Thus, yaw torque and color become equivalent predictors of the punishing heat. This switch (sw)-mode learning causes a larger aftereffect than yaw torque learning. This increased effectiveness of composite over purely operant or classical conditioning was observed previously, comparing classical conditioning to flight-simulator (fs) mode (Brembs and Heisenberg, 2000). In this setup, the angular velocity is calculated from the online torque signal that this momentum would give the fly. But instead of turning the fly, the yaw torque is made to turn the patterned arena around the fly in the opposite direction. This arrangement enables the fly to stabilize the arena, that is to fly straight with respect to the patterns on the arena wall, and to choose 'flight directions' with respect to these patterns. Before the training, the fly shows a moderate fixation of the patterns without a striking preference for one or the other. During training, the punishing heat beam is applied whenever the fly chooses a flight direction toward one of the pattern types. If after a few minutes of training the heat is permanently switched off the fly still prefers flight directions toward the previously 'cold' patterns.

Is visual pattern discrimination learning operant? The fly learns to associate the patterns (CS) with heat and 'no-heat' (US), meeting the definition of classical (Pavlovian) conditioning. Could the operant behavior in fs-learning merely accompany a learning process which in essence is classical? The standard experiment to answer this question is a 'yoked' control (Brembs and Heisenberg, 2000). Exact sequences of pattern position and heating periods are recorded during operant training and are played back to a naïve fly. The replay control is a purely classical conditioning experiment, since the fly has no influence on the stimuli presentation. This kind of training is considerably less effective than the training operantly controlled by the fly. Thus, just as the sw-mode learning is more effective than the yt-learning, the training in fs-learning is more effective than in replay control. In other words, in both cases the three-term contingency (CS → US, BH → US) is more efficient than the two-term contingency (BH → US in yt-learning; CS → US in the yoked control of fs-learning). The interesting difference is that in fs-learning the behavior is not directly modified (Brembs and Heisenberg, 2000), whereas in sw-learning the spontaneous yt-distribution is altered just as in yt-learning. This allowed investigating which of the components are learned in the three-term contingency (Heisenberg *et al.*, 2001). These studies showed that in both fs- and sw-learning only the classical but not the operant association is separately accessible, ruling out that the two act additively. Not summation but rather interaction between the operant and classical components characterizes the three-term contingency. Apparently, composite conditioning is so effective because learning about sensory stimuli is enhanced once the fly can control them by its own behavior, whereas behavioral modifications tend to be avoided (Heisenberg *et al.*, 2001). Experiments with robots have yielded similar results, supporting the notion of a general synergistic mechanism (Verschure *et al.*, 2003).

Similar questions can now be asked in a newly developed preparation in *Aplysia*. It was described above how the feeding behavior of *Aplysia* can be conditioned classically and operantly (Nargeot *et al.*, 1997, 1999a, 1999b, 1999c; Lechner *et al.*, 2000a, 2000b; Brembs *et al.*, 2002, 2004; Brembs, 2003a, 2003b; Mozzachiodi *et al.*, 2003). Taking advantage of the greater physiological accessibility, a reduced preparation of the isolated buccal and cerebral ganglia was used. This preparation still produces spontaneous nervous activity, which can be recorded as patterned motor output at selected motor nerves. These neuronal patterns can be related to feeding behavior in the intact animal. Stimulation of sensory nerves serves as CS and US input. Thus, all elements of the three-term contingency are present: BH, CS, and US. The preparation has been shown to produce robust learning effects; it

will be interesting to see if results can be found that are analogous to those in *Drosophila* and, if so, what their neural basis is (Brembs *et al.*, 2004).

Susswein and colleagues have developed another promising composite paradigm using *Aplysia* feeding behavior (Susswein and Schwarz, 1983; Schwarz and Susswein, 1984, 1986, 1992; Susswein *et al.*, 1986; Schwarz *et al.*, 1991; Chiel and Susswein, 1993; Botzer *et al.*, 1998; Katzoff *et al.*, 2002). Food touching the lips of *Aplysia* initiates biting, which causes food to enter the mouth. Swallowing is triggered by food within the mouth. Food or nonfood objects in the mouth can also trigger rejection (Kupfermann, 1974). In this aversive procedure, animals learn to avoid biting on inedible food items. Food is made inedible by wrapping it in a plastic net that the animals can neither swallow nor break. During training, the netted food touches the lips and the animal tastes the food through holes in the net. The animal bites, food enters the mouth and elicits swallows, which fail to convey the tough food to the gut. The food eventually is rejected. The food continues to stimulate the lips and elicit bites, which again lead to failed swallows. As training proceeds, the response of the animal to the food gradually changes. Food stays within the mouth for progressively shorter periods, eliciting fewer swallows and more rejections. Animals eventually stop responding to the food (Susswein *et al.*, 1986).

An instrument more suitable than the fs for using the powerful genetic techniques in *Drosophila* for operant conditioning is the heat box (Wustmann *et al.*, 1996; Wustmann and Heisenberg, 1997; Putz and Heisenberg, 2002). In the tiny, dark chamber, every time the fly walks into a designated half, the whole chamber is heated. As soon as the animal leaves the punished half, the chamber temperature reverts to normal. Even if the heat is switched off after a few minutes, the animals still restrict their movements to only one half of the chamber. Because it is completely dark in the chamber, the animal most likely relies on idiothetic cues to orient itself, thus minimizing the contamination with potential classical predictors. It can be shown that the operant memory consists of two components, a spatial component and a "stay-where-you-are" component (Putz and Heisenberg, 2002). One of the mutants found using the heat box is the 'ignorant' gene (Putz, 2003; Putz *et al.*, 2004). Interestingly, it appears that 'ignorant' has very different effects on operant and classical conditioning. The original mutant allele (ignP1) shows a sexual dimorphism in the heat box, where males are impaired but females appear normal (Putz, 2003; Putz *et al.*, 2004). Both males and females of that line are statistically indistinguishable from the wild-type controls in olfactory classical conditioning (Bertolucci, 2003). The null mutant ($ign^{58/1}$) shows decreased learning and memory in the classical case (Bertolucci, 2003), but is normal in the heat box (Putz, 2003; Putz *et al.*, 2004). Finally, several partial deletions of the *ignorant* gene are defective in the heat box (Putz, 2003; Putz *et al.*, 2004), but these lines have not yet been tested for classical conditioning. Apparently, different mutations of the 'ignorant' gene have different effects on operant and classical conditioning, indicating fairly well-separated mechanisms for both forms of learning.

Another composite paradigm in honeybees and bumblebees is visual discrimination learning in the Y-maze. The freely flying bees enter a triangular decision chamber on one side and can see two target objects at the ends of the two arms of the 'Y' attached to the other two sides of the triangular chamber. Only one of the two targets contains a sucrose reward and thus animals can be trained to associate one of the two targets with the reward. Repeating the procedure with the targets at both arms in random sequence establishes a visual discrimination memory that is independent of the location of the target and hence of the turning maneuvers needed to reach it. In this situation, bees learn to associate a given target with reward and the alternative target with the absence of reward such that the former is excitatory and the latter inhibitory (Giurfa *et al.*, 1999). Furthermore, bees are rewarded for every correct choice such that operant and classical associations certainly drive their choice within the maze. Several variants are known of this kind of visual learning in bees: from the simple visual target conditioning in which a bee has to land on a single rewarded stimulus, to training with a complex maze made from several connected boxes, at the end of which they get rewarded with sucrose solution (Zhang *et al.*, 1999), bees efficiently learn this kind of task which has allowed further insights into higher-order forms of learning (see Section 1.26.4).

1.26.4 Nonelemental Forms of Associative Learning

In previous sections we reviewed different forms of associative learning that can be described as elemental forms of learning. These forms have in common that they can be formalized as links that connect specific stimuli (in the case of Pavlovian conditioning), or a stimulus and a response (in the case of

operant conditioning). In most of the paradigms described, such links are established in such a way that they connect directly and unambiguously well-defined events in the animal's environment (Rescorla and Wagner, 1972). Simple links between an event (A or B) and reinforcement (+) (or its absence: –) allow solving elemental problems such as absolute conditioning (A+) and differential conditioning (A+ vs. B−). In the former, an animal has to learn to respond to A, which is unambiguously associated with reinforcement; in the latter, it has to learn to respond to A and not to B because A is unambiguously associated with reinforcement and B is unambiguously associated with the absence of reinforcement.

However, invertebrates are also capable of more complex forms of associative learning, which can be described as nonelemental forms of learning, and which in contraposition to elemental ones, do not rely on simple links between events. In the study of nonelemental learning, paradigms have been developed for which the associative strength of a specific event, stimulus or reaction, is ambiguous and therefore cannot be predictive for solving a problem. Such problems cannot be formalized, therefore, in terms of simple connectivity between events and their respective outcomes, because one or various events involved in the problem usually allow opposed outcomes (Pearce, 1994). For example: if a given stimulus, A, is rewarded as often as not rewarded, and the key for solving the problem is that it is rewarded whenever it is presented together with a different stimulus B, whereas it is nonrewarded when presented together with a third stimulus C (AB+ vs. AC−), the animal cannot rely on the pure associative strength of A to solve the problem. Two possibilities appear: (1) the nonelemental one, in which the animal learns that it is the configuration (AB or AC) which counts and which has to be learned, independent of the associative strength of the elemental stimuli A, B, C; and (2) the elemental one in which the animal learns to focus on B, which is always rewarded, and/or on C, which is always nonrewarded. This example, and its two possible interpretations which differ drastically, show that in studying whether animals exhibit nonelemental forms of learning, experiments must be carefully designed in order to avoid dual interpretations for the same behavioral outcome.

Standard paradigms have been developed by experimental psychologists to address this problem in an efficient manner (Rudy and Sutherland, 1992, 1995). Simple links between a stimulus and reinforcement do not allow solving nonelemental problems like negative patterning, biconditional discrimination, and the neutral discrimination feature (see discussion below). In these problems, each stimulus appears rewarded as often as nonrewarded such that linear solutions do not apply. In negative patterning, for instance, the subject has to learn to respond to the single stimuli A and B but not to their compound AB (A+, B+, AB−). This problem does not admit elemental solutions, since the animals learn that AB has to be different from the linear sum of A and B. In biconditional discrimination, the subject has to learn to respond to the compounds AB and CD and not to the compounds AC and BD (AB+, CD+, AC−, BD−). As in the previous problem, each element A, B, C, or D appears rewarded as often as nonrewarded such that it is impossible to rely on the associative strength of a given stimulus to solve the task. Finally, in feature neutral discrimination, the animal has to learn to respond to B and to the compound AC but not to C and the compound AB (B+, AC+, C−, AB−). In this case, each element is again ambiguous such that the animal has to learn the predictive value of the compounds AB and AC, independent of its composing elements, and the fact that B and C alone produce different outcomes than when in compound. These examples show to which extent more elaborated computational strategies are necessary in the case of nonelemental discrimination problems. In Section 1.26.6, other elaborated forms of nonelemental learning are discussed such as contextual learning, or rule learning, but here we focus on more formalized problems such as the ones just introduced, which allow discerning between linear and nonlinear problem solving. This distinction is pertinent not only with respect to the nature of established associations, but also with respect to the neural substrate subtending these different forms of learning (Rudy and Sutherland, 1995; Sutherland and Rudy, 1989). It has been suggested that different neuronal circuits and structures underlie elemental versus nonelemental learning in vertebrates, with a particular role assigned to the hippocampus and the cortical circuitry associated to it in the formation of nonelemental stimulus representations (Lachnit *et al.*, 2004; Rudy and Sutherland, 1995).

Lobsters placed in an aquarium can be aversively conditioned to stop searching in it by pairing an olfactory stimulus delivered in water with a mechanosensory disturbance produced by the experimenter (Livermore *et al.*, 1997). Lobsters were trained in this way with an olfactory compound AX reinforced (AX+). Conditioning was either absolute (AX+) or differential (AX+ vs. AY−). Both conditioning procedures yielded different results, thus showing that depending on the

conditioning schedule, lobsters treated and learned the compound differently (Livermore *et al.*, 1997). After absolute conditioning, lobsters inhibited their searching behavior when presented with AX but still searched when presented with A, X or with a novel odor Y. Similarly, a novel compound AY did not inhibit searching behavior. This result is consistent with learning the compound AX as an entity different from its components A and X (the configural view; Pearce, 1994). After differential conditioning, lobsters inhibited their searching behavior when presented with AX but not with AY. Interestingly, they also inhibited search when presented with the element X but not with the element Y. A was not useful as it was common to the reinforced and the nonreinforced compounds AX+ and AY−, respectively (Livermore *et al.*, 1997). In this case, lobsters seem to have learned the compounds AX and AY in elemental terms, thus being able to fully generalize their respective responses to X and Y.

In the honeybee, several recent studies have addressed the issue of elemental versus nonelemental learning, using visual training of free-flying animals or olfactory conditioning of harnessed animals. In both modalities, bees are able to solve a biconditional discrimination (AB+, CD+, AC−, BD−) – visual (Schubert *et al.*, 2005); olfactory (Chandra and Smith, 1998; Hellstern *et al.*, 1995). This capacity demonstrates that both visual and olfactory compounds are learned under certain circumstances as entities different from the simple sum of their elements. Similarly, negative patterning experiments (A+, B+, AB−) have also been performed both in the visual (Schubert *et al.*, 2005) and the olfactory modality (Deisig *et al.*, 2001, 2002). Such discrimination can only be explained if the compound AB is treated as being different from the simple sum of its elements. Two essential theories can be invoked for explaining this result; both are different from a purely linear approach based solely on the elements of a compound: the configural theory, mentioned above, which proposes that a mixture constitutes an entity different from its components ($AB = X \neq A + B$) (Pearce, 1994); and the unique-cue theory, which proposes that a mixture is processed as the lineal sum of its components plus a stimulus (u) that is unique to the joint presentation of the elements in the mixture ($AB = A + B + u$) (Whitlow and Wagner, 1972). In the latter case, the unique cue supports the inhibitory strength assigned to the compound. In the case of honeybee olfactory learning, computer simulations and experiments such as negative patterning and its variants (Deisig *et al.*, 2001, 2002, 2003) showed that olfactory compound learning and processing in bees was consistent with the unique-cue theory. A unique cue is generated when bees receive olfactory input to both brain sides, since bilateral olfactory input is required to solve a negative patterning task (Komischke *et al.*, 2003). Assuming that the elements interfered with each other is implied in a modified unique-cue theory (Redhead and Pearce, 1995), which provided the best account for all the behavioral results available (Deisig *et al.*, 2003; Lachnit *et al.*, 2004).

Another paradigm used to study nonelemental olfactory learning in bees is the side-specific olfactory conditioning (Sandoz and Menzel, 2001). In this case, a thin plastic wall separates the honeybee's antennas during olfactory stimulation. Bees were differentially conditioned using two odors: A and B. Bees were conditioned with A+ versus B− on one antenna and with A− versus B+ on the other. This discrimination resembles a form of contextual learning (see next section), since the context of each antennal side (left vs. right) determines the contingency of the stimuli. Bees learned to respond appropriately to the rewarded odor and to inhibit their reaction to the nonrewarded odor on each side (Sandoz and Menzel, 2001). They thus solved this side-specific, nonelemental discrimination.

This and two other paradigms were recently used to test mushroom body (MB)-ablated honeybees and to determine whether intact MBs are necessary to solve nonelemental olfactory discriminations (Komischke *et al.*, 2005). Bees with unilateral lesions of the MBs generated by larval treatment with hydroxyurea (Malun, 1998) were trained under different olfactory conditioning designs. When odorants were delivered in a side-specific manner, bees with unilateral MB lesions could not solve an unambiguous double discrimination (paradigm 1: A+, B− on one antenna, C+, D− on the other; $A + B-/C + D-$), whereas they could solve at least one of both discriminations of an ambiguous problem (paradigm 2: A+, B− on one antenna , A−, B+ on the other; $A + B-/A - B+$). In the latter case, they solved the discrimination proposed to their intact brain side. Nonablated bees could learn both side-specific discriminations. When odorants were delivered simultaneously to both antennas (paradigm 3: $A + B - C + D-$), ablated bees learned slower than normal bees. Thus, in all three cases, the unilateral loss of a median calyx affected olfactory learning (Komischke *et al.*, 2005). It was proposed that MBs are required for solving elemental olfactory tasks whose complexity is enhanced by virtue of the number of stimuli involved (paradigms 1

and 3: four stimuli) and that MB ablations could have an effect on the inhibition of information exchange between brain hemispheres. This exchange may or may not occur in normal circumstances, depending on the information stored in each brain side. MB lesions would impede the ablated side to block the transfer of information from the intact side in the side-specific ambiguous problem (paradigm 2).

Interestingly, cumulative experience seems to play a critical role for adopting elemental or nonelemental learning strategies (Giurfa *et al.*, 2003). Giurfa *et al.* (2003) trained free-flying bees to fly into a Y-maze to collect sucrose solution on a rewarded stimulus presented in one of the arms of the maze. Stimuli were color disks, violet (V), green (G), or yellow (Y), which were of equal perceptual salience for honeybees. Training followed an A+, BC+ design, followed by an AC versus BC test. Training consisted of 6 (3 A+ and 3 BC+), 20 (10 A+ and 10 BC+), or 40 (20 A+ and 20 BC+) acquisition trials. Elemental models of compound processing predict that in the test, a preference for the nontrained stimulus AC should occur while configural models predict a preference for the trained stimulus BC. After six training trials, bees favored an elemental strategy and preferred AC to BC during the tests. Increasing the number of training trials resulted in an increase of the choice of BC. Thus, short training favored processing the compound as the sum of its elements (elemental account) while long training favored its processing as being different from the sum of its elements (configural account). Additionally, it was observed that the change in stimulus processing was also influenced by stimulus similarity. Color perceptual similarity favored configural processing with increasing experience (Giurfa *et al.*, 2003), a result that was consistent with the results of honeybee olfactory compound conditioning (Deisig *et al.*, 2002).

It thus seems that some invertebrates, at least lobsters and honeybees, are capable of nonelemental forms of learning in the strict sense, and that such forms of learning are highly dependent on the way in which animals are trained, on the number of trials, and on the similarity between elements in a compound. Further factors favoring nonelemental compound processing and learning could be the spatial and temporal proximity of elements and the animals' previous experience. Further research should ask whether or not other invertebrate models can solve such nonlinear discrimination problems and determine the kind of processing underlying this problem solving.

1.26.5 Integration Across Sensory Modalities and Rule Learning

Animals, including invertebrates, can sometimes respond to novel stimuli that they have never before encountered or can generate novel responses that are adaptive given the context in which they are produced. In doing this, the animals exhibit a positive transfer of learning (Robertson, 2001), a capacity that cannot be referred to as an elemental form of learning because the responses are aimed toward stimuli that do not predict a specific outcome *per se* based on the animals' past experience. Moreover, animals can also learn to inhibit such a transfer in order to produce adaptive responses that can be linked to a specific context. They learn that, given a certain stimulus or condition, a particular response is appropriate, whereas, given a different stimulus or condition, the same response is no longer appropriate. This form of learning, usually referred as conditional learning or occasion setting, cannot be viewed as elemental learning, since a given stimulus may or not be predictive of a certain outcome, depending on the particular environment. Relying on its elemental outcome alone therefore does not help solving this kind of problem. In this section, we focus on these forms of nonelemental learning. We therefore deal with three main capacities: (1) selective attention, (2) rule learning, and (3) contextual learning. We start with selective attention, as it seems to be a necessary requisite for extracting the information that allows solving the other two problems.

1.26.5.1 Selective Attention

Selective attention consists of the ability to focus perceptual mechanisms on a particular stimulus and to actively process this information while ignoring nonrelevant stimuli (Zentall, 2005). It implies that the representation of the stimulus has been filtered or modified, presumably so that it can be processed or responded to more efficiently. Different approaches have been proposed to the notion of selective attention. Here we focus on the ecological notion of 'search image' (Tinbergen, 1960) and on the more traditional approach of discriminative learning to selective attention (Zentall and Riley, 2000).

Selective attention could be related to the notion of search image, since such images are assumed to exist in cruising animals in order to facilitate detection of relevant stimuli in the environment. Search images, which could be innate (see Section 1.26.2) or acquired through experience in the field, have been proposed to be a specific means for filtering

out sensory information and focusing perception on specific stimulus configurations as a way to more efficiently forage and avoid predation. Innate search images driving the first foraging flights are assumed in insect pollinators, in order to facilitate the detection of flower sources (Menzel, 1985). Indeed, naïve bees exhibit innate preferences for biologically relevant floral cues such as colors (Giurfa *et al.*, 1995; Gumbert, 2000) or bilateral symmetry (Rodriguez *et al.*, 2004). Salticid spiders have also an innate predisposition to form search images for preferred preys (spiders) rather than for nonpreferred preys (insects) (Jackson and Li, 2004).

Traditionally, selective attention has been studied through a discriminative learning approach. Such an approach posits that through selective attention animals gradually learn to attend to the dimension along which discriminative stimuli differ (Mackintosh, 1975). Honeybees (Giurfa, 2004) and bumblebees (Dyer and Chittka, 2004) discriminate differently a given color after absolute (training with a single reinforced color) and differential conditioning (training with a reinforced color and nonreinforced alternatives). They become progressively better in discriminating the trained color from colors that are perceptually close after prolonged differential conditioning while they are incapable of such discrimination after the same amount of absolute conditioning These results can be interpreted along the selective attention hypothesis such that insects gradually learned to attend to the spectral dimension along which discriminative stimuli differed. An alternative interpretation posits that differentially conditioned animals form positive and negative generalization gradients to the rewarded and the unrewarded stimulus, respectively, and thus develop a sharper generalization profile for the learned stimulus. An attentional account may also apply to pattern discrimination experiments in which bees do or do not discriminate the same two patterns depending on the kind of training used, absolute or differential conditioning (Giurfa *et al.*, 1999). Absolute conditioning promoted recognition based on local cues (the lower half of a disk made of different sectors) while differential conditioning expanded it to the whole pattern.

Arguments in favor of selective attention in invertebrates come from research on the fruit fly *D. melanogaster* (van Swinderen and Greenspan, 2003). A fly flying stationary within a circular arena and tracking a visual moving object (a vertical black bar) exhibits behavioral and neural processes consistent with selective attention for visual stimuli. Local field potentials recorded in the central brain of these flies show that activity in the 20–30 Hz range increases as a response to the moving bar when it appears in the visual field of the fly and before the insect initiates tracking. This neural response, which is interpreted as being related to the perceptual event occurring at the onset of stimulus tracking, can be retraced to the mushroom bodies. It increases by novelty and odor-evoked salience, it is anticipatory, and it is reduced when the fly is in a sleep-like state. These results suggest that selective attention underlies visual tracking in flies.

1.26.5.2 Rule Learning

Selective attention constitutes the very basis of rule learning, because in such a process animals learn to focus attention on the relevant information that allows detecting the rule underlying the problem to be solved. Rule learning presupposes positive transfer of an appropriate response from a known set to a novel set of stimuli. In this case, the animal bases its choice not on the perceptual similarity between the novel and the known stimuli, which might not share any common feature, but on a rule which transcends the stimuli used to train it. Examples of such rules are 'larger than', or 'on top of', which may apply to stimuli which do not share any common feature but which can nevertheless be classified according to the rule. Other examples are the so-called principles of sameness and of difference. These rules are uncovered through delayed matching to sample (DMTS) and the delayed nonmatching to sample (DNMTS) experiments, respectively. In DMTS, animals are presented with a sample and then with a set of stimuli, one of which is identical to the sample and which is reinforced. Since the sample is regularly changed, they must learn the sameness rule 'always choose what is shown to you (the sample), independent of what else is shown to you'. In DNMTS, the animal has to learn the opposite, that is 'always choose the opposite of what is shown to you (the sample)'.

Honeybees foraging in a Y-maze learn both rules (Giurfa *et al.*, 2001). Bees were trained in a DMTS problem in which they were presented with a changing nonrewarded sample (i.e., one of two different color disks or one of two different black-and-white gratings, vertical or horizontal) at the entrance of a maze. The bees were rewarded only if they chose the stimulus identical to the sample once within the maze. Bees trained with colors and presented in transfer tests with gratings that they have not experienced before solved the problem and chose the grating identical to the sample at the entrance of the maze. Similarly, bees trained with the gratings

and tested with colors in transfer tests also solved the problem and chose the novel color corresponding to that of the sample grating at the maze entrance. Transfer was not limited to different kinds of modalities (pattern vs. color) within the visual domain, but could also operate between drastically different domains such as olfaction and vision (Giurfa *et al.*, 2001). Furthermore, bees also mastered a DNMTS task, thus showing that they also learned a principle of difference between stimuli (Giurfa *et al.*, 2001). These results document that bees learn rules relating stimuli in their environment. The capacity of honeybees to solve DMTS tasks has recently been verified (Zhang *et al.*, 2004, 2005). It was found that the working memory for the sample underlying the solving of DMTS is ~5 s (Zhang *et al.*, 2005) and thus coincides with the duration of other visual and olfactory short-term memories characterized in simpler forms of associative learning in honeybees (see Section 1.26.6). Moreover, bees trained in a DMTS task can learn to pay attention to one of two different samples presented successively in a flight tunnel (either to the first or to the second) and can transfer the learning of this sequence weight to novel samples (Zhang *et al.*, 2005).

Despite the honeybees' evident capacity to solve relational problems such as the DMTS or the DNMTS tasks, such capacities are not unlimited. In some cases, biological constraints may impede the solving of a particular problem for which rule extraction is necessary. It is therefore interesting to focus on a different example of rule learning which bees could not master, the transitive inference problem (Benard and Giurfa, 2004). In this problem, animals have to learn a transitive rule, that is, A > B, B > C, then A > C. Preference for A over C in this context can be explained by two strategies: (1) deductive reasoning (Fersen *et al.*, 1990) in which the experimental subjects construct and manipulate a unitary and linear representation of the implicit hierarchy A > B > C; or (2) responding as a function of reinforced and not reinforced experiences (Terrace and McGonigle, 1994), in which case animals choose among stimuli based on their associative strength, that is, on the effective number of reinforced and nonreinforced experiences with the stimuli.

To determine whether bees learn the transitive rule, they were trained using five different visual stimuli A, B, C, D, and E in a multiple discrimination task A+ versus B−, B+ versus C−, C+ versus D−, and D+ versus E− (Benard and Giurfa, 2004). Training therefore involved overlapping of adjacent premise pairs (A > B, B > C, C > D, D > E), which underlie a linear hierarchy A > B > C > D > E. After training, bees were tested with B versus D, a nonadjacent pair of stimuli that were never explicitly trained together. In theory, B and D have equivalent associative strengths because they are, in principle, equally associated with reinforcement or absence of it during training. Thus, if bees were guided by the stimulus' associative strength, they should choose randomly between B and D. If, however, bees used a transitive rule, they should prefer B to D.

Honeybees learned the premise pairs as long as these were trained as uninterrupted, consecutive blocks of trials (Benard and Giurfa, 2004). But if shorter and interspersed blocks of trials were used, such that bees had to master all pairs practically simultaneously, performance collapsed and bees did not learn the premise pairs. The bees' choice was significantly influenced by their experience with the last pair of stimuli (D+ vs. E−) such that they preferred D and avoided E. In the tests, no preference for B to D was found. Although this result agrees with an evaluation of stimuli in terms of their associative strength (see above), during training bees visited more B when it was rewarding than D, such that a preference for B should have been expected if only the associative strength were guiding the bees' choices. It was then concluded that bees do not establish transitive inferences between stimuli but rather guide their choices by the joint action of a recency effect (preference of the last rewarded stimulus, D) and by an evaluation of the associative strength of the stimuli (in which case preference for B should be evident). As the former supports choice of D while the latter supports choice of B, equal choice of B and D in the tests could be explained (Benard and Giurfa, 2004). In any case, memory constraints (in this case the fact that simultaneous mastering of the different premise pairs was not possible and the fact that the last excitatory memory seems to predominate over previous memories) impeded learning the transitive rule.

1.26.5.3 Conditional Discriminations: Occasion Setting and Contextual Learning

Contextual learning is a term widely used for describing conditional discriminations which can be subsumed in the so-called occasion setting problem (Schmajuk and Holland, 1998). In this problem, a given stimulus, the occasion setter, informs the animal about the outcome of its choice (for instance, given stimulus C, the occasion setter, the animal has to choose A and not B because the former but not the latter is rewarded). This basic

form of conditional learning admits different variants depending on the number of occasion setters and discriminations involved, which have received different names. For instance, another form of occasion setting involving two occasion setters is the so-called transwitching problem. In this problem, an animal is trained differentially with two stimuli, A and B, and with two different occasion setters, C1 and C2. When C1 is available, stimulus A is rewarded while stimulus B is not (A+ vs. B−), while it is the opposite (A− vs. B+) with C2. Focusing on the elements alone does not allow solving the problem as each element (A, B) appears equally as often rewarded and nonrewarded. Each occasion setter (C1, C2) is, in the same way, simultaneously rewarded and nonrewarded, depending on its association with A or B. Animals have, therefore, to learn that C1 and C2 define the valid contingency. The transwitching problem is considered a form of contextual learning because the occasion setters C1 and C2 can be viewed as contexts determining the appropriateness of each choice. Note that a problem like the biconditional discrimination (AB+, CD+, BC−, AD−; see Section 1.26.4) is amenable to a transwitching problem, and thus to an occasion-setting problem, if one assumes that A and C act as occasion setters for B and D (i.e., given A, B+ vs. D−, and given C, B− vs. D+). This is so because all these problems are forms of conditional learning in which a stimulus can have different associates depending on the conditions in which it is presented.

Despite semantic confusions, conditional discriminations viewed as contextual learning have been studied in several invertebrate models. We will keep the term 'contextual learning' as it appears recurrently in the works that we will discuss from here on. In the nematode *Caenorhabditis elegans*, retention of habituation of an escape response is aided by contextual associations formed during training (Rankin, 2000; see also Section 1.26.6). Nematodes were trained and tested in the presence of a chemosensory cue ($NaCH_3COO$) which was used as the general context surrounding the animals. Animals trained and tested in different chemosensory environments showed lower retention than animals that stayed in the same context. In the same animal, Law *et al.* (2004) used taste cues to create distinct contexts for olfactory adaptation assays and showed that performance in this associative learning paradigm is sensitive to context manipulations. In *Aplysia californica*, Colwill *et al.* (1988) showed that animals exposed to two different contexts, a smooth, round bowl containing lemon-flavored seawater and a rectangular chamber with a ridged surface containing unscented seawater that was gently vibrated by an aerator located in one corner, and receiving a series of moderate electric shocks (US) in one of these two contexts, established an association between the context and the shock. The context alone elicited a defensive reaction which was exclusive for the reinforced context. In the freshwater pond snail *Lymnaea stagnalis*, aerial respiratory behavior was operantly conditioned so that the animals performed aerial respiration significantly less often (Lukowiak *et al.*, 1996; see also Section 1.26.3). Recall of the learned behavior was dependent on the context in which memory was established. Animals trained in water containing food odorant (the contextual cue) exhibited recall only in the presence of such food odorant context (Haney and Lukowiak, 2001).

Arthropods have provided reliable evidence of contextual learning. Hermitte *et al.* (1999) found that in the crab *Chasmagnathus*, spaced and massed training with an opaque screen moving overhead produced different forms of long-term habituation (LTH) of an escape response: LTH acquired by spaced but not by massed training was affected negatively by a change in context, provided by different visual cues around the bowl in which animals are kept. In the fruit fly *D. melanogaster*, the incidence of context on visual learning was analyzed using the flight simulator (see Section 1.26.3) in which a fly has to learn to fly stationary toward surrounding T-shaped patterns (upright and inverted) used as landmarks and presented on a surrounding screen. Context variation in this aversive learning paradigm was provided by a change in the color of the illuminating light (Liu *et al.*, 1999). Normal flies transferred appropriate responses toward patterns from a trained to a new context providing that the chromatic differences between the two contextual lights were not too large (Liu *et al.*, 1999). MB defective flies were unable to realize such a transfer and could not therefore remember the information learned in one context in a different one. These results suggest that the MBs help to stabilize visual memory with respect to context changes and thus allow the memory of an event to be stored and then retrieved in different situations (Liu *et al.*, 1999).

Matsumoto and Mizunami (2004) have shown that crickets *Gryllus bimaculatus* associate one of a pair of odors with water reward (appetitive US) and another odor with saline solution (aversive US) under illumination, and learn the reversed contingency in the dark. Thus, crickets solved this variant of the transwitching problem (see above) and the visual context affected learning performance only

when crickets were requested to use it to disambiguate the meaning of stimuli and to predict the nature of reinforcement.

Bumblebees have also been trained in a trans-switching problem to choose a 45° grating and to avoid a 135° grating to reach a feeder, and to do the opposite to reach their nest (Fauria *et al.*, 2002). They can also learn that an annular or a radial disk must be chosen, depending on the disk's association with a 45° or a 135° grating either at the feeder or the nest entrance: in one context, the nest, access to it was allowed by the combinations 45° + radial disk and 135° + annular disk, but not by the combinations 45° + annular disk and 135° + radial disk; at the feeder, the opposite was true (Fauria *et al.*, 2000). In both cases, the potentially competing visuomotor associations were insulated from each other because they were set in different contexts. Comparable behavior was found in honeybees where distinct odors or times of the day (Menzel *et al.*, 1998) were the occasion setters for a given flight vector.

Further examples for contextual learning in honeybees could be provided (e.g., Gerber and Menzel, 2000). The rich Russian literature on this subject is summarized in Kartsev (1996) and Mazokhin-Porschnyakov and Kartsev (1994), but they would be redundant for the main conclusion of this section which is that there is abundant evidence of conditional learning in invertebrates, which can be described as occasion setting or contextual learning. However, the nature of the associations underlying this kind of learning and its neural substrates remain unclear.

1.26.6 Memory Systems

Memories exist in multiple forms and functions. They are categorized according to their physiological substrates along a timescale as short-term, mid-term, and long-term memory (STM, MTM, LTM), referring to ongoing neural activity as the storage device of STM, intercellular signaling cascades leading to MTM, and gene activation, protein synthesis, and new structures underlying LTM. The transitions between these memory stages or phases can be sequential or parallel, processes referred to as physiological correlates of consolidation, a phenomenon originally known from human psychological studies (see Human Cognitive Specializations) capturing the fact that over time early, vulnerable forms of memory are converted into more stable and long-lasting forms (Ebbinghaus, 1964; Müller and Pilzecker, 1900).

Two processes of memory formation have already been introduced: the distinction between phylogenetic (the species' memory) and individually acquired memory (memory from learning). The relationship between these two forms of memory concerning their different molecular and cellular substrates – but also their corresponding mechanisms of expression (Fox *et al.*, 1998) – is of utmost importance in the study of behavior and cognition, but is poorly understood. Here we focus on experience-dependent memory.

The term experience-dependent memory subsumes two processes in the brain, storing and retrieving information. A content of memory that is potentially retrievable but not actually retrieved is often referred to as 'remote memory', whereas 'working memory' captures the fact that retrieved and updated information exists in an active form that allows predictions to be made about potential outcomes of actions. Working memory is probably the most important concept in describing cognitive processes, since it is considered the interface between evaluated earlier events and future events in the context of the animal's current needs and motivations. Memory systems are also categorized according to their contents, and in vertebrates particular brain structures are related to it, for example procedural memory (e.g., cerebellum), episodic memory (hippocampus, prefrontal cortex), and emotional memory (amygdala). Whereas procedural memory certainly exists in invertebrates and may be distributed over their ganglionic nervous systems, it is debatable whether any animal possesses episodic memory, the ability to carry out long-term recall of sequences of events or narratives (see, for instance, Suddendorf and Busby, 2003, and reply by Clayton *et al.*, 2003a). In humans, this property is intimately related to the functions of the hippocampus and cerebral cortex. It is argued that food-storing birds may develop an episodic-like memory about a kind of food stored at a certain place and at a certain time. Pollinating insects certainly control their foraging activities according to the kind of food they collect at a particular place and at a specific time of day, but it is unknown whether they make decisions between options integrating the what, where, and when of potential food sites (see Section 1.26.7).

Memory systems are highly dynamic and content sensitive. Any retrieval from the memory store will change its content due to the updating process in working memory. It is this updating process that may lead to extracting rules which underlie generalization, categorization, and implicit forms of abstraction (see Section 1.26.7). Furthermore, retrieval from memory store also induces new learning, and consequently consolidation into new

memory, a process referred to as 'reconsolidation' (see discussion below).

It may be expected that the structure and dynamics of memory systems have been shaped by the evolutionary history of the species, and may thus reflect species-specific adaptations to the requirements posed by the environment. Little is known about these ecological adaptations of the memory system, because animal models of memory are rarely studied with respect to their natural behavior in order to detect possible correlates between behavioral and memory dynamics. However, appetitive learning in the honeybee provides certain insights into this question (see discussion below).

1.26.6.1 Physiological Correlates of Memory Systems

In bacteria and ciliates the memory for sensory adaptation and habituation of innate responses lies in the temporal dynamics of second messengers and their targets (see Section 1.26.2). The nematode *C. elegans* habituates to mechanical stimulation, and the duration of the memory depends on the number and intervals of the stimuli. Since retention of habituation differs for different chemical context conditions, it has been proposed that some form of associative learning may be involved (Steidl and Rankin, 2002). The 24 h memory, but not an early memory, depends on protein synthesis, thus indicating an early and a late memory phases. *C. elegans* provides excellent opportunities to study the molecular, cellular, and network properties of learning and memory, but since associative learning and its memory phases have not yet been convincingly documented, this model system awaits further progress before it will become useful for such studies (see Rankin, 2004 for further discussion).

The memories of various forms of learning have been intensively studied in several species of mollusks (*Tritonia diomedea, Hermissenda crassicornis, Limax maximus, L. stagnalis, Helix pomatia, Pleurobranchaea californica, A. californica*; Byrne, 2002). The focus in these studies lies on nonassociative forms of learning such as habituation and sensitization and on their physiological correlates, depression and facilitation. Pavlovian conditioning was studied in *Hermissenda, Limax*, and *Lymnaea*, and as in nonassociative learning, two major phases of memory were found, STM and LTM. The cellular correlates of short-term and long-term sensitization have been studied in greatest detail in *Aplysia* (Kandel, 2001). Short-term sensitization lasts for seconds and minutes and involves the modification of neuronal membrane properties and synaptic efficacy, often through the alteration of the phosphorylation state of existing proteins. Long-term sensitization lasts from days to weeks, depending on the training protocol (spaced trials lead to longer memory than massed trials). It requires synthesis of new macromolecules, since the inhibition of either gene transcription into mRNA or translation of mRNA into protein blocks long-term sensitization. In its most persistent form, long-term sensitization involves morphological changes and neuronal growth. In all these respects, the studies of *Aplysia* sensitization are exemplary and paradigmatic for any research on the cellular basis of learning and memory (Milner *et al.*, 1998). In the context of the relationship between phylogenetic and experience-dependent memory, studies on the mechanistic relationship between development and learning are particularly interesting (Carew, 2002). In *Aplysia*, the nonassociative forms of plasticity in the sensory–motor connection of the siphon withdrawal response appear sequentially during early development: first habituation, then dishabituation, and then sensitization, indicating that the two facilitatory processes dishabituation and sensitization are mechanistically different. This interpretation is supported by the finding that the sequential appearance of plasticity phenomena at the sensory motor synapses corresponds to these forms of plasticity. Synaptic decrement of the sensory–motor synapse appears first in development, then facilitation of the decremented EPSPs, then facilitation of the nondecremented EPSPs, and, finally, an inhibition of nondecremented EPSPs appears in stage 12 of *Aplysia* development. The development of the inhibitory process can be nicely related to a maturation effect of sensitization in which strong stimuli become less effective in later developmental stages. Follow-up studies (Nolen and Carew, 1988) also demonstrated in adult *Aplysia* that dishabituation and sensitization are two different forms of facilitatory plasticity, at both the behavioral and the neural levels. Furthermore, it was found that long-term forms of sensitization emerge at the same time of development as short-term forms (stage 12), indicating that STM and LTM memory are mechanistically interrelated (Wright *et al.*, 1996). These studies are of general importance because they document that the nonassociative forms of learning (habituation, sensitization) reflect not only two opposing processes but rather are composed of four behavioral processes, two decrementing (habituation, inhibition) and two facilitatory.

Using molecular genetic tools in *Drosophila*, four distinct memory stages have been found following

aversive olfactory conditioning: STM, MTM, anesthesia-resistant memory (ARM), and LTM (Tully *et al.*, 1994). Among the several mutants that have been analyzed with respect to their memories, rutabaga (rut) and amnesiac (amn) yielded the most information about their cellular mechanisms and the localization of STM and LTM in the *Drosophila* brain (Heisenberg, 2003; Isabel *et al.*, 2004). Rut encodes a calcium-sensitive adenylylcyclase, and amnesiac encodes a neuropeptide similar to vertebrate PACAP (pituitary adenylylcyclase-activating peptide). Rut mutants learn normally, but suffer from reduced STM that can be rescued in transgenic flies expressing the gene in a subpopulation of mushroom body neurons (γ-lobe neurons; Zars *et al.*, 2000). Amn mutants lack PACAP-expressing neurons, and two of these neurons extrinsic to the mushroom body are essential for the transition to LTM (Feany and Quinn, 1995). Structural mutants with lesions restricted to the alpha lobe of the mushroom body specifically abolish LTM (Pascual and Preat, 2001; Dubnau *et al.*, 2001). Taken together with the analysis of several other single-gene mutants, these observations suggest that olfactory learning and memory depend, at least in part, on the activity of MB neurons. Different MB neurons may be involved in storing different memories, but currently nothing is known about how the transfer occurs between these neurons during consolidation from STM to LTM. So far only very few of the many neurons certainly involved in memory formation in *Drosophila* have been studied. It is estimated that more than 1000 genes are transcriptionally regulated during olfactory LTM formation (Dudai, 2002).

Reward learning in honeybees initiates a sequence of memory phases which lead to long-lasting memory passing through at least four forms of memory (Menzel and Müller, 1996; Menzel, 1999). An associative-learning trial induces an early form of short-term memory (eSTM) in the seconds range. This memory is highly dominated by appetitive arousal and sensitization, is rather unspecific, and is quickly converted into a late STM (lSTM). At the cellular level, stimulus association is reflected in the convergence of excitation of the conditioned stimulus pathway of the (odor) via nicotinic acetylcholine receptors (nAChRs) in the antennal lobe and the MB, and the pathway for the unconditioned stimulus, the putatively octopaminergic neuron VUM_{mx1}, most likely acting on octopamine receptor II receptors. In the antennal lobe, both cAMP/PKA and Ca^{2+}-dependent PKC are upregulated during STM, and the cAMP/PKA signaling cascade is indicative of the associative component (Müller, 2000). However, unlike in *Drosophila*, blocking the cAMP/PKA pathway does not interfere with learning and STM. The transition to MTM is a rather slow process and makes the memory trace unsusceptible to retrograde amnesic treatments. Single and multiple learning trials lead to different long-term forms of memory (LTM, see discussion below). An important molecular component in the transition to LTM formation is enhanced and prolonged PKA activity after multiple learning trials. Two lines of evidence support the conclusion that LTM formation requires enhanced PKA activity shortly after multiple trial learning, but is not involved in the learning process itself: (1) blocking NO synthase during lSTM reduces PKA activity and impedes LTM formation (Müller, 1996); (2) enhancing PKA activity by uncaging cAMP in the antennal lobe after a single learning trial facilitates the formation of LTM in the same way as multiple trials do (Müller, 2000). MTM is characterized by a first wave of PKC activity (Grünbaum and Müller, 1998). The constitutive activation of PKC is a proteolytic process of formation of PKM that lasts for several hours. Inhibition of proteases in the whole brain reduces the formation of PKM and blocks retention during the MTM phase. LTM formation is also blocked in this way, indicating that protein synthesis-dependent LTM and high levels of long-lasting PKC activity (until the third day after conditioning) are formed parallel to PKM-dependent MTM.

Two forms of LTM must be distinguished in honeybees: early LTM (1–2 days) characterized by translation-dependent retention and constitutively active PKC, and late LTM ($\geq$3 days) characterized by transcription-dependent retention and no more enhanced PKC activity. The two forms of LTM arise differently, after massed and spaced multiple learning trials (Menzel *et al.*, 2001). Memory resulting from spaced trials is blocked by protein synthesis inhibitors, whereas memory resulting from massed conditioning trials (intertrial interval 30 s) is independent of protein synthesis.

1.26.6.2 Working Memory: Capacity and Duration

The capacity and time span of working memory has been estimated in invertebrates only for the honeybee in the appetitive context of nectar foraging. As pointed out above, very short intervals ($<$1 min) between a learning and a test trial lead to high but rather unspecific responses, while long intervals lead to specific responses of the learned stimulus. Chittka *et al.* (1997) recorded the frequency of intervals between stay and shift flights made by bumblebees

foraging on more than two plant species. Stay flights appear at shorter intervals (~2 s) than shift flights, indicating that immediate choices are dominated by the most recent and the most effective STM, but reference to more remote memories needs more time. To interpret it from another perspective, one could say that longer intervals release working memory from the dominant memory of the last visit and allow for contributions from an earlier memory that has meanwhile been consolidated. Greggers and Menzel (1993) found for bees foraging in a patch of four feeders that delivered different flow rates of sucrose solution that they store the reward properties of these feeders in working memory. Similar results were found for eight feeders, indicating that the reward properties of eight feeders can also be stored in feeder-specific memories. The capacity of working memory is, therefore, at least eight items. The time range of these specific working memories could be estimated as lying around 6 min.

Recently, working memory in foraging honeybees has also been estimated using the DMTS procedure (see Section 1.26.4 and Giurfa *et al.*, 2001), in which a free-flying bee is exposed to a visual stimulus (the sample) through which it should fly to then subsequently choose between two options, one of which corresponds to the sample. If the bee matches its choice to the sample it is rewarded with sucrose solution (Giurfa *et al.*, 2001). Using this paradigm, Zhang *et al.* (2005) estimated the duration of the working memory for the sample, which allows a bee to choose between alternatives after passing though it. The duration of such a working memory was around 5 s. Longer delays between exposure to the sample and subsequent choice of stimuli result in random choices.

1.26.6.3 Reconsolidation

When memory is retrieved from a remote store by exposing the animal to the learned stimulus without reinforcement (extinction), two processes are initiated: extinction learning, due to the fact that the stimulus originally associated with reinforcement is now presented without it, and reminder learning, which may either recruit the original stable memory about the learned stimulus and bring it back into an unstable form (trace dominance hypothesis, see discussion below), or it initiates a new learning trial based on the fact that a learned stimulus also activates the reward system and thus initiates new learning (internal reinforcement hypothesis, see below). Both extinction and reminder learning are followed by their respective consolidation processes: consolidation of an extinction memory and reconsolidation of the reminder memory. Studies in the crab *Chasmaganathus* (Pedreira and Maldonado, 2003) and the honeybee (Stollhoff *et al.*, 2005) showed that the strength of these two consolidation processes depends on both the strength of the reminder memory and the strength or number of extinction trials. These observations resemble findings in vertebrates showing that the presentation of an extinction trial does not always result in extinction alone, rather it may lead to an opposing behavioral phenomenon, the stabilization of the response learned earlier. In this case, the extinction trial transfers the old memory from an inactive, stable protein synthesis-independent memory into an active and unstable protein synthesis-dependent reminder memory (Nader, 2003; Dudai and Eisenberg, 2004). In the honeybee, the balance between the extinction and reminder learning processes and their respective consolidation processes depends on the number of extinction trials. Extinction learning is dominant after two extinction trials, whereas reminder learning is expressed in a recovery from extinction and is induced by many (five) extinction trials. Consolidation of reminder learning (reconsolidation) depends on protein synthesis, which indicates that a new learning process is going on, as shown in the vertebrate studies. Nader (2003) and Dudai (2004) proposed the hypothesis of "trace dominance" and interpret the results as a competition between consolidation of extinction memory and reconsolidation of reminder memory "with the dominant one being the one most affected by protein synthesis inhibition." This hypothesis can explain the observations on aversive learning in the crab *Chasmaganathus* (Pedreira and Maldonado, 2003) but not those on appetitive learning in the honeybee (Stollhoff *et al.*, 2005). In *Chasmaganathus* extinction memory and its consolidation are dominant after either one strong or many extinction trials, whereas a weak or only one extinction trial leads to dominance of reminder learning and of its corresponding reconsolidation. Exactly the opposite was found in bees. One reason might be a difference between consolidation processes induced by aversive or appetitive learning. Furthermore, the trace dominance hypothesis proposes that either the reconsolidation of the reminder memory or the consolidation of the extinction memory occurs according to an 'all-or-none' rule. The bee data do not support such a conclusion, because inhibiting consolidation of extinction memory results in an opposite behavioral effect than the inhibition of reconsolidation of reminder memory. It was hypothesized that in bees these consolidation

processes take place in parallel, rather than following an 'all-or-none' rule (Stollhoff *et al.*, 2005).

The results on reconsolidation in the honeybee can be explained via properties of the reward system in the bee brain. Hammer (1993) found that the reward neuron VUM_{mx1} not only codes for appetitive reinforcement of odors, but also learns to respond to the conditioned odor stimulus, and an extinction trial does indeed activate the VUM neuron (Hammer, 1997). This means that an extinction trial will lead to new appetitive learning without an external reinforcing stimulus. This is exactly what was found: two forms of learning, one based on the lack of external reinforcement (extinction learning), and one based on the internal existence of reinforcement (reminder learning). These conditions are best captured by the 'internal reinforcement hypothesis', which states that the balance between extinction and reminder learning depends on the relative strength of the internal reinforcement. The balance will be shifted to the internal reinforcement with more unrewarded presentations of the learned stimulus.

1.26.6.4 Ecology of Memory Systems

From an evolutionary point of view one may expect that memory dynamics are adapted to choice behavior under natural conditions. Foraging in pollinating insects is a behavior with a highly regular sequential structure of events ranging from actions within seconds to those separated by months and thus may offer the opportunity to relate memory structure and ecological demands (Menzel, 1999). Different memories are consulted during the sequence of events during foraging. In the bee, the time courses of successive behaviors during foraging match the temporal dynamics of memory stages. Choices between flowers within the same patch quickly succeed each other and are performed during eSTM. Choices between flowers of different patches occur after the transition to lSTM. Successive bouts are interrupted by the return to the hive such that flower choices in a subsequent bout require retrieving information from MTM. The separation between the two forms of LTM may be related to the periods when flower patches are in bloom (Menzel, 2001).

Although these ecological considerations are highly speculative, they indicate, on the one hand, that sequences of natural behavior need to be examined with respect to the intrinsic properties of the neural machinery underlying memory formation. On the other hand, they emphasize the necessity of considering the results of laboratory studies on memory formation in the context of natural behavior. Only comparative studies will help us discern which properties reflect general mechanisms and which indicate species-specific adaptations, and invertebrates offer a huge range of ecological adaptations. This conclusion supports the fact that more studies on the natural behavior of animal models of memory are necessary. To which extent do the different memory phases of *Drosophila* or *Aplysia* correlate with specific sequences of their behavior? To which extent are aversive and appetitive memories coincident with respect to their dynamics, given the fact that they respond to different natural sequences and behavioral timing? These questions require comparative studies of memory that allow for the natural context in which memory is to be employed, a requirement that is seldom met by studies on invertebrate memory.

1.26.7 Representation and Planning, Observatory Learning; Navigation; Communication and Individual Recognition

One of the most important steps in conceptualizing cognition is the distinction between implicit and explicit knowledge. Explicit knowledge may – in a strict sense – exist only in humans. Animals (other than primates) might possess only implicit knowledge, but this does not rule out the possibility that certain forms of knowledge might reach an 'explicit-like' status specific for the animal species in question in the sense that internal operations on memories (or representations) are performed without motor expression of such operations. A telling example of this form of knowledge is the choice behavior of food-storing birds which makes their choices dependent on what kind of food they have stored and when and where they have stored it (Clayton *et al.*, 2003b). Thus, expectation, attention, planning, and decision making could be useful terms for animals, even if we assume that they possess only implicit knowledge. If such terms are used, we assume that the animal retrieves a memory whose structure allows two or more alternative outcomes to be evaluated before any motor action is performed. We shall address the question here whether it is reasonable to assume such forms of internal processing in invertebrates, in particular, in insects (e.g., the honeybee) and cephalopods (octopus) (for *Drosophila* see Greenspan and van Swinderen, 2004). Do these animals have a memory in the sense that its contents can be recollected outside of the immediate sensory–motor control, and used for internal operations?

There are good reasons for rejecting this kind of questioning when it comes to understanding invertebrate behavior. Too often, mentalistic reasoning has led to obscure arguments and untestable hypotheses. On the other hand, searching for experimental conditions that would allow us to address the internal processing of the brain devoid of sensory–motor control, its spontaneity and creativity, in even such a tiny brain as that of insects, may help to do better justice to the complexity of neural functions in bigger brains. In most behavioral studies, inference about internal processing can be made only indirectly by observing changes in behavior. In the honeybee, one can study a communicative process between foraging animals transcending the usual limitations of behavioral studies in invertebrates, and 'read' their reports on what they perceived, what they learned, and what they consider to be worth reporting. This unique situation was ingeniously exploited by von Frisch (1967), who discovered through it a whole range of sensory capacities and experience-dependent faculties. More recently, work on honeybees has benefited from cognitive perspectives (Menzel and Giurfa, 2001; Giurfa, 2003), and it is on this aspect that we focus in this article.

The topics of observatory learning, navigation and communication are addressed here because it is under these conditions that learning transcends elementary forms of association in particularly clear ways. The evaluating signal for storing experience must come from internal nervous system conditions at the time of learning, depends considerably on the motivational level, requires attention to a subset of stimuli, and is adjusted to the animal's own behavior in an intricate way. The signals learned are usually composed of multimodal inputs which cannot be isolated from each other, and the motor performances involve sophisticated sequences of motor programs. As will be seen, it is still very difficult to prove the cognitive nature of the processes, because more elementary interpretations need to be carefully considered. Close sensory–motor connections, simple partial matching strategies between experienced and remembered constellations, sequences of picture memories, and other forms of elementary solutions to complex conditions must be discarded before elaborating on an animal's cognitive sophistication.

1.26.7.1 Observatory Learning

Observatory learning differs from associative learning in the lack of an obvious external reinforcing stimulus. Thus, an animal capable of observatory learning should be able to internally evaluate environmental conditions as well as its own actions by activating a circuit that informs it about the value of signals and actions to which the animal is simply exposed.

The cephalopod mollusk *Octopus vulgaris* is able to learn a visual discrimination by observing the behavior of conspecifics (Fiorito and Scotto, 1992; Fiorito and Chichery, 1995; Fiorito *et al.*, 1998). In the experiment, one group of animals (demonstrators) was first trained to attack one of two simultaneously presented colored balls. When the animal attacked the correct ball, it was rewarded with food which was attached to the opposite side of the ball. If the animal chose the wrong ball, it was punished with an electric shock. In a second phase, untrained octopuses (observers) watched demonstrators attack that stimulus from an adjacent tank. No contingent reward or punishment was given to the demonstrator during this observation period. In the final phase, the observers were exposed to the two balls. In over 80% of all cases, they only attacked the ball of the same color as that attacked by the demonstrator. Untrained animals do not exhibit such a strong preference. This vicariously acquired discriminatory behavior is stable for at least 5 days after the observational phase.

The salticid jumping spider of the genus *Portia* was exposed to a three-dimensional maze which the animal could oversee in its entirety (Tarsitano and Jackson, 1997). One of the two wire paths led to a food lure, the other did not. After visually scanning the entire track, at the first trial the animal already made 75% correct choices at that vantage point. This remarkable display of problem solving requires planning and expectation, performed by a brain of only several hundred micrometers in diameter. Honeybees in a swarm searching for a new nest site inspect potential locations by running in various directions along the walls of the cavity and performing recruitment dances at the surface of the swarm according to the suitability of the cavity as a nest site (its size, humidity, proximity, and possibly other parameters (Seeley, 1977)). The kind of individual learning during these behaviors can only be addressed as observatory learning, because no reward is provided to the animal inspecting a cavity. The search is focused exclusively on potential nest sites: the animal has to integrate a large range of sensory inputs which are accessible only by its own exploratory behavior, and must evaluate them according to an innate template, which cannot be totally fixed, because otherwise a bee would never find a suitable new nest site. Many more such forms

of observatory learning exist in insects, crustaceans, and cephalopods, but only few have been studied carefully, because natural conditions are required for the animal to initiate its exploratory behavior, and it is often difficult to evaluate the learning during observation.

1.26.7.2 Navigation

Navigation is an orientation strategy that allows animals to travel between defined locations without having direct sensory access to these locations. In this sense navigation differs from general orientation and guidance, which include all forms of spatial relationships between the animals' body positions and trajectories of movement relative to sensory conditions. Navigation allows animals to travel over rather long distances relative to their body size and sensory range, according to their knowledge of the structure of their surroundings, which they acquire by sequential experience. Several classifications of navigation have been proposed, reflecting different conceptual frameworks, for example, route and local navigation (O'Keefe and Nadel, 1978), cognitive mapping, piloting and dead reckoning (Gallistel, 1990), egocentric and allocentric navigation (Wehner, 1992; Thinus-Blanc, 1987), random navigation, taxon navigation, praxis navigation, route navigation and local navigation, graph and map navigation (Gillner and Mallot, 1998). Irrespective of whether the classification focuses on sensory, neural, or mental processes, or whether the structure of the external signals was referred to, two basic navigation strategies emerge from these various viewpoints: route strategy (also called graph structure of navigation; Gillner and Mallot, 1998) and local navigation or map strategy (also called cognitive mapping; Tolman, 1948; Gallistel, 1990; Pastergue-Ruiz *et al.*, 1995). Besides these strict definitions, the term navigation is often used in loose terms, for example, when *C. elegans* explores its environment, and genes are characterized that control elements of this exploratory behavior in different ways (Gray *et al.*, 2005; Rodger *et al.*, 2004), or when *Drosophila* runs between two or more visual marks in a rather stereotypic way (Götz, 1975). It is an interesting but yet unresolved question whether genes identified by testing these very simple forms of movement may also be involved in controlling the ontogenetic setup and switching the circuits that underlie navigation in the strict sense. A hint in this direction comes from the observation that the foraging gene (Engel *et al.*, 2000) involved in exploratory behavior in *Drosophila* larvae appears to be more strongly expressed when honeybees switch to foraging behavior (Ben Shahar *et al.*, 2002).

Place learning in a small environment like the Morris water tank provides a useful paradigm for studying components of navigation in small mammals like rats, and their neuronal underpinnings (Morris, 1982). A similar attempt has been followed for the cockroach by providing a cool place on a hot platform (Mizunami *et al.*, 1998). In such a paradigm, called 'the Tennessee Williams paradigm' for obvious literary reasons, a cockroach learns to choose the cooler place using the spatial relationships between landmarks in the environment, though in a less robust way than rodents. In addition, it was found that bilateral lesions in the MB region reduce place learning. Coincidently, crickets may learn to use responses to olfactory stimuli at two different contexts (Matsumoto and Mizunami, 2004), or to combine visual and olfactory cues in choosing a particular exit hole among several identical holes in order to escape from a circular arena (Scotto-Lomassese *et al.*, 2003). MB neurogenesis seems to be crucial for the last task, since irradiating the dividing neuroblasts around this structure suppresses this ability.

In studies with rodents, an essential aspect relates to the distinction between navigation according to landmarks (true 'spatial-based' navigation), a hippocampus-dependent form of behavior, and 'cue-based' navigation, a behavior which in mammals is independent of the hippocampus (Morris, 1982). It is not yet known whether structures of the arthropod brain are differentially involved in these two paradigmatically different forms of navigation, and in particular whether the MB in the insect brain may be a structure necessary for relational, landmark-based navigation. However, a comparison between studies of the cockroach (Mizunami *et al.*, 1998), and the cricket (Scotto-Lomassese *et al.*, 2003) may provide an insight into these questions, because the cockroach study would reflect a case of spatial-based navigation, since the safe place in the arena had to be found on the basis of spatial relationships between external landmarks. The cricket study, on the other hand, could be considered as a case of cue-based navigation, because the correct and the wrong exit holes were marked by nonambiguous cues. Interestingly, the same structure, the MBs, was crucial for both kind of performances, although in the case of crickets it was the formation of new MB-constitutive neurons that was affected, and not the MB circuit itself, as in the cockroach study (Mizunami *et al.*, 1998).

Below we focus on navigation in social Hymenoptera, because the relationship between

route strategy and map strategy has been the subject of intensive and controversial debates in these insects. Traditional thinking about navigation in insects was based on the notion that a toolbox of rather simple sensory–motor routines is at the animal's disposal and their stepwise application may lead to the solution of isolated, rather independent navigational tasks. These robot-like concepts were developed by experiments based on the analysis of route learning, sometimes even at such a small scale that target-related orientation – rather than navigation – was tested (Collett and Collett, 2002). Bees and ants traveling between their nest and a food source learn the vector components of their movements (direction and distance) by a dead reckoning (path integration) process (Wehner, 1992). Landmarks experienced en route may serve to calibrate measured distances (Srinivasan *et al.*, 2000a), thus reducing the rotatory and translatory errors that may accumulate during path integration (Wehner *et al.*, 1996; Graham and Collett, 2002). Furthermore landmarks may provide procedural information about turns to make and distances to travel next (Collett, 1996, 1998; Collett and Collett, 2000; Kohler and Wehner, 2005) such that apparently complex performances could be based on simple rules of learning sensory–motor connections. Honeybees can, for instance, learn to negotiate complex mazes of adjacent boxes by associating colored disks with right or left turns (Zhang *et al.*, 2000; Srinivasan and Zhang, 2004). Since bees are able to refer to a compass direction even when the sun is not available (e.g., under an overcast sky and as measured by their dance performance; von Frisch, 1967; Dyer and Gould, 1981), landmarks also serve as compass-related directional cues that could be understood as a backup system, but it could also mean that landmarks are incorporated into a geostable map-like memory of the experienced environment (see discussion below). The redundancy of landmarks is thought to be reduced by reference to contextual information such as the visual panorama (Collett *et al.*, 2002; Kohler and Wehner, 2005). Consequently, 'isolated' landmarks may not be sufficient for successful navigation, or their information might be suppressed under competition conditions, as often occurs in experiments where bees are transported to an unexpected release site. The animal may then shift its reference to salient signals like farther-ranging landmarks. Furthermore, a dissociation of behavior may occur that seems to indicate the combined action of separate navigational cues. For example, bees flying in a narrow tunnel search at the correct distance if both local and contextual cues are available. If one of the two cues is shifted or removed, then the contextual cue appears to be the more important one (Collett *et al.*, 2002). Under more natural conditions, a dissociation of two forms of distance estimation, odometry by the visual flow field (Esch *et al.*, 2001; Srinivasan *et al.*, 2000b) and landmark sequences, can be found. Chittka and Geiger (1995) set up an experiment in which sequentially experienced landmarks guided free-flying bees over hundreds of meters to a feeding station. The bees searched both at the absolute distance as measured by odometry and at the location designated by the sequence of landmarks. The directional component of flight is also controlled by landmarks (Collett, 1996), and even novel directions can be traveled if a particular landmark constellation simultaneously triggers the retrieval of memories for two different flight directions (Menzel *et al.*, 1998).

Training bees along a route will establish a particular spatial memory structure, and very different forms of spatial memory may result from exploratory orientation flights that bees perform before beginning their foraging life (von Frisch, 1967; Capaldi *et al.*, 2000). Indeed, it was found that bees lacking route training were able to return to the nest from sites all around the hive, located several hundred meters away (Menzel *et al.*, 2000), a range over which orientation flights are performed (Capaldi *et al.*, 2000). Comparing such bees with those trained along a route showed that the working memory of the route dominated initial navigation, leading the animals in the wrong direction and first suppressing the memory that later quickly leads the animals back to their goal (the hive). Since only the initial flight path was monitored in these experiments, and vanishing bearings were taken as measures of navigation, inadequate concepts were derived because the multitude of spatial memories was not considered (Wehner and Menzel, 1990; Dyer, 1991; Wehner, 1992).

Most of the experiments on the role of landmarks on path integration, compass-driven sequential pictorial learning, and the relationship between 'isolated' landmarks and contextual landmarks were carried out in miniature environments which forced bees to fly through narrow entrances into small boxes or mazes (Collett and Collett, 2002). All these tests made forced route training necessary, avoided testing the sequential experience of natural landmarks in unhindered flight, and did not expose the bees to the temporal and spatial structures they would normally experience in free flight. Certainly, such experiments have the advantage of observing the bee over the full flight close to the goal, but the limitations for generalization to natural conditions

are obvious, since they reflect only the approaching components and orientation strategies that bees may adopt when close to the goal. Navigation in the real bee flight range (several kilometers) must be guided by compass-related learning of multiple sequences of landmarks that are visible under different perspectives and over different ranges of the flight path, some of which are only visible when the bees get close to them. The essence of the navigational problem – whether an animal is able to orient itself and infer a direction of movement along a novel path aiming toward another location that is not directly accessible – cannot be addressed by such reduced experimental setups. Questions that are not asked in an experiment cannot be answered by it. Working under natural conditions, however, limits the possibility of untangling the relevant parameters underlying navigation strategies. Therefore, such 'naturalistic' experiments have a different quality. They are necessarily more descriptive and less analytical, but they provide clues for behavioral capacities while allowing a rather limited mechanistic interpretation. Recently, a method based on harmonic radar detection of free-flying bees was applied that allows researchers to observe a bee when it travels over natural distances (see below).

By far most of the experiments on insect navigation were carried out with ants (the wood ant *Formica rufa*, the desert ant *Cataglyphis*, the Australian desert ant *Melophorus bagoti*) and the honeybee. The data were often used to transfer the concepts developed for ants to bees (and even to arthropods in general; Wehner, 1992), implying that navigation strategies are similar in running and in flying insects. Bees fly over distances of a few kilometers, cruising well above ground, with a bird's-eye view; ants run a few tens of meters. It would be rather strange if the navigation systems of running and flying insects, in particular their 'cognitive' (integrative) levels, were similar. Experiments addressing distance estimation in ants and bees clearly show, for instance, that these two insects differ dramatically in their manners of estimating distance. While bees seem to use the optic flow field (the relative displacement of the visual field on the insect retina) experienced en route to the goal, ants do not seem to rely on optic flow to the same extent (Ronacher *et al.*, 2000). Recently it was shown that ants can estimate the distance to travel within a tunnel in the dark without any kind of visual information (Thielin-Bescond and Beugnon, 2005), thus underlying the role of proprioceptive cues for distance estimation in ants.

1.26.7.2.1 The map concept and its experimental support Local navigation or map-based strategy allows goal-directed decisions at any place and toward any intended location in the experienced area, thus resulting in a transfer between routes and inference of novel routes. Such a strategy has until recently not been convincingly documented for insects, and is the subject of lively debate (Giurfa and Capaldi, 1999; Collett and Collett, 2002). Navigational memories that are more flexible than route memories are found in experiments that avoided route training in bees and proved that bees are able to return to the hive from any place around the hive within a rather short time (Menzel *et al.*, 2000). It was concluded that bees learn features of the landscape during their orientation flights (Capaldi *et al.*, 2000) and establish a special 'landscape memory' that relates landmarks to the bees' central place, the hive. It was only recently that the structure of this 'landscape memory' could be critically tested using harmonic radar technology to track individual bees (Menzel *et al.*, 2005). In this study, three test groups were studied and their flight paths recorded after they were released at many different release sites around the hive. The three test groups were: bees that were trained to a feeder placed at variable locations in close vicinity to the hive that was moved around the hive at a constant distance (VF bees) and that therefore did not develop a route memory; bees that were trained to a stationary feeder 200 m to the east of the hive (SF bees) and thus developed a route memory; and bees that were recruited by foragers that collected food at the stationary feeder (R bees). It was found that all bees returned to the hive along fast and straight flights from all regions around the hive. SF and R bees did so after they had performed the vector (distance and direction) components of their trained or instructed route flights; VF bees returned after searching for a while. Most importantly, SF bees performed either direct flights back to the hive or via the feeder to the hive.

Several operations must be at the animal's disposal: (1) recalling memories of these vectors (segments with defined headings and distances) pointing toward the hive with a large number of landmarks all around the hive that are recognized from different viewpoints, (2) a shift in motivation (fly toward the hive or toward the feeder), (3) reference to the outbound vector components of the route flight from hive to feeder, and (4) addition and subtraction of the flight vectors for at least two sets of vector memories; those which would lead directly back to the hive, and those that lead from the hive to the feeder. It is difficult to imagine that these

operations can be done without reference to vectors that relate locations to each other, and thus make up a map. The question now in bee navigation is not so much whether there is a map-like spatial memory, but rather 'What structure does this map-like memory have and how is it used?'. In any case, the map-like memory in bees is rich and can be used in a flexible way. Any model of bee navigation thus has to incorporate a strategy based on a map-like representation of the bees' large-scale home range and a freedom to choose between at least two goals. This further suggests that spatial relations between environmental features appear to be coherently represented in a map-like memory in insects as they are in other animals and humans (Gallistel, 1990; Shelton and McNamara, 1997; Klatzky, 1998).

Parsimony is a strong argument in the interpretation of experimental data, and has been applied rather strictly in studies of insect navigation (Wehner, 1992). However, it should not be overlooked that radical forms of parsimony as applied to behavioral science were (and may still, at least partially be), a historical burden. New approaches were required to correct for eliminating the brain in behaviorism and making too simple assumptions about the brain's functions in ethology. These cognitive approaches (cognitive ethology, psychology, neuroscience) provide us with novel avenues to brain function (Kandel and Squire, 1992). A frequently used argument in navigation studies, which states that reference to cognitive processing (a cognitive or mental map) must be avoided as long as 'simpler' explanations are at hand (Bennett, 1996), may warn us about potential traps, but should not be accepted as a ban. Furthermore, it is argued (Collett and Collett, 2002) that small brains, like that of the bee, need to solve their tasks with less 'cognition', meaning with a toolbox of loosely interrelated elementary functions rather than an integrated, allocentric level of spatial representation (see discussion above). It should be recognized that we simply do not know whether the integration of the multiple and complex sensory and procedural neural processes into a common spatial memory with geometric organization (a map) may not be a more economical and thus simpler way of representing sequential experiences during navigation (Griffin, 1984).

1.26.7.3 Communication

All sensory channels (besides magnetic sense) are used in invertebrates for communication, and elaborate sender–receiver systems have evolved for intersex communication, predator–prey relationships, and social interactions. Communication by pheromones has been addressed above (see Section 1.26.2). Optical signals include light emission by luminance organs in night-dwelling invertebrates ranging from unicellular organisms to crustaceans and insects (Lall, 1993; Buck and Case, 2002). Sex-specific coloration and movement patterns of body appendages or of the whole animal are often used to attract the other sex, and specific photoreceptors or visual interneurons have evolved (e.g., the flight maneuvers and the 'love spot' in diptera; Hornstein *et al.*, 2000; Egelhaaf and Kern, 2002). Airborne sound and substrate vibration are most important signals for all three behavioral conditions of communication (intersex, predator–prey, social interactions; Cocroft and Rodriguez, 2005). Although the sensory, ecological, and evolutionary aspects of these communication systems have been often studied in great detail in invertebrates (e.g., sound communication in insects: Henry (1994), Gerhardt and Huber (2002); substrate vibration in spiders: Barth (2002), and in insects: Hölldobler and Roces (2000), Cocroft (2003); visual signaling in butterflies: Eisner and Aneshansley (1973); chemical signaling: (Hölldobler and Wilson (1990), Eisner and Meinwald (1995); see also Section 1.26.2), very little is known about the cognitive dimensions of these communication systems, for example, how the innate mechanisms interact with experience-dependent developmental processes, how the innate mechanisms are related to internal and external context conditions, whether learning shapes the communication process, and if communication leads to individual recognition (see discussion below). More is known in these respects about the ritualized movements ('waggle dance') used by honeybees to communicate distance and direction from the hive or the swarm to important places (potential nest sites, feeding, water or resin places). We therefore consider this form of communication in more detail.

1.26.7.3.1 The cognitive dimensions of dance communication in honeybees In the waggle dance, a dancing bee (*Apis mellifera*) executes fast and short forward movements straight ahead on the comb surface, returns in a semicircle in the opposite direction, and starts the cycle again in regular alternation (each waggle dance involves several of these cycles; von Frisch, 1967). The straight portion of this course, called a waggle-run, consists of a single stride (Tautz *et al.*, 1996) emphasized by lateral waggling motions of the abdomen. The length of the single waggle-runs increases with the distance flown to reach the source, and their angles relative to gravity correlate with the direction of the

foraging flights relative to the sun's azimuth in the field and sun-linked patterns of polarized skylight. Thus, by encoding the visually measured distance (Esch and Burns, 1995; Srinivasan *et al.*, 2000a; Tautz *et al.*, 2004) and the direction toward the goal, the waggle dance allows colony members to share information about the distance and direction toward a desirable goal (von Frisch, 1967; Seeley, 1995; Dyer, 2002). Although Karl von Frisch used the term "dance language," Premack and Premack (1983) correctly stated that the honeybee dances should not be called a language, based on the argument that there is no evidence that the bees can judge whether their dances conform to anything in their surroundings. This question can be addressed by asking whether a bee receiving information from the dance responds differently to the information depending on its own experience. Such experiments have yet to be performed. There is also no evidence yet that honeybees employ chain communication whereby an animal picks up on the received information without experiencing itself the primary signals inducing the dance. In his studies of dance communication within a swarm, Lindauer (1955) did not observe a bee changing its dance pattern until it had actually visited the second cavity, and these observations were verified more recently by Visscher and Camazine (1999), who observed no higher attraction of bees to dances which indicated the same location as the one for which they had previously been dancing. The authors also found that it takes a swarm longer to get started with the flight to a new nest site if the decision must be made between alternative nest sites, and they present arguments for some form of collective "quorum sensing" (Seeley and Visscher, 2004; see also below). Furthermore, the term "language" is also misleading, because there is (as far as we know) no semantics or grammar in the ritualized movements of the dance. "Indexical" or "iconic" (Bermudez, 2003) would be better descriptive terms to characterize the informational status of the dance.

Since navigating bees benefit from path integration (Wehner and Menzel, 1990; Dyer, 1998; Collett and Collett, 2002; Wehner, 2003), vector memories derived from recent flight paths might be recalled in the dance context. Indeed, path integration (which requires working memory in order to continuously record the angular and linear components of the animal's movements) provides ants and most likely also bees with 'global' vectors at the ends of their outbound paths, which allow them to follow straight trajectories of the appropriate distance and direction during their inbound path. Although the global vector is emptied each time the animal returns to the nest, desert ants can store a short-lived 180°-reversed form of a recently experienced homing path, and use it to guide their outbound paths toward previously visited locations (Wehner, 1992). Moreover, when trained bees arriving at a foraging target are held captive for several hours, they subsequently fly farther outward away from the hive along the same hive-target direction (Dyer *et al.*, 2002). We may therefore assume that forager bees also store a form of global vector that they later recall in the context of the waggle dance. But does the waggle dance encode a global vector, an integrated form of the measures of distance and direction, and/or even code for a location?

Early detour experiments by von Frisch and colleagues (reviewed in von Frisch, 1967) indicated that the bees' visually driven odometer is primarily decoupled from the processing of directional information, indicating that no global flight vector is reported in the context of the waggle dance. When bees are compelled to fly a two-legged detour path to reach the goal, their dances indicate the direction of the straight line toward the goal (computed from the two legs of the detour), even when they followed the detour on the way back to the colony; but they signal the distances actually flown, and not the distance of the straight segment connecting the target and the hive's entrance. These early findings were recently confirmed by manipulating the navigational information provided to a dancing bee (De Marco and Menzel, 2005). Thus, one might ask whether the waggle dance encodes spatial information provided only by the actual flight path. The role of landmarks so far has been considered only in the context of resetting (Srinivasan *et al.*, 2000b) or calibrating the odometer (Tautz *et al.*, 2004) but not in the communicative process. The detour experiments by von Frisch suggest that the directional component reported in the waggle dance may also be derived from stored path integration coordinates of visually defined locations (landmarks). This idea is not without precursors. Early experiments showed that with increasing experience of the terrain, directional information available during the inbound flight may be computed for the purpose of directional indication in the waggle dance (Otto, 1959). If the waggle dance computes directional information which depends not only on the current state of the animal's path integrator, but also on information that the animal has associated with landmark views, that is, local vectors associated with landmarks (Etienne *et al.*, 2004), navigating bees would rely not only on an egocentric, but also on a geocentric system of reference.

It has been argued in the section on navigation (see Section 1.26.7.3) that the navigational strategies applied by foraging bees cannot be fully appreciated if one assumes a hive-centered egocentric form of spatial memory. Instead, it seems that the orientation flights of young or reorienting bees lead to a map-like spatial memory that appears to be derived from repetitive exposure to the same landmarks from different viewpoints. Given this capacity and the fact that bees are recruited by a dancing bee only after they performed their orientation flights, it is tempting to assume that bees attending a dance might recall from its memory of landmarks and homing vectors a corresponding outbound vector that is related to expected landmarks. Under these conditions neither the dance behavior nor the flight path of a recruited bee would be guided solely by two independent measures (direction and distance) but rather by an 'expectation' to arrive at a particular location. A component of this 'expectation' would be the route to be followed, as embedded in the map-like memory including sequences of landmarks. Indeed, already von Frisch (1968) stated that the effectiveness of waggle dances (in terms of successful recruitment) depends upon the foraging experience of the dance followers. When two groups of fellow bees have visited two different (and currently exhausted) unscented feeding places, contact with a dancer indicating the accustomed goal is much more effective than contact with a dancer indicating the unfamiliar one. In spite of these early findings, however, the role of stored navigational information on the decoding process involved in the waggle dance remains entirely unknown.

1.26.7.4 Individual Recognition

Cricket males perform rivalry songs, defend their territories, and fight against each other (Alexander, 1961; Adamo and Hoy, 1995); however, no clear evidence exists yet that winners and losers learn to recognize each other on an individual basis (Paul Stevenson, personal communication). The yellow–black patterns of the faces and the abdomen of the paper wasp *Polistes fuscatus* vary considerably, making it possible that individual animals in these small colonies might recognize each other (Tibbetts, 2002). More variable patterns with larger black components were found to be carried by individuals ranking higher in the nest hierarchy. Altering these facial and/or abdominal color patterns induces aggression against such animals, irrespective of whether their patterns were made to signal higher or lower ranking, and arguments – though not fully conclusive – have been provided stating that this altered aggressiveness may indicate individual recognition (Tibbetts and Dale, 2004). Although this study appears to indicate the first case of individual recognition in insects, it is rather likely (but not yet proven) that other invertebrates, in particular territorial cephalopods like octopus, should recognize each other on an individual basis.

An additional but in no way necessary component of individual recognition is 'self-recognition', the discrimination between signals from the animal's own body from those from the outside world. Do invertebrates experience 'pain', a form of self-recognition that includes an emotional and a warning component that points to the future? Locusts and crabs cast off body appendages when attacked. Do they experience different forms of sensory input when they perform these actions themselves or when the same appendages are removed? When honeybees lose their stinger the abdomen is damaged so much that the animal will die. It has been observed that alarm pheromone, which usually triggers an attack flight, induces stress analgesia via an opioid system in the honeybee (Nunez *et al.*, 1997), potentially indicating that a preparatory response of the nervous system leads to a reduction of the strong sensory input from the body distraction. Opioids, which are usually associated with stress-induced analgesia, have been found in other invertebrates such as crickets (Jaffe and Blanco, 1994) and the praying mantis (Zabala *et al.*, 1984), thus suggesting that their presence may serve to counteract the effect of nociceptive stimuli as in vertebrates.

E. O. Wilson states on the final page of *The Insect Societies*:

> The insect societies are, for the most part, impersonal. The small, relatively primitive colonies of bumblebees and Polistes wasps are based on dominance hierarchies, and individuals appear to recognize one another to a limited extent. In other kinds of social insects, however, personalized relationships play little or no role. The sheer size of the colonies and the short life of the members make it inefficient, if not impossible, to establish individual bonds (Wilson, 1971).

As pointed out above (Section 1.26.2), the sheer unlimited capacity of honeybees to discriminate odors provides the potential for discrimination of a very large number of group constellations, even to the level of individual recognition. Only recently has it become possible to separately mark all individuals in a bee colony for automatic detection, and it will now be possible to track life history, behavior, and social contacts on an individual basis continuously over long periods of time.

Sociobiological arguments have been put forward to argue that due to haplodiploid reproduction in

hymenopteran societies their members should be characterized by less individuality, more gene-related group effects, and some form of individuality of the society (Queller and Strassmann, 2002). From a cognitive point of view, individuals are defined by the operations within their nervous systems, and their experience that this nervous system belongs to a particular body. As Churchland (2002, p. 310) points out "Body-state signals have to be integrated, options evaluated, and choices made, since the organism needs to act as a coherent whole, not as a group of independent systems with competing interests." Body-state signals are continuously integrated in members of an insect society, options are implicitly evaluated as indicated in the communicative processes, and the individual organism acts within the society. As in the case of implicit operations on working memories (representations), individual recognition within the society does not require the assumption of any form of explicit (personal) recognition of oneself and another member of the society. In this sense, one may use the modern techniques to search for discrimination on the individual level in communication within insect societies.

1.26.7.5 Collective Cognition

So far, we have concentrated on individual cognitive capabilities, because learning and memory are properties which depend on individual experience and which have to be studied, therefore, at the individual level. However, individuals of many species live in societies or form groups, and therefore face problems that require coordination, task sharing, and collective decision making. From this perspective, it is legitimate to ask whether collective behaviors reflect or even surpass individual plasticity, due, for instance, to the possible additive effect of individual cognitive capacities.

This question has been the subject of debate in social insects in which colonies were considered 'superorganisms' (Seeley, 1989; Southwick, 1983; Wilson and Sober, 1989). From this point of view, it has been argued that the 'superorganism' protects and constitutes itself thanks to colony recognition systems based on cuticular hydrocarbons which are transferred between individuals within the colony, thus obscuring, in theory, individual identity (Queller and Strassmann, 2002). The metaphor of the 'superorganism' may be in a sense misleading, because an individual, behaving organism is made from cells and structures tightly interconnected by complex neuronal, circulatory, and regulatory networks, and has a central brain that commands and produces behavior; the 'superorganism', on the other hand, is made up of individuals which may be interconnected by complex chemical interactions but which are rather autonomous and can be hardly compared to constituent cells. The essential difference, however, is that although an insect colony produces collective behavior, it does not have a central brain to command and control such behavior. On the contrary, studies on collective decision making in social insects show that collective behavioral patterns can arise without any central control. How, then, can these patterns of collective behavior emerge? This question has been intensively tackled by studies focusing on self-organization models of collective behavior (Camazine *et al.*, 2003). Such studies deal with pattern formation processes in the physical and biological world, which determine the arising of global order and structure based on simple interactions between lower-level components, in this case, individuals (see above: decision making in a bee swarm).

Such studies have allowed researchers to understand that – despite their potential complexity – many collective insect behavior patterns emerge from simple interactions between individuals, which seem to act on the basis of extremely simple behavioral rules. For instance, cockroaches and other insects seeking refuge exhibit complex aggregation phenomena and group distribution between available shelters. Instead of resulting from individual decision making and evaluation and comparison of each refuge's characteristics, collective aggregation results from individual probabilistic rules determining when to stop walking or when to resume. Factors like the presence of borders or of a co-specific may amplify these probabilities like a snowball (i.e., the larger the cluster, the higher the probability of attracting even more individuals; see Theraulaz *et al.*, 2003) but the collective behavioral pattern emerges without central control or individual comparison strategies (Jeanson *et al.*, 2003, 2005). Similarly, complex architectural structures characterizing ant or termite nests arise without central control, simply based on a restricted set of individual behaviors performed in a rather automatic way, without 'knowledge' of the behavioral patterns exhibited by other individuals (Buhl *et al.*, 2004). Ants facing a diamond-shaped bridge with two branches choose randomly between branches if both are identical but collectively orient their choice and select one branch if it has a lateral wall along its edge. Pharaoh ants walking the wrong way along a trail are unable to reorient at a trail bifurcation if the angle is 120°, but can if the angle is smaller (Ratnieks, 2005). Such a collective systematic

choice does not result from a sum of individual evaluations and decisions but from amplification processes based on physical heterogeneities and chemical communication (Dussoutour *et al.*, 2005). Thus, a preference is set without a clear incidence of individual decision making and comparison. Differences in individual thresholds for reacting to environmental sensory stimuli seem to be a critical factor for the emergence of collective behaviors based on task partitioning. Not all individuals within a group will respond similarly to the same set of environmental stimuli such that differences in reaction thresholds may be the basis for behavioral specializations and thus for the emergence of sociality.

The conclusion emerging from these studies on insect collective behavior is that individuals, which may be viewed as extremely sophisticated at the cognitive level when performing some individual tasks, appear as automatons with limited cognitive capacities when performing collective tasks. This difference may seem puzzling, because it could be that cognitive richness is lost or at least temporally inhibited in a social context. What, for instance, drives a honeybee scout, which is able to learn space in the form of a cognitive map (Menzel *et al.*, 2005) and learns the localization of a potential nest site and its physical characteristics (Seeley, 1982), to follow dances once, at random, in the hive, instead of crossing over and comparing dances for selected sites in the process of nest selection (Visscher and Camazine, 1999)? In fact, it can be argued that the question does not actually make sense from the point of view of the individual insect: in an individual and in a social context, the animal will adopt the behavioral strategies leading to adaptive solutions either boosting or sacrificing what researchers would view as cognitive sophistication. The critical question in this context is therefore what determines the adoption of one or the other level of cognitive complexity? Which factors are responsible for the fact that an ant or a bee that can learn and memorize several cues while foraging solve complex discriminations and generate novel behaviors leading to adaptive solutions, behave like an automaton following a reduced set of repetitive patterns and simple rules in a social context? Which physiological changes, if any, determine the passage from one state to the other? Do social regulation pheromones intervene in the expression or inhibition of behavioral autonomy in a social context by acting on neurotransmitter levels in the insect nervous system? So far, we have no answers to these questions, but they can be approached on an experimental level. Studying whether or not individual learning and memory are modified by exposure to social pheromones or by chemosensory cues within a group and whether or not biogenic amine and neurotransmitter levels are changed in the presence of a group of co-specifics are just some of the paths that can be followed to provide some answers to these questions.

1.26.8 Conclusion

The environment poses the same basic demands on animals with small and with large brains. How do mates find each other, how much effort, risk, energy, and time must be invested to find food, what is the best way for the animal to protect itself and avoid predators or hazardous conditions, and how does the animal find its way around are questions that have to be answered both by vertebrates and invertebrates in order to survive. It is usually concluded that invertebrates, with their smaller brains, apply local solutions to these problems, that is, solutions that are specific and isolated, local in a verbal, a mechanistic, and a behavioral sense. Verbally, a localized solution may mean that it may suit only a problem at a particular location in space and time. Mechanistically, local solutions may mean that only specific sensory–motor connections in the nervous system may exist with no cross-talk between them and no common level of memory storage. Behaviorally, local solutions refer to independent behavior routines that are applied without gaining from each other in different environmental conditions. In other words, the concept of information transfer between sensory–motor routines, translated in the rise of novel behaviors that allow responding to novel environmental demands, seems to be neglected or minimized in the case of invertebrates.

Our review provides multiple indications as evidence against such an understanding, which ignores the enormous richness of invertebrate behavior, its high flexibility, and the cross-talk between different behavioral routines. Rather, it is appropriate to conceptualize invertebrate behavior from a cognitive perspective, meaning that small nervous systems also interconnect the sensory and motor systems, and install memories not only into specific sensory–motor circuits but into a common reference system as well. As a consequence, many invertebrate species that were studied in this respect were found to extract rules from particular sequences of experience and transfer these rules to other sensory domains. Furthermore, the structure of the memory established in this way represents the multiple sensory inputs and related behaviors in an integrated

way such that a representation of complex environmental conditions is formed. Such capacities may not be surprising when the underlying neural substrate subserving them is explored in detail. As in larger brains, two basic neural architectural principles of many invertebrate brains are: the existence of specialized neuropiles, which refer to specific sensory domains, and higher-order integration centers, in which information pertaining to these different domains converges and is integrated, thus allowing cross-talking and information transfer. In this sense, both modularity and central integration seem to be basic building principles adopted by different nervous systems to provide flexible solutions to a changing environment. Navigation and communication in social Hymenoptera are particularly telling examples in this respect, but it is fair to conclude that similar integrated forms of dealing with the environment will be found in other invertebrates when they are looked at more closely. In this sense, research addressing behavioral complexity and its underlying neural substrates is necessary to characterize the real potential of invertebrate learning and memory. Usually such an approach has been used to characterize behavioral simplicity rather than complexity. It therefore seems timely to focus on the latter by studying problem solving besides elemental forms of learning.

One of the major questions of behavioral biology in general is also unresolved for invertebrate behavior: the relationship between innate and acquired behavior. Innate behavior can often be considered highly integrated in the sense that rather complex motor performances are adequately expressed in particular stimulus combinations. The 'intelligence' of these integrated systems stems from the specific activation of such innate behavioral components in the animal's motivational conditions and the often less obvious experience-dependent adaptations. We have tried to capture these complex interactions between innate components, motivation, and acquired memory by coining the terms 'phylogenetic memory' and 'experience-dependent individual memory'. Indeed, both forms of stored information are based on the intimate connectivity of the nervous system, and from the point of view of how memory is retrieved it may not matter how the relevant memory has been stored. Rather, it seems crucial for the organization of memories and the expression of resulting behaviors how memory is acquired, that is, to which natural conditions do memories provide solutions. The intimacy between phylogenetic and experience-dependent memory is reflected in the fact that innate and acquired information can often not be separated in any given behavior. Although we have stressed the point that the richness and flexibility of acquiring information through experience is the key issue in a cognitive perspective of invertebrate behavior, it should not be overlooked that innate components cannot be considered less cognitive if they are closely connected with flexibility and adaptiveness. To which extent innate components constitute real limitations of invertebrate behavior must be analyzed in an ecological and evolutionary perspective which takes into account the evolutionary consequences of innate behaviors. Invertebrates provide us with an enormous range of solutions to these integrated forms of dealing with changing environmental conditions and the animal's needs. Ideally, more studies on invertebrate behavior will be inspired by this cognitive perspective.

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1.27 Aggression in Invertebrates: The Emergence and Nature of Agonistic Behavioral Patterns

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1.27.1 Introduction

The study of behavior generally begins with watching. It is hard to say it better than Yogi Berra in his famous quote "You can observe a lot just by watching!" With repeated observations of social rituals, patterns often emerge that are easily recognizable and highly stereotypical. However, like the digits on a hand that differ from each other even though all are called fingers, behavioral patterns differ each time they are performed depending on the social context. In addition, variability exists in (1) when during social rituals patterns appear and (2) how one pattern links to the next. Despite such variability, statistical techniques can reveal behavior's inherent structure and show when patterns are most likely to appear and how predictably they link to each other. But is all behavior of all animal species modular and does this extend to human behavior? Moreover, if behavior is modular, how do patterns of this sort get established within nervous systems and what rules govern the likelihood that one will transition to the next? Modular construction implies that superimposed on the hard wiring diagrams of nervous systems, codes exist specifying that particular combinations of neurons and neuronal circuits transiently link together to form recognizable behavioral patterns at appropriate times during behavioral rituals. The observed behavioral patterns overlap each other, can utilize identical sets of muscles in different ways, are species- and/or genus-selective, and must be understandable by conspecifics in communicating with each other. In this article, we examine studies of aggression using invertebrate models: (1) to illustrate the highly organized nature of this complex behavior, (2) to explore the roles of neurohormones such as amines in the behavior, and (3) to begin an exploration of the role of specific genes in establishing behavioral patterns in nervous systems. A comprehensive review of aggression in invertebrates has appeared recently (Kravitz, and Huber, 2003; see also Cognition in Invertebrates, Sleep in Invertebrates). In this article, therefore, the focus will be solely on studies with fruit flies and with two decapod crustacean species, crayfish and lobsters.

1.27.1.1 A Short History: Nature versus Nurture

Although the presence of stereotyped elements in behavior has been recognized for more than 50 years, the interpretation of how these come about has given rise to acrimonious debate. The presence of stereotyped behavioral elements and their formation became an ultimate battleground when it became inextricably linked with the dispute over nature versus nurture. Early ethologists noticed that many distinct, species-specific displays appear to develop fully intact, even in individuals raised without access to tutors (cf. Heinroth and Heinroth, 1933; Lorenz, 1941). They attributed the action of inherited developmental programs to

fixed action patterns and argued that such behavior would lend itself to phylogenetic analysis, akin to the use of morphological or physiological characteristics. Lehrman (1953) delivered a stinging critique, arguing that any discussion about the genetic basis of behavioral traits is a distraction from learning more about the developmental processes that produce them. Subsequently, the emergence of any sort of comprehensive view with respect to behavioral phenomena was held up for decades by this dispute. J. P. Scott's work on the roles of genetic components in mammalian social behavior highlighted the genetic contributions to core processes in behavior and helped to establish the field of behavioral genetics (Scott and Fuller, 1965). The practical solution now posits that behavioral development inevitably forms as a complex interchange of genes and environment and that the emergence of some traits may owe more to the contributions of one than the other. Attempts at an either/or classification between nature and nurture simply lack heuristic value due to the complexity of their entanglement (Marler, 2004). These notions have been adopted by researchers in evolution, genetics, and neuroscience with their fields moving on to more important questions. Studies of behavior in ethology, psychology, and sociology, however, are only now beginning to emerge from this counterproductive clash of extreme positions. The present review aims more to discuss the characteristics and functioning of stereotyped elements in behavior that occur linked to specific elements in the nervous system, and less to contributions of how these systems got there.

1.27.1.2 Why Aggression?

Intraspecific aggression is a behavior that is particularly well suited to a comprehensive examination of its component structure and underlying mechanisms. In many taxa, agonistic encounters are readily evoked in dyadic interactions, making possible studies ranging from behavioral through physiological and ultimately to molecular and genetic levels. Animals with no previous social experience or tutors often are able to perform and interpret agonistic behavioral patterns and their structure. With a clear ability to decode the communication components of the behavior, animals are effectively able to take part in coordinated, agonistic encounters. Even with these innate abilities, however, the behavioral patterns are malleable and can be molded by experience.

1.27.2 The Fruit Fly Model of Aggression

1.27.2.1 Characterization

Male and female fruit flies (*Drosophila melanogaster*) both show aggression. Ethograms of the behavioral patterns involved have been constructed and first-order Markov chain analyses have been performed to explore the dynamics of the fights (Chen *et al.*, 2002; Nilsen *et al.*, 2004). In comparing the patterns of fighting behavior between pairs of male and pairs of female flies, some of the patterns seen are the same in the male and female fights, whereas others appear selective to male or female fights (Figure 1; Nilsen *et al.*, 2004). Thus, certain

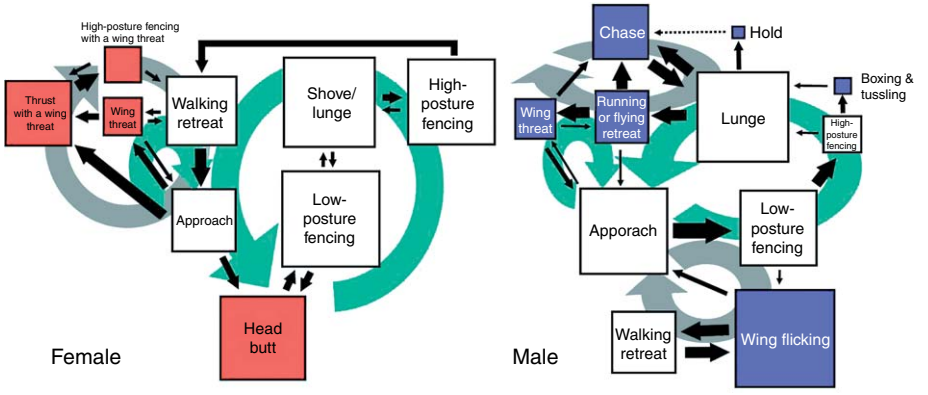

Figure 1 Behavioral patterns and transitions seen in fights between pairs of male and pairs of female *D. melanogaster*. Females and males share five common behavioral patterns (white boxes) while several gender-selective behavioral patterns also are seen (male (blue) and female (red) boxes). The box sizes represent the numbers of transitions to and from a pattern. The arrows between boxes represent the likelihood of transitions, with the addition that the dashed line between 'hold' and 'chase' in males illustrates a transition that approaches statistical likelihood. The large green arrows highlight similar transition loops, and the gray arrows highlight gender-selective transition loops. The female data is from 2597 behavioral transitions and the male data was collected from 2526 transitions from 376 encounters in 19 trials. Reprinted from Nilsen, S. P., Chan, Y. B., Huber, R., Kravitz, E. A. 2004. Gender-selective patterns of aggressive behavior in *Drosophila melanogaster. Proc. Natl. Acad. Sci. USA* 101, 12342–12347.

mid- or high-intensity components of fighting such as chasing, tussling, lunging, and boxing along with extended wing threats (greater than 2 s and up to several minutes) are seen predominantly in male fights, whereas head butting and very short wing threats (all under 2 s) are found mainly in encounters between females. Moreover, male fights are more likely to lead to hierarchical relationships than female fights (Nilsen *et al.*, 2004).

These patterns appear in full complexity without requiring a tutor, but also are malleable by experience. In all experiments, male and female flies are placed singly in isolation vials containing fly food when they first emerge as adults or as pupae that emerge as adults in isolation. Thus, the first time that these flies see another adult fly is when they are paired for fights, yet they use all the components that make up fighting behavior, they change intensity levels in appropriate ways in response to their opponents, and they establish hierarchical relationships (at least in males). This and genetic evidence suggest that the responsible neural circuits are established during embryonic, larval, or pupal life, and are already fully functional when flies emerge as adults. While the patterns are established early in development, they can be modified by experience that both alters the way male flies fight and changes the likelihood of their winning subsequent fights (A. Yurkovic, unpublished observations).

1.27.2.2 Genes and the Establishment of Behavioral Modules

In addressing the issue of how patterns of behavior get established in nervous systems, one approach is to ask how the patterns of female fighting behavior get established in female brains and the male patterns in male brains. Logical candidate genes for this purpose are the already established genes of the sex determination pathway (cf. Goodwin *et al.*, 1999; Baker *et al.*, 2001). The X-autosome ratio determines gender in fruit flies (females are XX and males XY), and early genes involved in the determination are: *sex lethal* (*sxl*), a splicing factor; *transformer* (*tra*), a second splicing factor that works in conjunction with a second transformer gene (*tra-2*), and *fruitless* (*fru*), a gene that codes for expression of members of the bric-a-brac-tram-trac-broad (BTB) complex family of zinc finger transcription factors (for reviews see Goodwin *et al.*, 1999; Baker *et al.*, 2001). The genes *sxl* and *tra* are transcribed and translated in females but not in males. This leads to splicing of *fru* to male- and female-specific transcripts, all of which are generated from the first of four promoter sites found in the gene. The male-specific transcripts generate FRU^M proteins, while no proteins are generated from the female-specific mRNAs (see Lee and Hall, 2000; Goodwin *et al.*, 2000). Mutant *fru* male flies cannot distinguish between male and female flies and will actively court both.

An indication that the emergence of behavioral modules might be influenced by genes is the report by Lee and Hall (2000), demonstrating enhanced levels of head-to-head interactions in *fru* mutant male flies. Such interactions are rarely seen in fights between pairs of wild-type male fruit flies, but they are commonly seen in fights between pairs of wild-type female flies (Nilsen *et al.*, 2004). Thus, it is possible that *fru* mutant male flies not only cannot distinguish between males and females during courtship rituals, but perhaps one of the behavioral patterns associated with female fighting behavior might have been introduced into the brains of male flies in the absence of *fru* transcripts. Recent studies by Nilsen (unpublished observations) suggest that *fru*-null male flies fight more like females than like males in that extended wing threats and mid- and high-intensity components of male fighting behavior are not observed in these animals, whereas head butting is common.

1.27.2.3 Genes and Mating Behavior

Mating behavior has been well studied in fruit flies, and as mentioned above, the genes of the sex-determination pathway have been defined (cf. Goodwin *et al.*, 1999; Baker *et al.*, 2001). Mating behavior also appears largely modular, with distinct recognizable components including approach and orienting, tapping, singing, licking, attempted copulation, and copulation taking place in a well-defined sequence that is compatible with a first-order Markov chain (Markow and Hanson, 1981). As mentioned above, *fru* mutant males cannot distinguish between males and females. The *fru* gene is expressed in about 20 groups of neurons in the fly brain and nerve cord (Goodwin *et al.*, 2000; Lee and Hall, 2000). Using an RNAi technique, Manoli and Baker (2004) eliminated the FRU^M protein isoforms in one subgroup of about 60 *fru*-expressing central nervous system neurons located in the subesophageal ganglion. These neurons send processes from that site in a major projection through the median bundle to terminate in the dorsal protocerebrum of the brain. The phenotype of the resultant male flies was that they attempted to court females, but the first two modules associated with courtship behavior were not observed (orienting and tapping): instead flies immediately began singing, licking females, and

attempting to copulate all at the same time and at an accelerated rate. These male flies were unsuccessful at copulating with females. In some thus far unknown way, the absence of FRU^M expression in this one subgroup of neurons leads to the absence of two of the early components of the courtship ritual, and the speeding up and disordering of the later ones.

1.27.2.4 Hormones and the Modulation of Behavioral Modules

Singing is usually not observed during aggression between pairs of males in *D. melanogaster*. In other species, however, such as *D. pseudoobscura*, singing is part of the fighting ritual (S. Nilsen unpublished observations). Singing also can be introduced into fights between male *D. melanogaster* by altering levels of the amine octopamine (S. J. Certel *et al.* unpublished observations). Using fly lines with mutations in the enhancer region for the gene tyramine-β-hydroxylase (the key enzyme in the biosynthesis of octopamine) generated by Monasterioti *et al.* (1996), it was found that flies with low or normal levels of octopamine would not sing during fights, while flies with double the level of octopamine would. This effect seemed related to selective effects of the amine on all modules of behavior associated with wing movements, as wing threats also increase in occurrence with increases in the levels of octopamine in the flies. These studies raise the interesting possibility that different neurohormones might have selective effects on different patterns of behavior and that switching between patterns during behavioral interactions might depend on the release of such substances associated with the social context at the time.

1.27.2.4.1 The role of genes, hormones, and experience in molding behavior in fruit flies There is little doubt that genes play an important role in laying down behavioral patterns in fruit fly central nervous systems. In addition, with fruit fly studies leading the way, much recent work has focused on defining the sequences of genes involved in specifying neuronal identity and in establishing pathways of neuronal connectivity in the developing nervous system (cf. Skeath and Thor, 2003). Much less work, however, has been done on the role of genes in establishing the behavioral patterns that are of concern here. Here again, however, elegant work with fruit flies is leading the way. For example, Bate and colleagues (Suster and Bate, 2002; Langraf *et al.*, 2003) have explored how the central pattern generator (CPG), which governs movements of larval fruit flies, gets established. Their findings are that rather than motor neuron cell body position determining the organization of these motor systems, the dendritic arbors of motor neurons appear to form a myotopic map within the larval ventral nerve cord. These endings form a central representation of the peripheral musculature and they do not require the muscles themselves, properly differentiated glial cells or sensory input to form their organizational domains. While sensory input is not required to form the central motor patterns, without sensory input the polarity of the movement patterns is abnormal. They found further that even though muscles are organized segmentally, the arbors of endings of the motor neurons are in the embryonic parasegmental organization lined up with the anterior margin of *engrailed* gene expression in the nervous system. They suggest that neurons of the ventral nerve cord can "differentiate autonomously to produce the CPG for larval peristalsis in Drosophila," and that this may be an essential feature of the formation of patterns within the fruit fly central nervous system (Suster and Bate, 2002).

1.27.3 Crustaceans as Model Organisms for the Study of Aggression: Exploring the Role of Amines in Aggression

1.27.3.1 Characterization of the System

Crustacean species, including crayfish and lobsters, represent excellent study systems to explore the structure of complex behaviors and their causation. Paired fighting between socially naïve animals features an exchange of highly stereotypical behaviors that escalate through different intensity levels and that ultimately result in a decision with behavioral consequences for both winners and losers (Bruski and Dunham, 1987; Huber and Kravitz, 1995; Pave and Fielder, 1996; Barki *et al.*, 1997). Quantitative analysis of agonistic (fighting) behavior in lobsters and crayfish allowed the construction of ethograms of the common behavioral patterns and their temporal structure (Huber and Kravitz, 1995; Huber *et al.*, 2004). Fighting proceeds according to strict rules of conduct. All animals exhibit a series of common behavioral patterns in a stereotypical manner. A temporal sequence of these patterns was evident, representing an increase in intensity during confrontations. The typical scenario of an encounter begins with extensive threat displays upon first contact, continues with periods of ritualized aggression and restrained use of the claws, and terminates in a brief session of unrestrained combat. Predictions of game theory

(i.e., assessment strategies) provide a useful framework for the understanding of fighting in lobsters (Parker and Rubenstein, 1981; Leimar and Enquist, 1984). The presence of a highly structured behavioral system may reduce the potential for damage in fights among conspecifics, and may prove useful in attempts to study the neurobiological causes of complex behavioral patterns such as aggression.

1.27.3.2 Neurohormones and Aggression

A common theme in these studies is that amines, peptides, and steroid hormones, substances that function as neuromodulators and as neurohormones, serve as important modulators of aggression at the behavioral level. Amine systems have been extensively studied in crustaceans. Beginning with studies showing that injections of octopamine and serotonin into animals triggered the appearance of opposing postures that resembled those seen in winning and losing animals during fights (Livingstone *et al.*, 1980), and continuing with demonstrations of the localization of amine neurons in the nervous system (Beltz and Kravitz, 1983; Schneider *et al.*, 1993), learning how these neurons function (Beltz and Kravitz, 1987; Ma *et al.*, 1992; Heinrich *et al.*, 2000; Kravitz, 2000), and recent demonstrations of changes in the functioning of these neurons with changes in social status (Yeh *et al.*, 1996, 1997; Edwards *et al.*, 1999), much has been learned about the functioning of amine neurons in aggression in crustacean systems. Several recent reviews describe the results of these experiments (Kravitz, 2000; Panksepp *et al.*, 2003; Kravitz and Huber, 2003; Huber *et al.*, 2004; Huber, 2005). The focus here, therefore, will be on the behavioral effects of amine infusion and/or on the consequences of pharmacological manipulations that alter amine levels in these species.

In one set of studies, fighting behavior has been examined in crayfish pairs with large size asymmetries (>30%), where the smaller (subordinate) animal received acute serotonin infusions via a fine-bore, fused silica cannula (Huber *et al.*, 1997a, 1997b; Huber and Delago, 1998; Figure 2). During such treatment, infused animals re-engaged their larger opponents, resulting in longer fights that reached higher levels of intensity compared with controls. Multivariate techniques (e.g., discriminant function analysis) revealed that serotonin treatment specifically altered the decision to retreat from an opponent, without affecting how likely the animal was to initiate fights, how individual fights progressed to higher intensities, or in the case of large size asymmetries, the eventual social rank that was achieved. In these studies, therefore, serotonin appears to act on key sites for agonistic decision making in the crayfish central nervous system. It is likely that serotonin reuptake mechanisms play an essential role in this behavioral change as the effects associated with acute serotonin infusion were significantly reduced in the presence of fluoxetine (Huber *et al.*, 1997a, 1997b; Huber and Delago, 1998). This finding suggests that a functional high-affinity serotonin reuptake mechanism (Livingstone *et al.*, 1981) is involved, and that serotonin-associated behavioral plasticity requires the reloading of synaptic terminals (which can be blocked by fluoxetine). Little information is available on the categories of serotonin receptors involved.

In studies with juvenile lobsters, although arriving at similar empirical outcomes after injections of serotonin, other investigators offered alternative interpretations of the observed effects, suggesting that an inhibition of retreat simply reflects global down-regulation of motor activity or motor coordination (see Peeke *et al.*, 2000). To address such criticism and to further distinguish between specific aggression-enhancing effects of serotonin and more global effects on motor activity or coordination, levels of activity, movement patterns, and space utilization were studied in crayfish receiving serotonin in ways that corresponded in dose, mode, site, and time course of infusion to those used in the earlier fighting studies. Under such conditions and compared with controls, acute serotonin-infusion neither altered absolute levels of locomotion nor movement patterns (Huber, 2005).

Subsequent work using chronic augmentation or disruption of serotonin function explored to what degree considerations of timescales and dynamic properties enhance understanding of the links between amines and behavior. Pharmacological interventions that alter serotonin–neuron function appear to be accompanied by the rapid induction of compensatory mechanisms that counteract such treatments. Thus, the differences in fighting behavior resulting from chronic infusions of serotonin were initially accompanied by the anticipated effects on behavior, but these were followed by a steady decline in effectiveness of the amine (Panksepp and Huber, 2002; Panksepp *et al.*, 2003). Infusions from silastic tube implants containing serotonin (Panksepp and Huber, 2002) and lasting up to several weeks initially boosted absolute amine levels in the nervous system. Within a week, however, it appeared that adaptations took place in the system that counteracted the effects of constant infusion and absolute levels of serotonin returned to pretreatment levels. Moreover, with continued serotonin

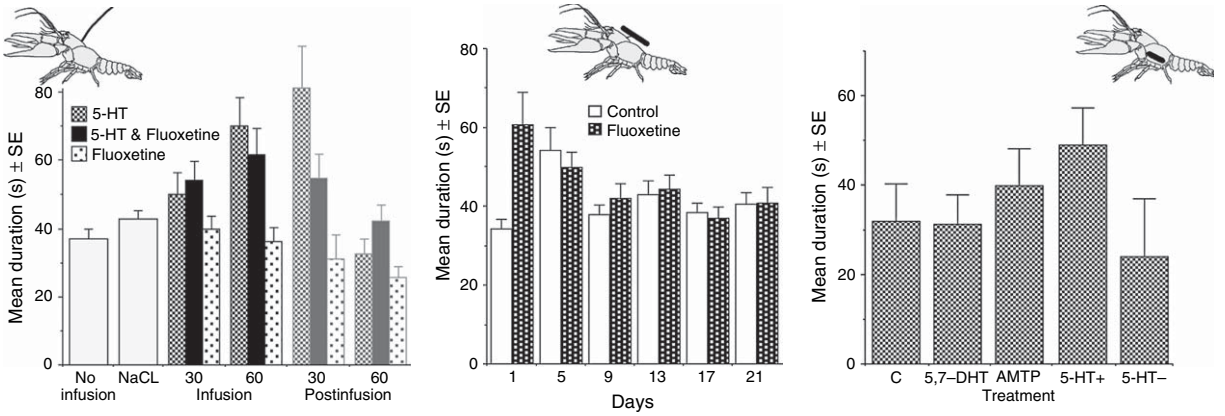

Figure 2 Composite figure illustrates differences in fight duration resulting from pharmacological manipulations of crayfish serotonin systems. Fight durations varied considerably with some lasting a few seconds and others several minutes. Encounters are graphed according to mean duration (in seconds) of fighting during the experimental period. a, Fine-bore fused silica capillaries were used to infuse serotonin, fluoxetine, or both substances together into freely moving, subordinate animals. Serotonin infusion (at 3 μg min^{-1}) resulted in longer fighting that persisted well after the infusion pump was turned off. Multivariate statistical techniques (i.e., discriminant function analysis) revealed that longer bouts of fighting resulted from a decreased likelihood of retreat. Infusion of fluoxetine alone did not enhance aggression, however, co-infused with serotonin resulted in a reduction of the fight-enhancing effects of serotonin. b, Chronic infusion of fluoxetine via osmotic minipumps increased duration of fighting during the early stages of treatment compared to animals receiving vehicle only. As with acute serotonin infusion, these differences in fighting were due to a decrease in the probability for retreat. c, Duration of fighting in animals that received chronic silastic implants containing either 5-HT synthesis inhibitors (5,7-dihydroxytryptamine or alpha-methyltryptamine) or serotonin at one of two different rates. No significant differences in fight duration existed among these groups. Reproduced from Huber, R., Panksepp, J. B., Yue, Z., Delago, A., and Moore, P. 2001. Dynamic interactions of behavior and amine neurochemistry during acquisition and maintenance of social rank in crayfish. *Brain Behav. Evol.* 57, 271–282, with permission from S. Karger AG, Basel.

infusion, some animals actually showed serotonin depletion likely resulting from overcompensation in the system. Such neuronal compensation could involve changes at many different levels, including altered synthesis (Stachowiak *et al.*, 1986; Sivam, 1995), amine release (Lent, 1984; Hall *et al.*, 1999), metabolic activity (Ase *et al.*, 2000), or receptor distribution or turnover (Patel *et al.*, 1996; Woo *et al.*, 1996). The studies utilizing chronic treatments did not generate increases in the aggression-enhancing effects of serotonin like those that result from acute infusions. Although this advises caution when discussing possible links between amines and behavior, it more likely reflects the constraints in working within a dynamic system in which critical components are the relative size of a signal relative to a given set point, and the timing of when a signal is triggered. Thus, the global systemic effects of pharmacological manipulations may be poor mimics of normal physiological processes in which neurons release neurohormones such as amines within nervous systems at precise times for actions on restricted sets of targets. To illustrate, raising or lowering levels of serotonin in lobsters by pharmacological manipulations generates the same behavioral phenotype of enhanced aggression (Doernberg *et al.*, 2001). It may well be that the more precise and selective control of amine neuron function that is available utilizing genetic methods such as the GAL4/UAS system (Kitamoto, 2001; Brand *et al.*, 1994), or recently developed methods for selectively activating neurons in behaving animals (Lima and Miesenbock, 2005), may provide more accurate information on how neurohormones function in complex behaviors such as aggression.

1.27.4 Summary

There is little doubt that in invertebrate animals, complex behavioral patterns exist that are stereotypical, recognizable by conspecifics, moldable by experience, and likely specified during development by genes. For the most part, there is little or no parental rearing in these species. Thus, as organisms emerge as adults, they must be fully capable of functioning on their own in order to survive and procreate. The fruit flies and crustacean species described in this article know all the rules of how to fight and make decisions the first time they meet a conspecific, and the decisions made as a result of agonistic interactions and probably other social experiences as well have profound consequences on their subsequent behavior. Neurohormonal substances such as amines serve important and essential roles in behaviors such as aggression in these species and much elegant experimental work has focused on

defining the roles served by these substances. In vertebrate species, with their extended periods of rearing, extensive learning or refining of behavioral patterns undoubtedly takes place shortly after pups emerge. How much of the rearing builds on patterns already defined, at least in outline form, by genes, during embryonic life, and how much defines new behaviors and patterns, remains an area of great contention. This discussion gets even more contentious when one relates it to human beings and to higher human functions such as cognition. Our goal is not to engage in this debate in this article. Instead, we aim to establish that there is much to learn from simpler forms such as invertebrates about important and essential processes that relate directly to human life and its complexities. Aggression is a serious problem in human society, a problem that undoubtedly has a biological basis. Invertebrate models offer insights into how complex behavioral patterns get established in nervous systems that are difficult to impossible when addressed in higher forms with the methodologies available at the present time, and offer elegantly detailed information on how social experience in turn molds behavior and the nervous system at levels of detail that are equally inaccessible with vertebrate studies. Those facts alone justify and warrant continuing studies of the amazing invertebrate organisms and the rich worlds in which they function and have functioned for far longer periods of evolutionary history than humans.

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1.28 Sleep in Invertebrates

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Glossary

arousal threshold	The level of stimulation required to elicit a behavioral response.
circadian rhythms	Daily patterns of behavioral activity.
homeostasis	Physiological balance for the purpose of survival.
LFPs	Local field potentials, recordings of summated brain activity.
MPC	Medial protecerebrum, part of the central brain of arthropods.
micro-behaviors	Movements of parts of the body, excluding locomotion.
phylogeny	The evolutionary relationships, through time, of all species.
quiescence	Behavioral immobility, awake or asleep.
unimodal	Described by a single peak.

1.28.1 Why Identifying Sleep in Invertebrates is Important

Sleep is universal among vertebrates, from fish to amphibians, reptiles, birds, and mammals (Campbell and Tobler, 1984). In these animals, sleep is regulatory in addition to being a behavioral state of quiescence. Similar evidence of sleep in invertebrates, on the other hand, was weak until fairly recently, partially because invertebrates have been relatively poorly studied experimentally for sleep criteria. It now appears the invertebrate realm may be partitioned between animals that require sleep and animals that do not sleep. For example, some invertebrates, such as flies, sleep as much as humans do, whereas others, such as certain nematodes, are never entirely quiescent. Although it is tenuous to make any premature claims about which invertebrates do not sleep (the case for flies sleeping is itself fairly recent; Shaw *et al*., 2000; Hendricks *et al*., 2000), one may predict more firmly that some invertebrates, perhaps certain jellyfish, roundworms, and sponges, among others, will be rigorously shown not to require any sleep at all. Much research in that realm remains to be done. Sleep has been rigorously demonstrated so far in only one class of invertebrates – arthropods.

Yet, deducing the phylogenetic emergence of sleep among invertebrates is interesting and important because it will immediately suggest which anatomical structures, neural systems, or behavioral repertoires are associated with sleep-like behavior. For example, is sleep associated with attention-like mechanisms or demonstrations of long-term memory? Is it associated with dopaminergic neurotransmitter systems? With a brain? These phenomena are all likely to have emerged within invertebrate evolution, alongside the need for sleep. Thus, by identifying sleep (or the lack thereof) in invertebrates, we are by the same token identifying which other aspects of their biology may be tied to sleep, thereby providing a valuable perspective on the possible function of sleep and its mechanism.

Sleep must accomplish an important function in both vertebrates and invertebrates: both flies and humans, for example, sleep on average a third of their life away, time during which they are highly unresponsive and cannot accomplish other behaviors which, during waking, are crucial to survival. This

function is most likely ancient and unrelated to the subjective, conscious associations we have with sleep in humans. Although our own sleep may be tied to altered conscious states, the formal demonstration of sleep relies purely on behavioral measures. These include observational studies, such as measures of daily cycles of quiescence as well as postural changes or sleeping sites. In addition, experimental studies probing the regulatory aspect of sleep include tests for behavioral responsiveness (arousal thresholds) as well as tests for increased sleep need following sleep deprivation. Finally, neural correlates of sleep can be identified in the brain activity of animals displaying the preceding behavioral states. Key invertebrates have been shown to demonstrate all of these criteria for sleep.

1.28.2 Gross Behavioral Measures

1.28.2.1 Quiescence

Behavioral immobility, or 'quiescence', is the most obvious predictor of sleep in invertebrates. The classical literature of invertebrate natural history provides a number of meticulous descriptions of behavioral states involving periodic quiescence in arthropods, annelids, and crustaceans (Rau and Rau, 1916). However, as we shall see in the following, these observations remain by and large insufficient for convincingly identifying sleep in these organisms. While some invertebrates seem to never stop moving for very long (e.g., certain nematodes and jellyfish), others become quiescent for hours at a time. Fruit flies and honeybees, for example, display long periods of immobility during the night, and cockroaches and scorpions are mostly immobile during daylight hours. Such daily cycles of activity, or 'circadian rhythms', have been well studied in model invertebrates such as fruit flies, and the underlying cellular processes controlling these rhythms are quite well understood (Hall, 2003). However, circadian rhythms alone do not necessarily reflect sleep processes. On shorter timescales (minutes or seconds), an immobile animal is not necessarily sleeping; it may be quietly vigilant. This is especially true for predatory arthropods such as scorpions and spiders, and may be general for any invertebrate capable of extended quiescence. Such immobility may also be an effective strategy for some invertebrates to evade predators, as in the case of camouflaged moths or stick-insects. Thus, immobility alone is not a sufficient criterion for sleep even if in the long term (days) circadian rhythms and sleep patterns do correlate.

1.28.2.2 Posture

Many invertebrates display a specific posture during periods of extended quiescence, and sometimes this occurs in specific sites as well. One striking example of a posture and a site associated with extended immobility is shown in Figure 1 for a species of solitary bees. These insects will seek out a twig at dusk and clamp on to its extremity with their mandibles, groom for a while, and then remain largely immobile, thus perched until the first rays of sunlight strike them the following morning. It is thought that this bizarre sleep position protects the bee while it is most vulnerable (asleep) by minimizing potential contact or detection by predators (Kaiser, 1995). Other solitary bee species sleep in the protective environment of burrows. Social honeybees sleep within their hive, where they retreat to the outer regions of the combs during the night. Fruit flies have been seen to sleep close to, but not on, food in constrained experimental settings, but in the wild they usually take refuge underneath leaves or such substrate. Scorpions, cockroaches, and spiders similarly seek burrows or find rocks or vegetation under which to retire, as do certain marine invertebrates such as *Aplysia* or cephalopods. Crayfish have been shown in the laboratory to display a typical sleep posture, lying immobile on their side just under the surface of the water, with chelae (pincers) extended (Figure 2). In each of the preceding examples of sleep posture or position, animals are almost completely immobile and (where it has been measured) display

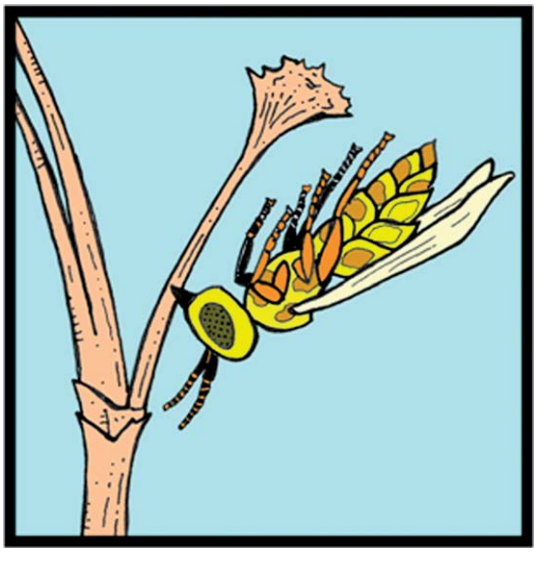

Figure 1 Sleeping posture in a solitary bee (*Nomada* sp.). At dusk, bees clasp twig extremities with their mandibles, usually with their head pointing downward and their legs off the substrate. They remain thus until the morning. Adapted from Kaiser, W. 1995. Rest at night in some solitary bees – a comparison with the sleep-like state of honeybees. *Apidologie* 26, 213–230, Elsevier.

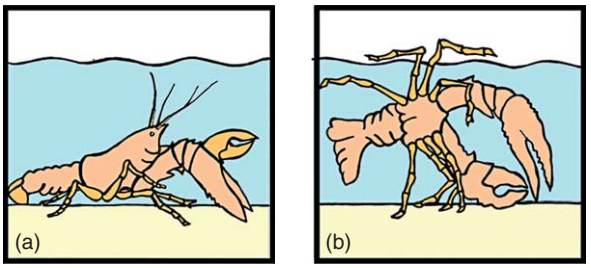

Figure 2 Sleep in the crayfish (*Procambarus clarkia*). a, Waking crayfish move, are upright, are responsive to mechanical stimuli, and display characteristic brain responses to visual stimuli. b, Sleeping crayfish lie on their side with appendages fully extended, are less responsive to mechanical stimuli, and display different levels of brain activity. Adapted from Ramon, F. *et al.* 2004. Slow wave sleep in crayfish. *Proc. Natl. Acad. Sci.* USA 101(32), 11857–11861. Copyright (2004) National Academy of Sciences, USA, with permission.

decreased muscle tone (Kaiser, 1988; Ramon *et al.*, 2004; Tobler and Stalder, 1988).

1.28.2.3 Arousal Thresholds

Still, postural immobility in itself is not a sufficient demonstration of sleep in invertebrates (as it should not be in humans either). The sleeping state can only really be put to the test by measuring behavioral responsiveness to stimuli – often irritating stimuli. During sleep, behavioral responsiveness (evidenced by movement or postural change) is always decreased (arousal thresholds are increased) compared to quiescent waking. Investigators have devised various methods of documenting increased arousal thresholds in immobile invertebrates, thereby formally associating the quiescent postural states with sleep. For example, an electric motor pounding on the thorax of a crayfish will require a stronger, longer on-time to evoke any movement while the crayfish is immobile in its sideways, extended posture (Figure 2), than when it is immobile but upright at the bottom of the observation tank (Ramon *et al.*, 2004). Arousal threshold studies in smaller invertebrates, such as flies, cockroaches, and scorpions, usually involve sporadically tapping or shaking the observation container housing the animal and quantifying subsequent whole-body movement by means of time-lapse video or infrared monitoring systems (see Cognition in Invertebrates, Aggression in Invertebrates: The Emergence and Nature of Agonistic Behavioral Patterns; Tobler, 1983). Although these studies have been relatively limited, considering arousal thresholds are the definitive indicator of sleep, they have all shown that a greater stimulus is required to evoke movement if a period of extended quiescence preceded the test stimulus. The amount of prior quiescence required for decreased behavioral responsiveness is probably variable for different invertebrates and has not been well examined. The most detailed, quantitative, studies to date on the relationship between arousal threshold and prior quiescence have been done in fruit flies, where it has been demonstrated that five minutes of immobility usually predicts decreased behavioral responsiveness, hence sleep (Shaw *et al.*, 2000; Huber *et al.*, 2004). This result argues for the possibility of a transitional state preceding sleep when the flies are still partially aroused, otherwise a shorter quiescent epoch might have been just as predictive of decreased responsiveness.

1.28.2.4 Sleep Homeostasis

The same methods which are used to test for behavioral responsiveness can also be used to deprive an animal of sleep. Sleep deprivation typically results in a 'sleep rebound' following the stimulation regime, where animals will enter extended quiescence with associated postures even during normal waking hours. Thus, a solitary bee deprived of twigs in a laboratory environment will lose sleep until it is provided with a twig, at which point it quickly engages in its bizarre sleep posture (Figure 1). In fruit flies, sleep rebounds are characterized by increased sleep intensity (higher arousal thresholds than usual) and fewer brief awakenings than are typical during a normal rest epoch. Crayfish deprived of sleep (by violently bubbling water) were shown to spend more time subsequently in their immobile 'sideways, floating' posture (Figure 2). Forced locomotion in cockroaches during the last three hours of their usual rest cycle enhanced immobility in the first four hours of the subsequent usual wake period (Tobler, 1983). Altogether, these experiments show that the regulatory or 'homeostatic' aspects of sleep common to vertebrates are present in invertebrates as well: sleep loss must be compensated by sleep recovery (Figure 3).

1.28.3 Micro-Behaviors During Sleep in the Honeybee

Studies of sleep and arousal in invertebrates have relied mainly on measures of whole-body movement, often for practical reasons since animals are mostly rather small. A few investigators have looked more closely at the micro-behaviors of individual body parts and aspects of invertebrate physiology during sleep, mainly in the honeybee. Several observations indicate that sleep in the honeybee is not a passive, homogenous state of continuous torpor-like immobility, but rather, is an active, dynamic process.

Close observation of marked individuals in a bee hive throughout the night revealed that honeybees

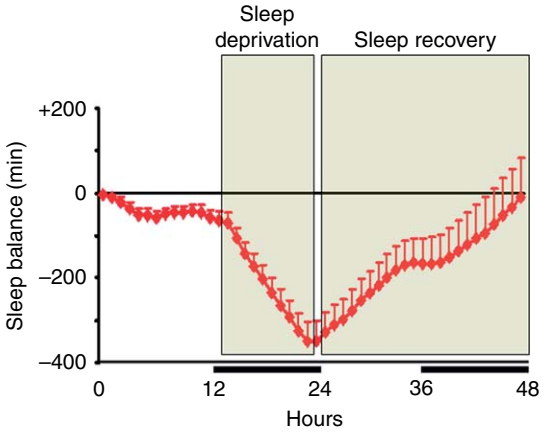

Figure 3 Sleep homeostasis in fruit flies. Cumulative rest is plotted for a population of *Drosophila melanogaster*, following a day of baseline activity (16 females, ± SEM). Fly activity is quantified by crossings of an infrared beam in a narrow tube containing food on one end. Cumulative rest (minutes of inactivity) is plotted for two days (a dark bar indicates night), during which flies are mechanically sleep-deprived during the first subjective night shown. Sleep recovery is evident in the following daylight hours, until all lost sleep has been recovered by the end of the second night, thus achieving sleep homeostasis. Data courtesy of Rozi Andretic.

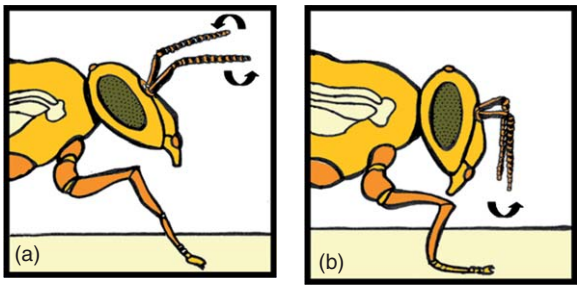

Figure 4 Micro-behaviors of sleep in the honeybee (*Apis mellifera*). a, In waking honeybees, the antenna move constantly by rotating (arrows) and orienting while the animal maintains an erect posture. b, During sleep, honeybees lose muscle tone in the neck and legs, but the antenna remain somewhat motile ('swaying', arrow) while pointing downward. Antennal motility gradually decreases until the middle of the night, when the antennas become mostly immobile. During this epoch, honeybees are most unresponsive to stimuli. Adapted from Kaiser, W. 1988. Busy bees need rest, too: Behavioural and electromyographical sleep signs in honeybees. *J. Comp. Physiol.* A 163, 565–584.

do not enter into a state of prolonged sleep immobilization, but instead show a gradual decrease in motility. Muscle tone in the legs is lost only late in the night, often culminating with the animal lying on its side in remote parts of the hive. Even closer observation achieved by video recordings of restrained bees tethered onto a treadmill further revealed that the antennas of bees show characteristic changes in motility and orientation during the night. Even while the insect is not moving its body, the antennas sway and rotate, typically ending in a downward-pointing orientation, whereupon the antennas themselves become immobilized (Figure 4). Overnight measures of antennal movement show that these appendages become gradually less motile until reaching a 'peak' of immobility in the middle of the night (hour 7 of the night cycle), thereby describing a unimodal behavioral dynamic. Arousal threshold and sleep-deprivation studies confirm that this peak of antennal immobility occurring in the middle of the bee's rest period represents the 'deepest' sleep state in the insect (Sauer *et al.*, 2003, 2004).

Additional observations of other honeybee micro-behaviors confirm that sleep is a dynamic and gradual process in this model invertebrate. Physiological recordings show that respiration, cardiac pulses, and neck muscle tone decrease gradually during the night, while behavioral measures indicate that various body parts (scape, flagella, and head) assume characteristic positions during deepest sleep. These physiological measures and body part positions all appear to co-vary significantly with antennal dynamics. Furthermore, the dynamics of some measures, such as the time between ventilatory cycles, follows exactly the same unimodal behavior as antennal immobility, centered with a peak in the middle of the night. Such comparable dynamics, as well as the co-variance among other behavioral and physiological measures, suggests that sleep in the bee is controlled by one process only. It is likely that a closer observation of micro-behaviors in other invertebrates will reveal that the dynamic, active, correlative aspects of rest in the honeybee are a general feature of sleep processes which have just never been investigated in other small invertebrates (Kaiser, 1988).

1.28.4 Electrophysiology of Sleep in Invertebrates

There are few studies of brain recordings during sleep (or wake) in invertebrates, even though this measure has become central to identifying sleep in humans (e.g., large-amplitude slow-EEG potentials are a signature of deep sleep in many mammals). Some probable reasons for the scarcity of brain activity recordings in invertebrates is their small size, the lack of neuroanatomical correlates or landmarks, and the fact that most larger invertebrates are water-dwelling, thus complicating electrophysiological recording paradigms. Sleep has nevertheless been examined in some brain recordings of insects and crayfish.

1.28.4.1 Crayfish

During epochs when crayfish are immobile and least responsive to arousing stimuli, floating sideways just beneath the water surface (see Figure 2), recordings of brain activity from their medial protocerebrum display a very regular 8 Hz oscillation. As the crayfish brain has little neuroanatomical homology with the mammalian brain, it is intriguing that any sleep-related oscillation occurs at all, even if it is not strictly 'slow'. Perhaps more relevant (than the exact frequency range of this signature) is the possibility that such a strong oscillation effectively reduces the complexity of recorded brain activity in the sleeping crayfish. Human slow waves are also less information-rich than waking brain activity.

In addition to spontaneous brain activity, the crayfish brain's responsiveness to visual stimuli was also measured during sleep. In vertebrates, trains of light pulses (e.g., 10 Hz) during waking evoke characteristic potentials upon stimulus termination, called 'omitted stimulus potentials' (OSPs), which have been described as a cognitive effect tied to expectancy. OSP amplitude in the crayfish was found to follow a diurnal cycle correlated with sleep. Behaviorally unresponsive crayfish floating on their side fail to elicit OSPs in their recorded brain activity. These evoked potentials immediately recover, however, when the crayfish is awakened by mechanical stimuli (Ramon *et al*., 2004).

1.28.4.2 Honeybees

Responsiveness to visual stimuli was also demonstrated to be dynamically tied to sleep in recordings from single neurons in the honeybee brain. 'HR' neurons in the optic lobes of insects typically respond to motion in a specific direction, by increased firing of action potentials. These neurons were found to respond much less (often not at all) to motion during the bee's rest cycle, compared to waking epochs. The spontaneous, unstimulated rate of firing also displayed a small rise and fall coinciding with wake and sleep. Critically, the neurons' responsiveness level during sleep could be 'awakened' by arousing stimuli such as air puffs or light flashes to the opposite eye. The rapid reversibility of neuronal unresponsiveness coinciding with wakefulness in the honeybee resembles similar effects found in crayfish brain recordings. The demonstration that other (nonvisual) modalities could instantly reset the responsiveness of a specific visual neuron suggests the existence of a 'general' arousal system in some invertebrates. This view is also consistent with behavioral studies in the bee showing that different sleep micro-behaviors, such as breathing and antennal immobility, seem to be under common control. The responsiveness of certain neurons in the brains of arthropods are thus tightly matched to behavioral responsiveness in general. These neurons may form part of a central system governing arousal, where an endogenous state prevents external stimuli from evoking full-fledged responses during sleep (Kaiser and Steiner-Kaiser, 1983).

1.28.4.3 Fruit Flies

Local field potential (LFP) recordings from the brains of tethered, yet intact and behaving fruit flies have further confirmed that neural correlates of sleep can indeed be identified in some invertebrates. Unlike crayfish recordings, no specific oscillations were observed in the medial protocerebrum of sleeping flies. Instead, brain activity was uniformly decreased across all frequencies measured (1–100 Hz) when flies were immobilized by sleep and showing decreased responsiveness to mechanical or visual stimuli. Similar to the crayfish OSP attenuation and the honeybee's HR neurons decreased responsiveness during sleep, a characteristic 20–30 Hz response to visual stimuli in the fly was found to be significantly attenuated during epochs of extended quiescence (Nitz *et al*., 2002; van Swinderen and Greenspan, 2003).

Similar to sleep in honeybees, sleep in fruit flies also appears to be a dynamic process marked by changes throughout the rest cycle. Correlation levels between ongoing brain activity and fly movement were found to change during the night, often decreasing to the lowest level in the middle of the night when brain activity became completely uncorrelated with any movements displayed by the insect (flies, like honeybees, move sporadically throughout the night). Furthermore, epochs of extended quiescence in the fruit fly were preceded by a few minutes of decreased correlation between brain activity and movement, providing some evidence that sleep processes may involve transitional states in these insects as well. Finally, such decreased correlation, or uncoupling, between brain and body measures of activity were associated with decreased behavioral responsiveness to a similar degree as extended quiescence itself (van Swinderen *et al*., 2004).

1.28.5 Conclusions

It is now undeniable that some invertebrates sleep. Combined data from behavioral and electrophysiological studies done almost exclusively in

arthropods (insects, arachnids, and crayfish) show that sleep is likely to be a central process in the brains of these simple animals. It is a dynamic process rather than just a state of inactivity, ultimately characterized by decreased behavioral responsiveness. As such, sleep in invertebrates is perhaps better described by an 'uncoupling' between the brain and external stimuli, instead of by single observations alone, such as brain signatures, behavioral postures, or even immobility. Taken together though, these disparate observations make a compelling argument for invertebrate sleep being a distinct behavioral state analogous to sleep in other animals. The function of sleep is likely to be the same in all animals, and perhaps the mechanism will be found to be similar as well.

The fruit fly *Drosophila melanogaster* has been pushed to the forefront as a model system in which to study sleep mechanisms and function since it is amenable to genetic analysis. Recent genetic and pharmacological studies in the fruit fly have suggested that similar neurotransmitter effects, such as neuromodulation by dopamine, may be central to controlling sleep processes in all animals. Combined with electrophysiological and behavioral data from flies and other simple arthropods, it is conceivable that invertebrates will provide valuable insights on the function and mechanisms of sleep applicable to all animals. Since invertebrates are likely to include a number of members which do not sleep, this sub-kingdom may prove to be a key resource to eventually understanding the phenomenon of sleep.

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1.29 Gene Expression in the Honeybee Mushroom Body and Its Gene Orthologues

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Glossary

cDNA microarray method — A powerful tool to analyze transcription levels of thousand of genes (~20 000) between different biological conditions with a few repeated experiments. cDNA microarray is the method to use cDNA clones fixed on a glass slide in an ordered two-dimensional matrix as probes. The transcription levels of each gene can be calculated by the intensity of hybridized targets (RNA, cDNA), which are derived from tissues of interest and labeled with florescent dye.

differential display method — A method to identify genes transcribed differentially between different biological conditions. The RNAs are prepared from tissues of interest and RT-PCR is performed using short primers (10 mers). By analysis on a sequencing gel, RT-PCR products derived from a gene of interest can be detected as a band, which shows different intensities. By subcloning of the RT-PCR product, partial cDNA sequences (~300–900 bp) of the genes are determined. As this method often generates false positives (over 50%), it is necessary to confirm the differential expression using other methods (Northern hybridization method, real-time RT-PCR method, or *in situ* hybridization).

in situ *hybridization* — A method to visualize the location of nucleic acid (RNA or DNA) in the cell or tissues. Using this method, a cell expressing a gene of interest can be identified in a tissue. To visualize transcripts of interest, the fixed tissues are prepared as sections or whole-mounted. The tissues are then hybridized to a labeled RNA or DNA probe, which has complimentary sequences to the transcripts. Finally, the labeled probe can be detected using antibody against it.

matrix-assisted laser desorption/ ionization with time-of-flight mass spectrometry (MALDI-TOF MS) — A powerful tool to determine molecular masses of biomolecules, such as proteins, peptides, and oligonucleotides. As it is a very sensitive method, low (10^{-15}–10^{-18} mole) quantities can be detected. It is also applied for analysis of a mixture of samples and can be used for direct analysis of peptide profiles from small pieces of dissected tissue and makes it possible to demonstrate the expression of bioactive peptides.

1.29.1 Introduction

The honeybee is a eusocial insect and the honeybee colony is comprised of three types of adults. Male honeybees are drones, whereas female honeybees have a genetically equal potential to differentiate into two castes, which is determined by nutritional factors: queens (reproductive caste) and workers (labor caste) (Winston, 1987). In the honeybee colonies, queens and drones are fed by the workers and have unique specializations, particularly in association with reproduction. Worker bees are sterile and perform diverse tasks to maintain colony activity. The life span of a worker bee is usually 30–40 days and their role changes depending on their age after eclosion (age-polyethism). Young workers (nurse bees)

labor at in-nest jobs such as cleaning the comb and nursing the brood, while older workers (forager bees) forage for nectar and pollen (Figure 1). A returning forager bee communicates by dance to inform other workers in the hive of the direction and distance of food. The molecular basis of honeybee social behavior, however, is largely unknown.

Mushroom bodies (MBs) are paired structures in the insect brain that are important for memory formation and are involved in higher-order processing for different sensory modalities (Heisenberg, 1998; Rybak and Menzel, 1998; Strausfeld, 2002). The MBs of the aculeate Hymenoptera, including the honeybee, are more prominent compared with most other insects (Mobbs, 1982, 1984) (Figure 2). In the honeybee, each MB has two calyces composed of 170 000 Kenyon cells, the intrinsic MB neurons (Mobbs, 1982) (Figures 2 and 3). In contrast, fruit fly MBs have only approximately 2500 Kenyon cells each (Balling *et al.*, 1987). Honeybee MBs are composed of two morphologically distinct, large (6–7 μm in diameter) and small (4–5 μm in diameter), Kenyon cells. The somata of the large-type Kenyon cells are located on the inside edges of each calyx. The small-type Kenyon cells are subdivided into two classes: the somata of class I Kenyon cells are located on the inner core of each calyx and those of class II Kenyon cells are located in the outer-bottom region of each calyx (Strausfeld, 2002; Farris *et al.*, 2004; Figure 3). The dendritic projection patterns also differ among the Kenyon cell types. The large-type Kenyon cells receive projections from olfactory and optic neurons at the lip or collar zone, respectively, of the calyces (see The Loss of Olfactory Receptor Genes in Human Evolution). The class I small-type Kenyon cells receive projections at the basal ring and collar zones, and the class II Kenyon cells receive sensory afferents from the entire calyx. Honeybee MBs have a high degree of structural plasticity and the volume of the neuropil varies according to the division of labor and sex (e.g., see Mobbs, 1985; Withers *et al.*, 1993; Durst *et al.*, 1994; Robinson *et al.*, 1997; Figure 1). These observations suggest that MB function is closely related to honeybee social behavior.

To identify genes involved in intrinsic MB function, some genes expressed preferentially in the MBs of the worker honeybee brain were identified using molecular biologic techniques (e.g., differential display method, cDNA microarray, and matrix-assisted

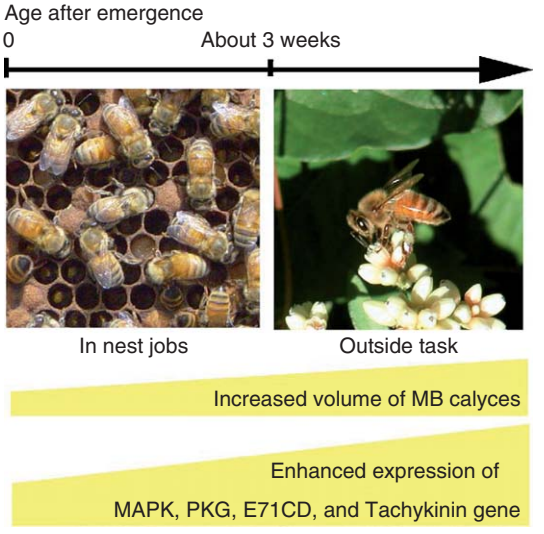

Figure 1 Age-based division of labor and gene expression in the worker brain. The many tasks required to maintain the hive are divided among worker bees in an age-based manner. Individual bees perform the tasks in an age-specific order (age-polyethism). Young bees clean the hive and nurse larvae in the nest. At around 3 weeks, the bees stand guard at the hive entrance, and after a few days the bees begin to collect pollen, water, and honey outside of the hive. The expression of some genes encoding mitogen-activated protein kinase (MAPK), methionine sulfoxide reductase, guanosine monophosphate-dependent protein kinase (PKG), and neuropeptides (tachykinin) are enhanced in forager bees, compared with nurse bees. Methionine sulfoxide reductase is encoded by an ecdysone-inducible gene, *E71CD* (Ben-Shahar *et al.*, 2002; Whitfield *et al.*, 2003; Takeuchi *et al.*, 2003). In the honeybee brain, the MBs undergo internal reorganization according to the division of labor, with the calyx volume increasing markedly between nursing and forager bees (Wither *et al.*, 1993). Photograph courtesy of T. Kubo.

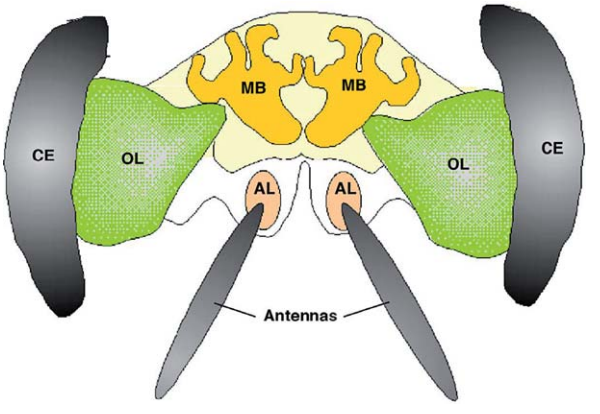

Figure 2 Schematic illustration of the front view of the honeybee brain. Honeybee brain consists of approximately 1 million neurons. The ratio of MB volume to the whole brain is approximately 12% (Mobbs, 1985). The MBs, paired protocerebral neuropils that receive processed sensory input from optic lobes (OL) and antennal lobes (AL), are thought to receive multiple inputs and have an important role in learning and memory.

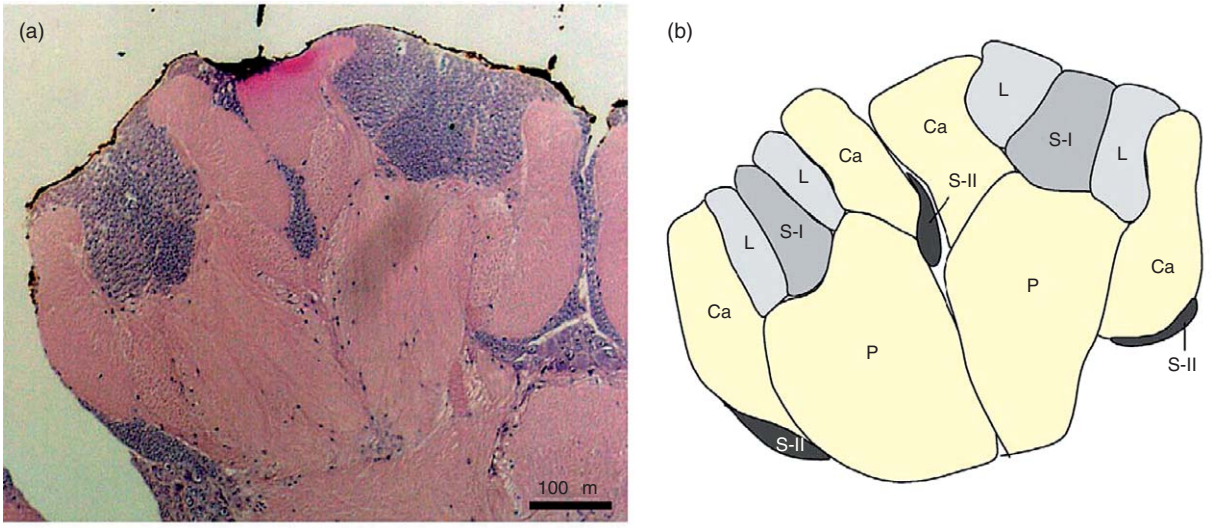

Figure 3 Honeybee MB structure. a, The left MB section with hematoxylin eosin staining. Each MB has two calyces composed of 170 000 intrinsic neurons of similar architecture called Kenyon cells (Mobbs, 1982); b, Organization in the MB. Dendrites of Kenyon cells compose the calyces (Ca), which comprise the lip (receives olfactory information from the antennoglomerular tracts), collar (receives projections from the visual medulla and lobula), and basal ring (receives dual olfactory and visual input). Kenyon cell axons compose the peduncles (P), which provide both the MB efferents and afferents (for review, see Strausfeld, 2001). Kenyon cells can be subdivided into two types: large (6–7 μm diameter) and small (4–5 μm diameter) Kenyon cells. The small-type Kenyon cells are subdivided into two classes: class I and class II (Strausfeld, 2002). In this figure, clusters of large type, and class I and class II small-type Kenyon cell bodies are indicated by L, S-I, and S-II, respectively. Farris *et al.* (1999) used another terminology to denote these main Kenyon cell subtypes: class I small-type Kenyon cells, inner compact cells (ICCs); Class II small-type Kenyon cells, outer compact cells (OCCs); large-type Kenyon cells, noncompact cells (NOCCs) (for review, see Robinson *et al.*, 1997). a, Modified from Takeuchi, H., Kage, E., Sawata, M., *et al.* 2001. Identification of a novel gene, Mblk-1, that encodes a putative transcription factor expressed preferentially in the large type Kenyon cells of the honey bee brain. *Insect Mol. Biol.* 10, 487–494, with permission from Blackwell Publishers Ltd.

laser desorption/ionization with time-of-flight mass spectrometry (MALDI-TOF MS)). In this chapter, the expression of these genes in the honeybee brain is described and their possible functions in honeybee MBs are discussed.

1.29.2 Expression of Genes for Ca^{2+}-Signaling Pathway Proteins in the MBs of the Honeybee Brain

To identify genes expressed preferentially in honeybee MBs, the differential display methods (Figure 4) and cDNA microarray methods were used to compare gene expression between the MBs and optic lobes, which were used as control tissue to detect genes expressed ubiquitously in neural tissues (see Genetic Analysis of Neural and Non-Neural Co-Evolution). Two genes were identified; one encoded an inositol 1,4,5-triphosphate (IP_3) receptor orthologue (Kamikouchi *et al.*, 1998) and the other a type I IP_3 5-phosphatase orthologue (Takeuchi *et al.*, 2002). *In situ* hybridization indicated that both of these genes were selectively expressed in the large-type Kenyon cells in the honeybee brain (Figures 5a and 5b). In mammalian brain, the IP_3 receptor is an endoplasmic reticulum protein required for mobilizing Ca^{2+} in response to IP_3-mediated extracellular signals (Mikoshiba, 1993) and type I IP_3 5-phosphatase is the major IP_3-hydrolyzing isoenzyme (De Smedt *et al.*, 1996). Thus, the function of IP_3-mediated Ca^{2+} signaling might be enhanced in large-type Kenyon cells in the honeybee brain (Figure 6).

In mammalian brain, the IP_3 receptor and type I IP_3 5-phosphatase genes are also co-expressed in cerebellar Purkinje neurons (Maeda *et al.*, 1990; De Smedt *et al.*, 1994). In Purkinje cells, some synaptic neurotransmitter receptors combine with G-proteins to activate phospholipase C (Figure 6). Phospholipase activity produces a pair of second messengers, diacylglycerol and IP_3. IP_3 mobilizes Ca^{2+} from the endoplasmic reticulum via IP_3 receptors and diacylglycerol activates protein kinase C (PKC) (Figure 5). The mammalian PKC family consists of 11 isoforms, at least 8 of which are expressed in cerebellar Purkinje cells (Barmack *et al.*, 2000).

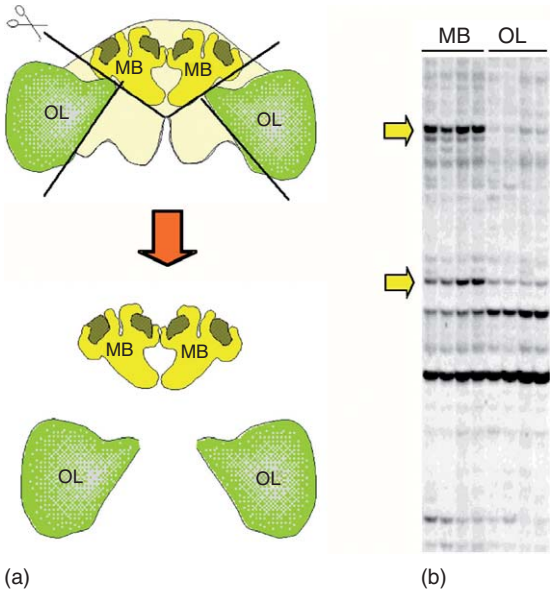

Figure 4 a, The dissection strategy used to isolate tissue prior to RNA extraction for differential display. Honeybee MBs can be easily dissected; b, Using the differential display method, the expression of each gene is detected as a band on a sequencing gel. Comparing bands in the MBs with those in the optic lobes, genes expressed preferentially in the MBs are identified as MB-selective bands as indicated by the arrows. a, Modified from Takeuchi, H., Kage, E., Sawata, M., *et al.* 2001. Identification of a novel gene, Mblk-1, that encodes a putative transcription factor expressed preferentially in the large-type Kenyon cells of the honey bee brain. *Insect Mol. Biol.* 10, 487–494, with permission from Blackwell Publishers Ltd.

PKC catalyzes the phosphorylation of various cellular proteins to affect both short-term biologic responses, such as synaptic modification, and long-lasting neuronal responses that require changes in gene expression (for review, see Clapham, 1995). In the cerebellum, the postsynaptic cascade in Purkinje cells is involved in synaptic plasticity (long-term depression), which occurs in certain forms of motor learning, such as associative eyelid conditioning and adaptation of the vestibulo-ocular reflex (for review, see Hansel *et al.*, 2001).

Therefore, the expression of other genes for proteins involved in the Ca^{2+} signaling pathway might also be enhanced in honeybee MBs. Consistently, the honeybee gene orthologues for Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and PKC are predominantly expressed in the MBs (Figures 5c and 5d). In particular, the CaMIIK gene is preferentially expressed in the large-type Kenyon cells. Furthermore, the enzymatic activities of CaMKII and PKC are higher in the MBs and central bodies than in the optic and antennal lobes of the worker bee brain (Figures 5e and 5f). In mammalian brain, CaMKII is a leading candidate as a synaptic memory molecule because it is persistently activated after the induction of long-term potentiation and because mutations that block this persistent activity prevent long-term potentiation and learning (for review, see Lisman *et al.*, 2002). Given that CaMKII is important for synaptic and behavioral plasticity, these findings strongly suggest that the genes for proteins involved in synaptic plasticity and/or behavioral plasticity are enhanced in honeybee MBs.

In fruit fly brain, the *dunce*, *rutabaga*, and *DCO* genes, which are necessary for olfactory learning, are preferentially expressed in the MBs. The *dunce*, *rutabaga*, and *DCO* genes encode cAMP phosphodiesterase, adenylyl cyclase, and a catalytic subunit of cAMP-dependent protein kinase A (PKA), respectively, all of which are involved in the intracellular cAMP signaling pathway (Figure 6). In fruit fly, mutations in the *dunce*, *rutabaga*, and *DCO* genes influence olfactory learning (for review, see Davis, 2004). PKA is also preferentially expressed in honeybee MBs, and the downregulation of PKA during olfactory learning using an antisense technique impairs long-term memory formation (Müller, 1997; Fiala *et al.*, 1999; Eisenhardt *et al.*, 2001). Thus, the expression of some genes involved in synaptic plasticity is enhanced in insect MB neurons. The fruit fly genes for IP_3 receptors and PKC, however, are not reported to be predominantly expressed in the MBs, unlike in the honeybee. The gene (*itp-r*) encoding fruit fly IP_3 receptors is a single copy gene and its expression occurs throughout the cortex of the brain (Hasan and Robash, 1992). Similarly, there are three isoforms of PKC (53E, 53(ey), and 98F) in fruit fly and their expression pattern differs from those of the honeybee orthologue (Rosenthal *et al.*, 1987; Shaeffer *et al.*, 1989). Thus, the expression pattern of some genes involved in Ca^{2+} signal transduction might be associated with the evolution of honeybee MB function. Although the role of Ca^{2+} signal transduction in the MBs is unknown, the function of Ca^{2+} signal transduction might be involved in synaptic plasticity in the honeybee MBs, based on the function of the mammalian and fruit fly orthologues.

What synaptic receptors modulate cAMP or Ca^{2+} signal transduction in the honeybee Kenyon cells? In the honeybee, there are genes for nicotinic acetylcholine receptor α-subunits (Thany *et al.*, 2005), metabotropic glutamate receptors (Funada *et al.*, 2004), dopamine receptors (Humphries *et al.*, 2003), tyramine receptors (Mustard *et al.*, 2005), and octopamine receptors (Farooqui *et al.*, 2004). In honeybees and fruit flies, octopamine and dopamine stimulate adenylyl cyclase via G-protein-coupled receptors (for review, see Davis, 2004). In honeybee, orthologue genes for fruit fly dopamine

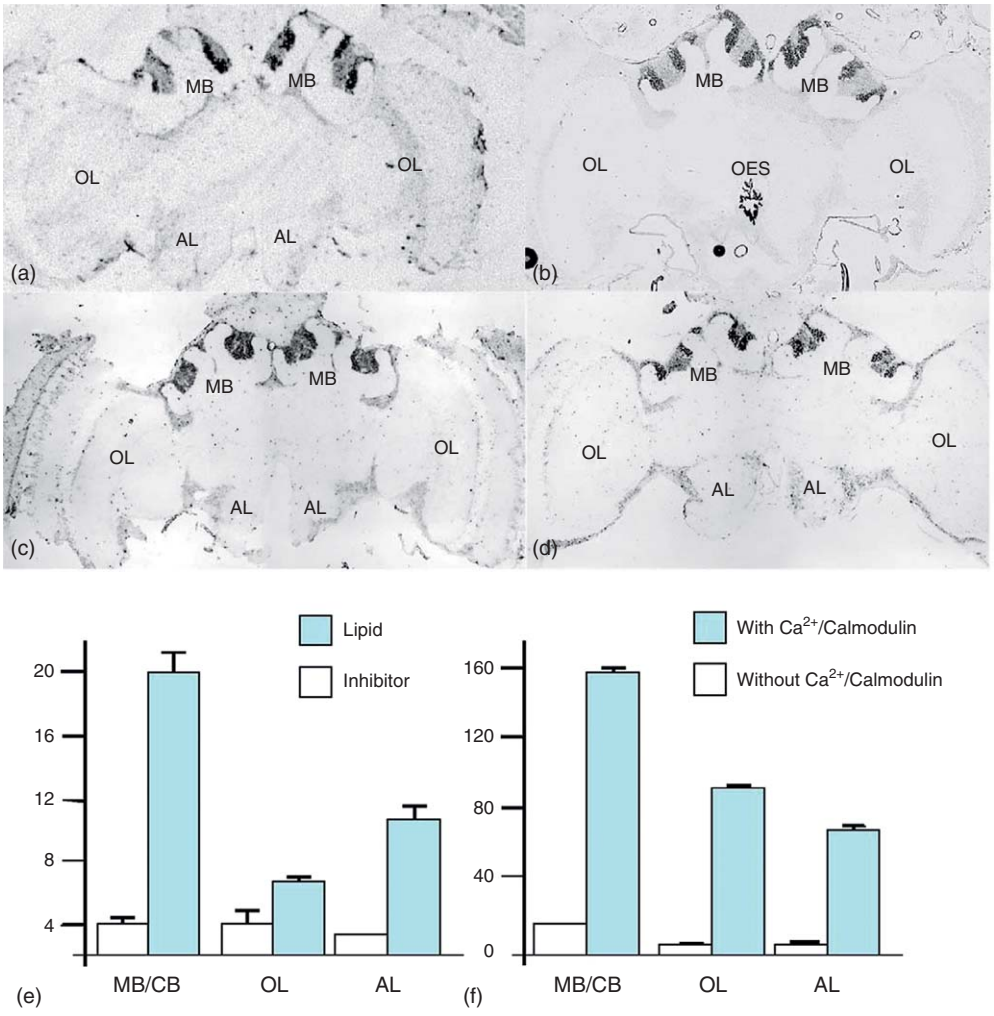

Figure 5 The expression pattern of genes for proteins involved in the Ca^{2+} signaling pathway in the honeybee MBs. a–d, *In situ* hybridization analysis was performed using frozen sections of worker bee brains with a DIG-labeled anti-sense RNA probe. Expression of genes encoding IP_3 receptors (a), IP_3 phosphatase (b), PKC (c), and CaMKII (d) was observed preferentially in the MBs. Furthermore, the expression of genes encoding the IP_3 receptor, IP_3 phosphatase, and CaMKII was observed preferentially in large-type Kenyon cell bodies, which are located at both inside edges of each calyx. e and f, Enzymatic activities of PKC and CaMKII in various regions of the worker bee brain. The *y*-axis indicates units per microgram protein. Enzymatic activities were determined using synthetic oligopeptides as specific substrates for these enzymes. The bars indicate duplicate values of the activities. The activity of PKC in the MB/central bodies (CBs) was higher than that in the optic lobes (OLs) (7.1 times) and antennal lobes (ALs) (2.5 times; e). Similarly, the activity of CaMKII in the MB/CBs was higher than that in the OLs (1.6 times; f) and ALs (2.2 times). These results indicated that the enzymatic activities of PKC and CaMKII are also enriched in the MB/CBs of the worker bee in comparison to other regions of the brain. a, Modified from Kamikouchi, A., Takeuchi, H., Sawata, M., Ohashi, K., Natori, S., and Kubo, T. 1998. Preferential expression of the gene for a putative inositol 1, 4, 5-trisphosphate receptor homologue in the mushroom bodies of the brain of the worker honeybee *Apis mellifera* L. *Biochem. Biophys. Res. Comm.* 242, 181–186, with permission from Elsevier. b, Reproduced from Takeuchi, H., Fujiyuki, T., Shirai, K., *et al.* 2002. Identification of genes expressed preferentially in the honeybee mushroom bodies by combination of differential display and cDNA microarray. *FEBS Lett.* 513, 230–234, with permission from Elsevier. c–f, Modified from Kamikouchi, A., Takeuchi, H., Sawata, M., Natori, S., and Kubo, T. 2000. Concentrated expression of Ca^{2+}/calmodulin-dependent protein kinase II and protein kinase C in the mushroom bodies of the brain of the honeybee *Apis mellifera* L. *J. Comp. Neurol.* 417, 501–510, with permission from John Wiley & Sons, Inc.

and octopamine receptors are also expressed in the MBs (Figure 5). Thus, the major components of the cAMP signaling system seem to be conserved between the two insects. To compare the molecular basis underlying MB functions between honeybee and other insects, it will be important to identify receptors that activate phospholipase C in the honeybee Kenyon cells.

1.29.3 Ecdysteroid Regulated Genes (*Mbk1-1/AmE93* and *AmE74*) in the MBs

A novel gene, *mblk-1* (mushroom bodies large-type Kenyon cells preferential gene -1) encoding a transcription factor was identified as a MB-preferential gene using the differential display method (Takeuchi *et al.*, 2001). *Mblk-1* is preferentially

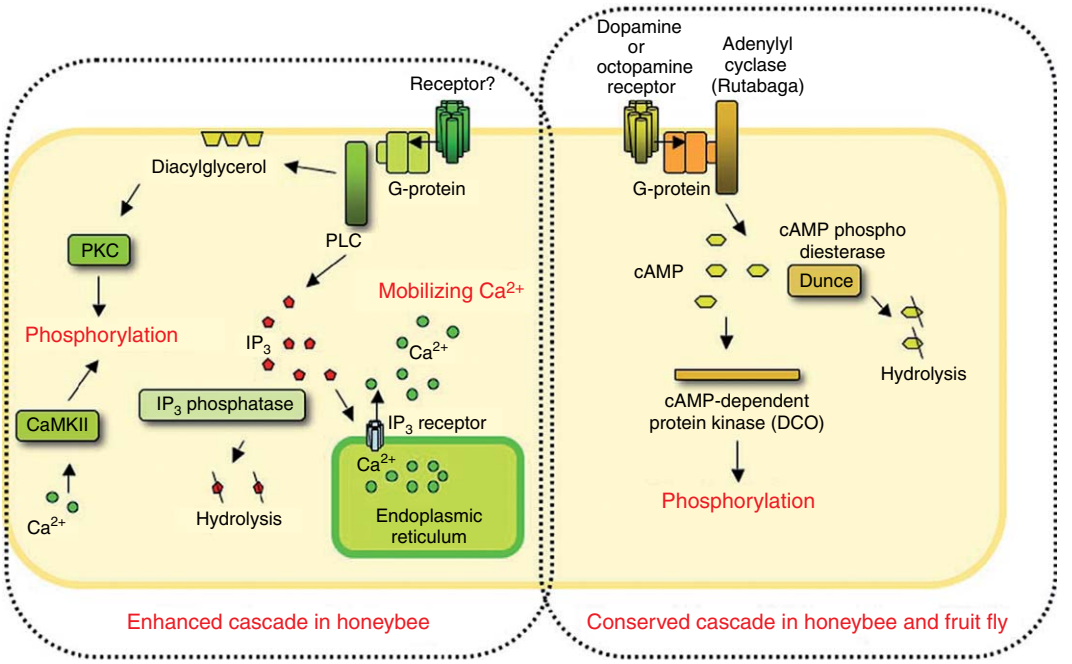

Figure 6 A possible molecular and cellular model as mediated by honeybee Kenyon cells. In fruit fly, plasticity of MB neurons, which underlies olfactory memory, requires the cAMP signaling pathway. Mutants and dominant negatives for the genes *dunce*, *rutabaga,* and *DCO* all had impaired olfactory memory. In the fruit fly, these genes are expressed predominantly in the MBs (for review, see Davis, 2004). The cAMP signaling pathway might be stimulated by neuromodulatory inputs from G-protein-coupled receptors, including dopamine D1-like receptors and octopamine receptors. In both honeybee and fruit fly MBs, PKA gene expression is enhanced. (Müller, 1997). In contrast, the expression of genes encoding PKC, CaMKII, IP_3 receptor, and IP_3 phosphatase are concentrated in the honeybee MBs. Thus, the function of the Ca^{2+} signaling pathway might be enhanced in the honeybee Kenyon cell.

expressed in the large-type Kenyon cell bodies of the MBs (Figures 7a and 7b). Mblk-1 encodes a novel transcription factor, which contains two helix-turn-helix DNA-binding motifs (Figure 8a). Park *et al.* (2002, 2003) demonstrated the biochemical properties of the honeybee Mblk-1. Honeybee Mblk-1 preferentially binds specific DNA sequences (MBE) and transactivates promoters containing MBEs (Park *et al.*, 2002, 2003). Furthermore, mitogen-activated protein kinase (MAPK) phosphorylates recombinant Mblk-1 at Ser-444 *in vitro* (Figure 8b) and Mblk-1-induced transactivation is stimulated by phosphorylation of Ser-444 by the Ras/MAPK pathway in the luciferase assay using insect culture cells (Figure 8c). Interestingly, MAPK gene expression changes according to the division of labor in worker brains (Figure 1). MAPK expression is increased over twofold in foragers compared to nurses (Whitfield *et al.*, 2003). Therefore, it is plausible that Mblk-1 functions as a transcriptional factor in the MB neuronal circuits and that its transcriptional activity might be regulated according to the division of labor (Figure 8d).

There are Mblk-1 orthologue genes in various animals, including fruit fly, nematode, mouse, and human (Figure 8a). Although orthologue genes in these species are also expressed in the nervous system, the involvement of these genes in neurons has not been characterized. In fruit fly and human, the orthologue genes are involved in steroid signaling. In fruit fly, an increase in steroid hormone (ecdysteroid) triggers metamorphosis and induces cell proliferation, cell death, and cell differentiation. The fruit fly orthologue gene of *mblk-1* (E93) is involved in programed cell death in the salivary gland in response to ecdysteroid (Baehrecke and Thummel, 1995; C. Y. Lee *et al.*, 2000). The human orthologue of Mblk-1 (LCoR) binds to the steroid hormone receptor via a LXXLL motif and the steroid hormone-receptor complex represses steroid-induced promoter activity (Fernandes *et al.*, 2003).

What is the expression pattern of other ecdysteroid-regulated genes in honeybee brain? Interestingly, the honeybee orthologue gene of fruit fly *E74* is also preferentially expressed in worker bee MBs, but in the type I small-type Kenyon cells (Figures 7c and 7d). Fruit fly *E74* encodes a transcriptional factor containing Ets DNA-binding domains and its expression is activated by ecdysteroids during metamorphosis. Like

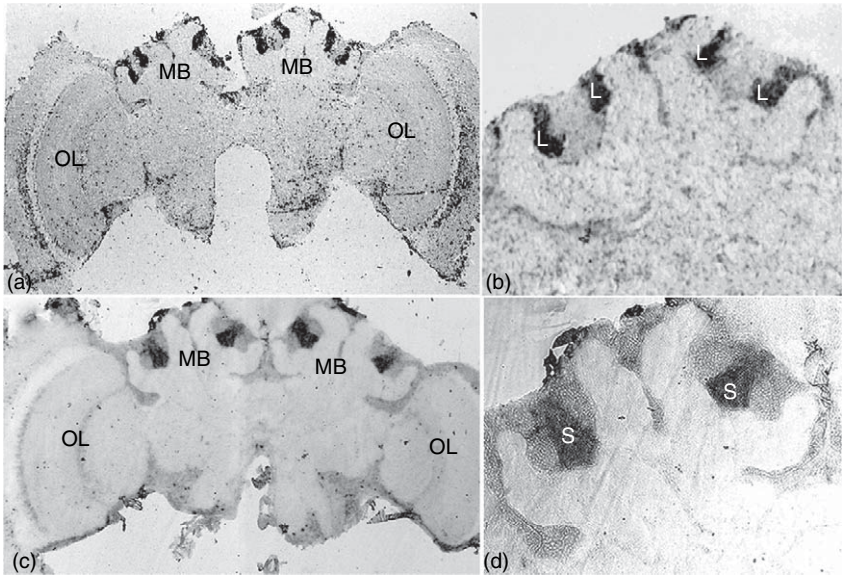

Figure 7 Kenyon cell subtype-selective expression of ecdysteroid-regulated genes (*Mblk-1/E93* and *E74*) in the honeybee MBs. In the worker brain, two ecdysteroid-regulated genes are expressed preferentially in a subset of Kenyon cells; *E74* is expressed selectively in the class I small-type Kenyon cells (S), while *Mblk-1/AmE93* is expressed selectively in the large-type Kenyon cells (L). *Mblk-1/E93* expression is shown in (a) and (b). *E74* expression is shown in (c) and (d). In fruit fly, both *E93* and *E74* are ecdysone-regulated genes, which are necessary for the regulation of ecdysone-triggered programmed cell death during metamorphosis. a and b, Modified from Takeuchi, H., Kage, E., Sawata, M., *et al.* 2001. Identification of a novel gene, Mblk-1, that encodes a putative transcription factor expressed preferentially in the large-type Kenyon cells of the honey bee brain. *Insect Mol. Biol.* 10, 487–494, with permission from Blackwell Publishers Ltd. c and d, Reproduced from Paul, R. K., Takeuchi, H., Matsuo, Y., and Kubo, T. 2005. Gene expression of ecdysteroid-regulated gene E74 of the honeybee in ovary and brain. *Insect Mol. Biol.* 14, 9–15, with permission from Blackwell Publishers Ltd.

E93, *E74* is also required for programed cell death in salivary glands (Lee *et al.*, 2000). In large-type Kenyon cells in the honeybee MBs, the expression of some genes for proteins involved in the Ca^{2+}-signaling pathway IP_3 receptor, IP_3 phosphatase, and CaMK II is enhanced (Figure 5). In the class I small-type Kenyon cells, the cGMP-dependent protein kinase (PKG) gene is preferentially expressed (Ben-Shahar *et al.*, 2002). Thus, some ecdysteroid-regulated genes (*AmE93* and *AmE74*) might regulate the transcription of genes preferentially expressed in a neural subtype-selective manner: *Mblk-1/E93* in the large-type Kenyon cells and *Am*E74 in the type I small-type Kenyon cells.

The endocrine system is important for modulating social status in eusocial Hymenoptera, including the honeybee. In the honeybee, juvenile hormone (JH) has a crucial role in caste differentiation. JH also has a role in age-polyethism of workers and acts as a behavioral pacemaker, modulating task transition (for review, see Robinson *et al.*, 1997). In contrast to the extensive studies of the effects of JH, little attention has been paid to ecdysteroids with respect to their possible involvement in regulating social behavior in the honeybee (Hartfelder *et al.*, 2002). In the honeybee, makisterone A is the principal ecdysteroid involved in molting and metamorphosis (Feldlaufer *et al.*, 1986). The hemolymph ecdysteroid titer is transiently elevated in young workers (Hartfelder *et al.*, 2002). Interestingly, the expression of the ecdysone-inducible orthologue gene *Eip71CD*, which encodes methionine-S-oxide reductase, is significantly enhanced in foragers compared to nurses (Figure 1). Thus, it is possible that ecdysteroid-regulated genes respond to ecdysteroids in the worker brain at a young age to alter neural function in adult worker honeybees. In some insects, ecdysteroids regulate insect neuronal remodeling (for review, see Tissot and Stocker, 2000). For example, in fruit fly brain, the projection of a subset of Kenyon cells changes during metamorphosis and the molecular mechanism underlying MB remodeling has also been characterized. A gene encoding an ecdysteroid receptor isoform (EcR-B1) is specifically expressed in Kenyon cells destined for remodeling, and mediates morphologic changes of the Kenyon cell axons during metamorphosis (T. Lee *et al.*, 2000; Zheng *et al.*, 2003). It might be possible that honeybee genes, whose orthologues are involved in neuronal remodeling

during metamorphosis in other insects, regulate the MB neuronal remodeling underlying the division of labor during the adult stage. Another possibility is that ecdysteroid-regulated genes (*Mblk-1/E93*, *E74*) function independently of ecdysteroids in the adult brain, as the hemolymph ecdysteroid titer of adult workers is much lower than that during metamorphosis.

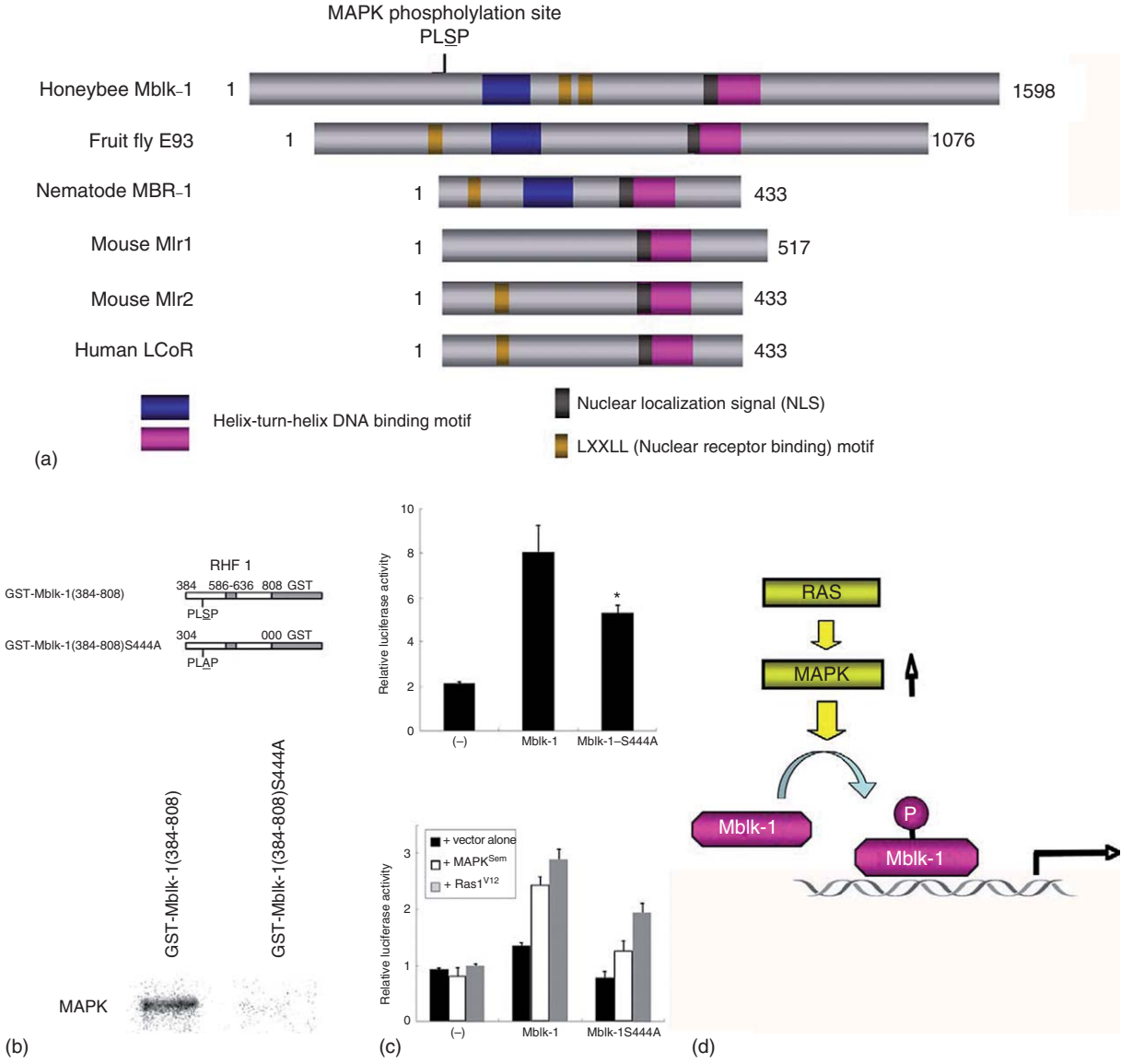

Figure 8 *Mblk-1* encodes a novel transcription factor. a, Comparison of the domain structures of Mblk-1 orthologues. The helix-turn-helix DNA binding motif of Mblk-1 has significant similarity with those of fruit fly E93 (Baehrecke and Thummel, 1995), nematode MBR-1 (Kage *et al.*, 2005), mouse Mlr1 and Mlr2 (Kunieda *et al.*, 2003), and human LCoR (Fernandes *et al.*, 2003). Human LCoR can interact with nuclear receptors (estrogen receptor) via the LXXLL motif, and this motif is also conserved; b, Mitogen-activated protein kinase (MAPK) phosphorylated Mblk-1 at Ser-444 *in vitro*. Mblk-1 contains a single consensus phosphorylation sequence (P*X*(S/T)P) for MAPK. The phosphorylated or mutant residue is underlined. The truncated Mblk-1 (GST-MBlk-384-808) can be phosphorylated by MAPK, while the mutant protein (GST-MBlk-1 384-808 S444A, where a phospho-acceptor residue, Ser-444, is changed to Ala), is not phosphorylated by MAPK; c, Mblk-1-induced transactivation is stimulated by phosphorylation of Ser-444 by the Ras/MAPK pathway in the luciferase assay using *Drosophila* Schneider's line 2 cells. Luciferase activity increases for the reporter vector containing six MBE elements, when exogenous Mblk-1 is expressed. The enhanced luciferase activity is significantly reduced using mutant Mblk-1S444A protein (upper panel). Co-expression of $MAPK^{Sem}$ or the $Ras1^{V12}$ plasmid, which are activated forms of *Drosophila* MAPK or Ras1, respectively, increases the transcriptional activity of intact Mblk-1 approximately twofold; d, The possible function of Mblk-1 in the honeybee MBs. Mblk-1 is a transcription factor that might function in the MBs neuronal circuits downstream of the Ras/MAPK pathway in the honeybee brain. As the expression of a gene encoding MAPK is enhanced in the forager bee, the Mblk -1 transactivation might be regulated according to the division of labor. b and c, Reproduced from Park, J. M., Kunieda, T., and Kubo, T. 2003. The activity of Mblk-1, a mushroom body-selective transcription factor from the honeybee, is modulated by the ras/MAPK pathway. *J. Biol. Chem.* 278, 18689–18694, with permission from American Society for Biochemistry and Molecular Biology.

1.29.4 Gene Expression of Preprotachykinin Gene in the Honeybee Brain and Other Insects

In some animals, neuropeptides are important for modulating social behaviors (for review, see Robinson, 2005). For example, in voles, two neuropeptides are critical mediators of partner-preference formation: oxytocin and arginine vasopression (for review, see Insel and Young, 2001). In nematode, social feeding is associated with the G-protein-coupled receptor NPR-1, which is activated by FMRFamide-related neuropeptide encoded by *flp-18* and *flp-21* (Rogers *et al.*, 2003).

What are the endogenous neuropeptides that function in the honeybee MBs? To determine the synthesis and expression of bioactive peptides in the honeybee MBs, the combined techniques of MALDI-TOF MS, molecular cloning, and online capillary reverse-phase high pressure liquid chromatography/quadrupole orthogonal acceleration time-of-flight (Q-Tof)-MS were applied. MALDI-TOF is a powerful tool for direct analysis of peptide profiles from small pieces of dissected tissue (for review, see Li *et al.*, 2000).

To identify neuropeptides from the honeybee MBs, MALDI-TOF MS was directly applied to slices of worker honeybee brain (Takeuchi *et al.*, 2003). Some peptides were detected as major peaks from the MB area. Based on Q-Tof MS/MS analysis, the sequence of a peak at mass *m/z* 994 was assigned as APMGFQGMRamide (Figure 9), which is a honeybee tachykinin-related peptide homologue (*Apis mellifera* tachykinin-related peptide, abbreviated AmTRP). cDNA cloning revealed that AmTRP is synthesized from precursor molecules that contain six forms of AmTRP. TRPs have been identified in various insects (for review, see Nässel, 1999, 2002). Although TRPs have neuromodulatory actions on central nervous system interneurons in locust (Lundquist and Nässel, 1997), the *in vivo* function of insect TRPs remains unclear. In blowfly, locust, cockroach, and *Drosophila*, mapping of TRP-immunoreactive neurons suggests a role for TRPs in central nervous system interneurons and in some other neurons that might regulate hormone release (for review, see Nässel, 1999). *In vitro* experiments indicate that most insect TRPs have a myostimulatory role in visceral (hindgut and oviduct) muscle (Kwok *et al.*, 1999).

In situ hybridization of the *AmTRP* gene revealed that the prepro-AmTRP gene is expressed preferentially in the honeybee MBs (Figure 9b). There are no reports of TRP-expression in the MB neurons (Kenyon cells) of other insect brains (Nässel, 1993, 1995; for review, see Nässel, 1999, 2002) Thus, prepro-TRP gene expression in Kenyon cells might be characteristic of the honeybee. In the MBs, the prepro-AmTRP gene is strongly expressed in the small-type Kenyon cells and weakly expressed in some of the large-type Kenyon cells with somata located at the inside edges of each calyx (Figure 10). Other than MBs, AmTRPs are expressed in the honeybee brain in a pattern that is similarly conserved among insect species (Nässel, 2002). In cockroach and locust brain, the TRP-immunoreactive neurons are interneurons that

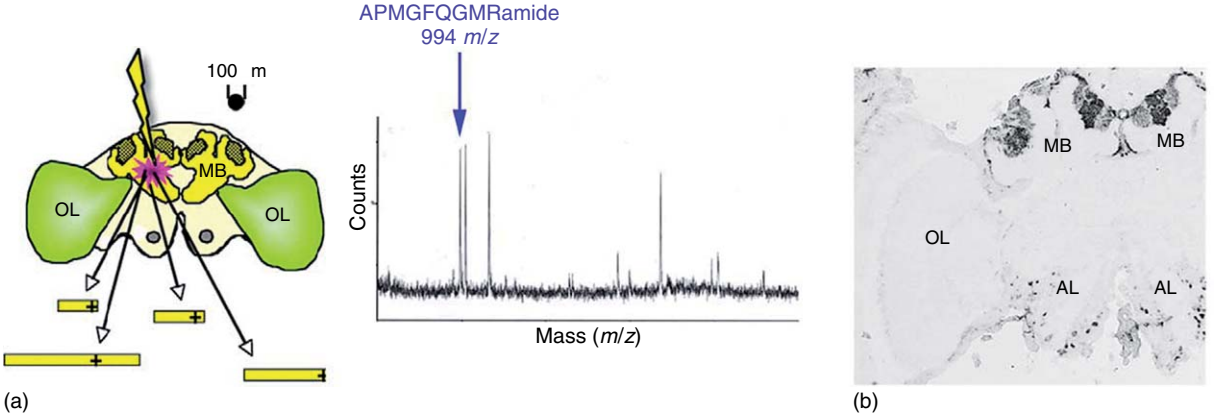

Figure 9 a, MALDI-TOF MS was directly applied to slices of worker honeybee brain. The matrix-coated brain section was mounted on a MALDI plate that can be moved within the MALDI source to expose the area of interest to the fixed position of the laser beam. Based on Q-Tof MS/MS analysis, the sequence of one of these peptides (mass *m/z* 994) was assigned as APMGFQGMRamide. Tachykinin-related peptides are characterized by the preserved C-terminal portion as FX1GX2Ramide (X1 and X2 are variable residues). As the extent of the laser beam is 100 μm, which is indicated by the black closed circle, the peptide profile from the MBs can be analyzed using this method; b, *In situ* hybridization indicates that the expression of the gene encoding the peptide is enhanced in the honeybee MBs.

have neurites projecting toward the antennal lobes, optic lobes, and central bodies. In the moth, TRPs are expressed in the cerebral neurosecretory cells (Kim *et al.*, 1998). The prepro-*Am*TRP gene is also expressed in the optic lobes, antennal lobes, and subesophageal ganglion, suggesting that the expression of TRPs in these brain regions is also conserved in the honeybee (Figure 10).

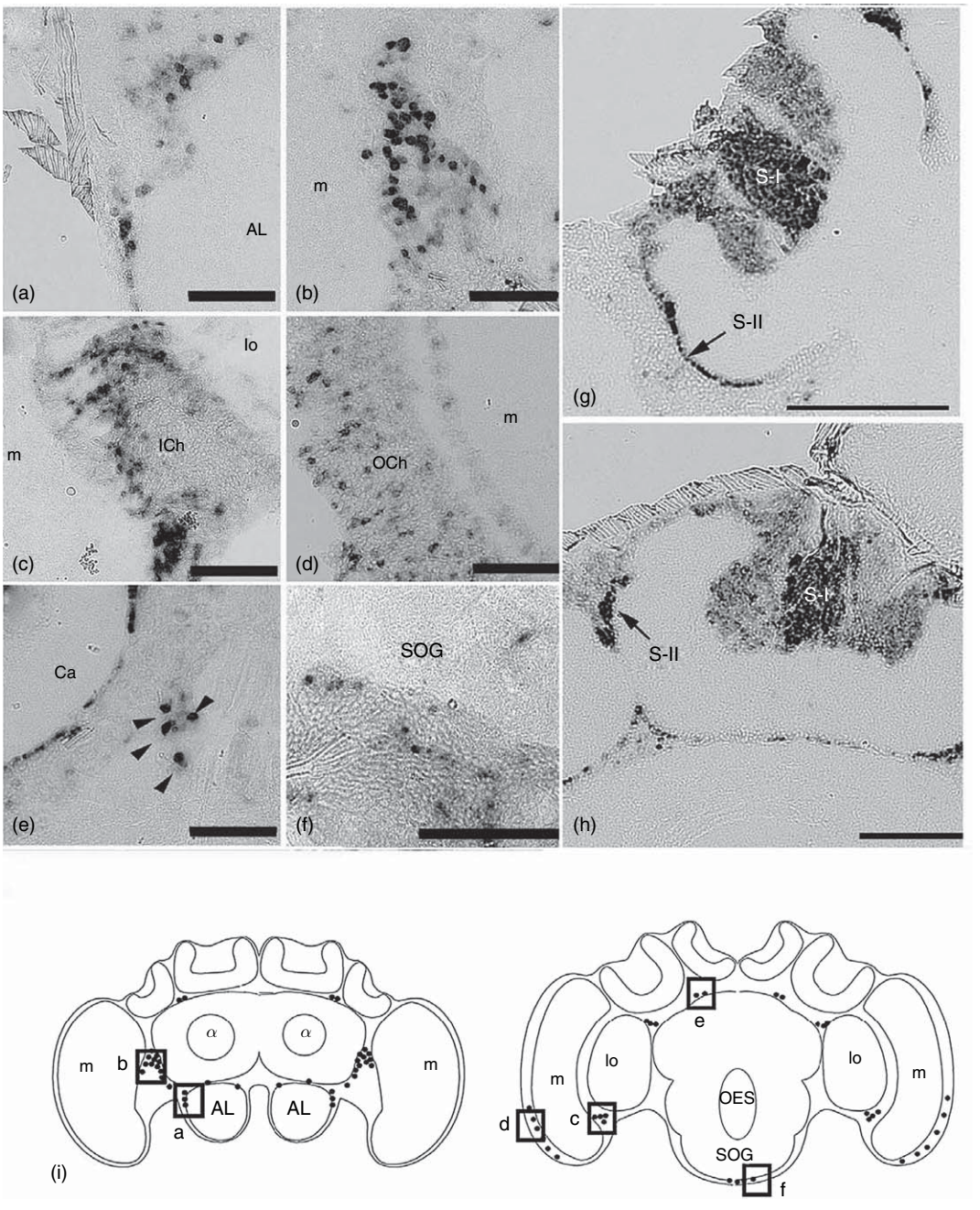

Figure 10 Expression of the tachykinin gene in the honeybee brain. (a–f), Tachykinin gene expression in some neurons of the antennal lobes (ALs), optic lobes, and other regions in the worker bee brain. a, Lateral soma layer of the ALs; b, Anterior surface of the medulla (m); c, Inner chiasma (Ich) connecting the lobula (lo) and medulla (m); d, Outer chiasm (Och) connecting the medulla (m) and lamina (l); e, Posterior view of the MB peduncles. Signals are indicated by the arrowheads; f, The somata in the ventral cortex of the subesophageal ganglion (SOG). (g, h), Gene expression in the MBs of the worker bee brain. g, Frontal section of lateral calyx; h, Frontal section of medial calyx. S-I, somata of class I small-type Kenyon cells; S-II, somata of class II small-type Kenyon cells. Bars indicate 100 μm; i, Schematic illustration of honeybee brain section (left, anterior part; right, posterior part) to show brain regions (a–f). a–i, Reproduced from *Cell Tissue Res.*, vol. 316, 2004, pp. 281–293, Prepro-tachykinin gene expression in the brain of the honeybee *Apis mellifera*, Takeuchi, H., Yasuda, A., Yasuda-Kamatani, Y., *et al.*, figures 2 and 3. With kind permission of Springer Science and Business Media.

How does *AmTRP* expression change according to age/division of labor, caste, and sex in honeybees? *In situ* hybridization indicates that there is no difference in *Am*TRP-expressing cells among worker, queen, and drone brains, suggesting that the cell types that express the prepro-*Am*TRP gene do not change according to division of labor, caste, or sex (Takeuchi *et al.*, 2004). Northern blot analysis of RNA from the head of nurse and forager bees, however, indicates that *AmTRP* expression in the head changes depending on age/division of labor and sex of the honeybees, suggesting that the AmTRP peptide functions as a neuromodulator associated with sex-specific or age/division of labor-selective behavior (Figure 1). Considering that *Am*TRP gene expression is enhanced in the MBs, which are believed to be associated with social behavior, *Am*TRP is a strong candidate neuropeptide involved in the social behavior of honeybees. In the honeybee, PKG gene expression is enhanced based on the division of labor of the workers, like the AmTRP gene, and enhanced PKG activity induces foraging behavior (Figure 1) (Ben-Shahar *et al.*, 2002). Interestingly, in honeybee MBs, the PKG gene is also selectively expressed in the small-type Kenyon cells (Ben-Shahar *et al.*, 2002). AmTRPs might be involved in the division of labor, associated with PKG function, in the small-type Kenyon cells.

1.29.5 Discussion and Future Prospects

In this article, the identification and expression of some genes expressed preferentially in honeybee MBs are described. These are strong candidate genes that are associated with honeybee social behavior and the evolution of honeybee MB function. Recently, analysis of honeybee molecular biology has greatly progressed and the Human Genome Sequencing Center at the Baylor College of Medicine is currently sequencing the honeybee genome, which has allowed us to identify more MB-preferential genes. For example, genome-wide screening of the genes of interest using DNA microarray is in progress (Takeuchi *et al.*, 2002; Whitfield *et al.*, 2003; Robinson *et al.*, 2005). The honeybee genome project has also helped to identify possible *cis*-regulatory elements involved in MB-selective expression, which could unveil the molecular mechanisms underlying transcriptional regulation of these genes. One possible strategy to investigate honeybee MB evolution is to compare the promoter regions of these genes with their orthologues in other insects and to identify the genomic regions underlying the unique expression pattern of genes for the Ca^{2+} signaling pathway and/or tachykinin in the honeybee brain.

Further studies are needed to clarify the molecular functions of MB-preferential genes in honeybees. Gene manipulation techniques such as RNA interference (Farooqui *et al.*, 2003, 2004) and in *vivo* electroporation (Kunieda and Kubo, 2004) can be applied to bees. A combination of behavioral and neurobiologic methods will contribute to clarifying the molecular mechanisms underlying honeybee social behavior. A comparison of the functions and expression patterns of the MB-preferential genes with their orthologues among insects will contribute to a better understanding of the evolution of honeybee brain and behavior.

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2.01 Evolution of the Deuterostome Central Nervous System: An Intercalation of Developmental Patterning Processes with Cellular Specification Processes

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Glossary

acrania	A chordate with no obvious head (such as the lancelet, Amphioxus).
axon	Neuronal process typically involved in emitting output.
BF1 (Foxg1)	Brain forkhead gene 1 (forkhead gene g1).
bHLH	Basic helix-loop-helix gene (transcription factors such as Atoh1, Neurog1).
BMP	Bone morphogenic protein.
cephalochordate	Animals in which the notochord extends throughout the body, including the most rostral of head part (see acrania).
chordate	Deuterostome animals with a notochord.
coelenterate	Radial symmetric, diploblastic animals (jellyfish, corrals, cnidarians).
craniate	A chordate with fully developed head (cyclostomes and gnathostomes).
cyclostomes	Jawlesss with a circular mouth (lampreys and hagfish).
dendrite	Neuronal process(es) typically involved in receiving input.
deuterostomes	Animals in which the invaginating gastroporus becomes the anus.
Dmbx	Diencephalon/mesencephalon homeobox 1.
ecdysozoans	Animals that use ecdyson for molting.
En	Engrailed gene.
FGF	Fibroblast growth factor.
GABA	Gamma-amino-butyric acid.
Gbx	Gastrulation brain homeobox gene.
genotype	The sequence of all or of specific genes.
gnathostomes	Jawed vertebrates (sharks, bony fish, tetrapods).
hemichordates	Animals with a notochord equivalent.
hodology	Connections between a complex set of neuronal entities.
Hox	Homeobox gene.
lophotrochozoans	Animals that develop larvae with a lophotroch.
MHB	Midbrain/hindbrain boundary.
nerve	Bundle of nerve fibers in the PNS.
Otx	Orthodenticle homologue gene.
Pax	Paired box gene.
phenotype	The endproduct of all genes.
progenitor(s)	An incompletely committed cell or population of cells.
protostomes	Animals in which the invaginating gastroporus becomes the mouth.
rhombomere	Compartment of the hindbrain.
topology	The position of structures such as nerve centers in a defined spatial relationship.
tract	Bundle of nerve fibers running in the CNS.

transcription factors	Proteins that bind to DNA to regulate gene transcription.
urochordates	Animals in which a notochord exists only in the tail (tunicates, ascidians).
Wnt	Wingless-type MMTV integration site family member.

2.01.1 Introduction

Multicellular animals other than sponges, coelenterates and some other basic taxa have a sizable portion of their nervous system grouped into a more or less continuous tissue aggregate, referred to as the central nervous system (or CNS: comprising the brain and spinal cord in vertebrates, the preoral and postoral chain of ganglia in invertebrates). Evolution of this centralized nervous system from a diffuse epidermal nerve network such as is present in more primitive taxa requires a reorganization or elaboration at two basic levels. First, patterning of the developing embryo must establish the position and size of the CNS anlage within the ectoderm, possibly by co-opting existing genes into a new developmental module. Second, the specification of neuronal phenotypes (which in taxa without a CNS is limited predominantly to simple neurosensory and neuromuscular cells whose connections provide limited integrative capacity) must provide molecular diversity to develop an expanded interneuron population that permits the sophisticated sensorimotor processing and integration necessary to govern the more complex motor repertoires exhibited by animals with a CNS.

Although some rudiments of the relevant molecular developmental modules are traceable to taxa without a CNS (Martindale *et al.*, 2004), the evolutionary elaboration has reached paramount status in animals in which the two basic levels have become interdependent features of an integrated process of neural patterning. In these animals, the developmental process that creates the neuronal phenotype diversity has become integrated into the patterning process to generate specific neurons in the correct places, to specify their migratory pathways and their dendritic and axonal growth patterns (in an interaction with environmental influences) to form a characteristic pattern of tracts and nerves, and to regulate proliferation and survival so that the appropriate adult number of neurons is established and maintained (Ghysen, 2003). The evolutionary transformation of a distributed subectodermal nerve net into a highly patterned CNS with numerous compartments, each containing a rich variety of characteristic neuronal types, must be explained as a well-coordinated progression of cell specification at the global and local levels. Moreover, evolution of the structure of the CNS also has to be integrated with the evolution of more sophisticated sensory apparatuses. Combination of the two would facilitate the extraction of more specific sensory information and its utilization to govern more graded and detailed motor responses, thus endowing the bearer of a given modification with enhanced survival.

A narrative of brain evolution can be assembled through a deconstruction process in which various nervous systems of extant animals are compared and a common set of principal features, or Bauplan is deduced. The validity but also the limitations of this approach are discussed in recent reviews (Butler and Hodos, 1996; Nieuwenhuys *et al.*, 1998). While successful at the level of the CNS of craniate vertebrates, it has only limited analytic power at the level of animals without a CNS such as coelenterates and basic bilaterians. Ultimately, the insights gained by this approach have to be related to the developmental patterning mechanisms that regionalize the neural anlage and specify the various populations of neurons within it (Puelles and Rubenstein, 2003). At the core of any model of brain evolution must therefore be the evolution of the transcription factors and intercellular signaling molecules that govern the patterning events necessary for CNS development (Ghysen, 2003; Holland, 2003; Lowe *et al.*, 2003; Meinhardt, 2004) and cell type-specifying transcription factors that govern the evolution of unique cell fates in specific locations (Bermingham *et al.*, 2001; Ghysen, 2003).

In this review we will outline first the basic brain organization of a cyclostome to highlight the differences from the epithelial nerve net found in hemichordate taxa (Figure 1), an outgroup of chordates among deuterostomes. We then briefly compare the major organizational differences in the CNS of adult chordates and other deuterostomes, and highlight what we consider to be the minimal steps in development necessary to generate a craniate nervous system. We then provide an overview of our understanding of the developmental patterning events that underlie CNS regionalization in major deuterostome phyla and then highlight the molecular interactions between regional patterning and neuronal phenotype determination. Finally, to underscore the importance of functional context in brain evolution, we briefly outline how the evolution of the CNS might be related to evolution of the peripheral nervous system (PNS), in particular the evolution of major sensory systems.

We hope that this review, as incomplete a snapshot of our current insight and ignorance as it is, will nevertheless provide a basis for critical and fruitful discussion of the hypotheses and ideas presented.

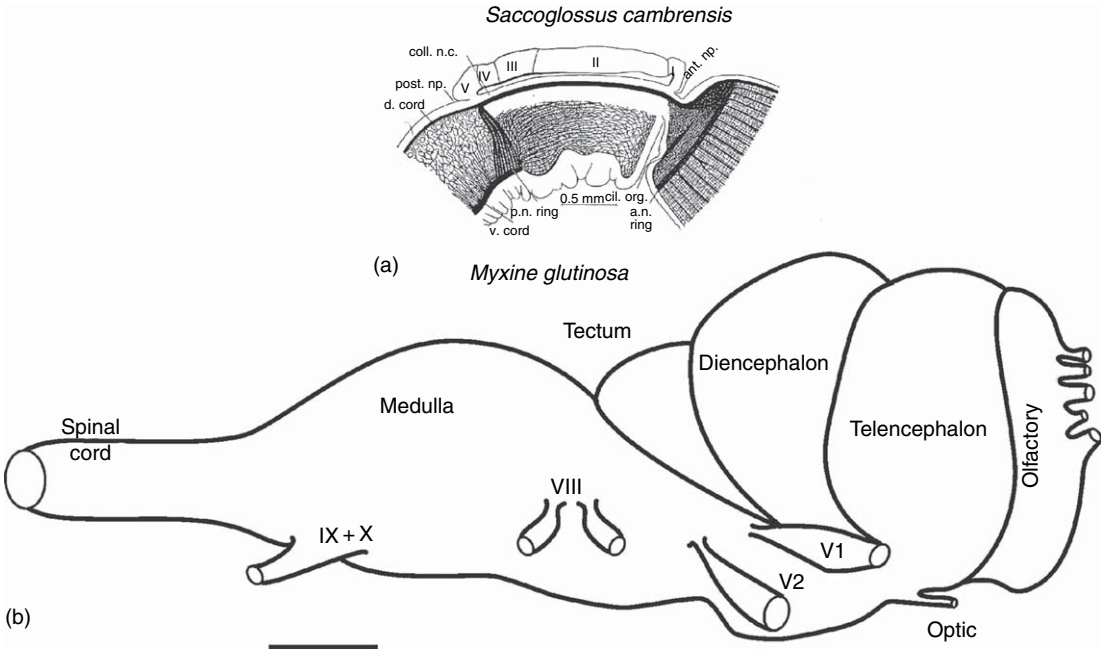

Figure 1 A hemichordate (a) and craniate (b) CNS compared at the same scale. Enteropneust hemichordates have an epidermal nerve network that shows condensations in certain areas. At the base of the proboscis is an anterior nerve ring (a.n. ring) that is next to the ciliary organ (cil. org), which is adjacent to the oral opening (org). The collar region has a collar nerve cord (coll. n.c.), an invaginated part of the epidermis with anterior and posterior neuropores (ant. np., post. np.) that lies dorsal to the buccal cavity. At the third body division, the metasome, the collar nerve cord becomes confluent with the dorsal nerve cord (d. cord) and, through the posterior nerve ring (p.n. ring), with the larger ventral cord (v. cord). Neither true nerves nor major sensory organs are apparent in this simple epithelial nerve net. In contrast, craniates (here shown is a hagfish) have a typical craniate brain that develops from invaginated ectoderm that becomes completely transformed into nervous tissue but remains confined within the former epithelial basement membrane. Only numerous distinct nerves pass through the basement membrane to connect the brain with various multisensory organs that provide chemical (olfaction and taste), mechanical (touch and vestibular sense), and visual (eyes) input for the brain to integrate into a motor output that is elicited via the brainstem and spinal cord. Adapted from Bullock and Horridge (1965) and Nieuwenhuys *et al.* (1998).

2.01.2 Cladistic Analysis of Major Differences in the Deuterostome Metazoan CNS

Analysis of CNS morphology has to be rooted in an independently corroborated cladistic analysis of the taxa to be investigated, for example, based on gene sequence data (Figure 2). Such analysis exists for only some genes, including the 18s ribosomal DNA, and relationships are likely to change as more data are considered. It should be stressed that the most recent molecular analysis has strongly supported the grouping of all extant jawless craniates into a single taxon, the cyclostomes (Winchell *et al.*, 2002; Takezaki *et al.*, 2003). This analysis also supported the sister taxon relationships of hemichordates and echinoderms and of cephalochordates and craniates. However, it only weakly supported a coherent chordate taxon, indicating that the apparent morphological similarities among chordates are imposed on deep divisions among extant deuterostome taxa (see Origins of the Chordate Central Nervous System: Insights from Hemichordates, Evolution of the Amphibian Nervous System).

Obviously, characters should be grouped to fit to such cladograms with minimal additional assumptions. Given that these separations in deuterostome phyla are approximately 600 million years old, it is to be expected that none of the crown taxa will in actuality reflect the ancestral features but rather will represent each its own idiosyncratic mix of characters retained in nearly ancestral states, characters transformed, and characters evolved anew. Such assumptions are warranted as genetic analysis has shown that about 30% of the genes of mammals are not shared with insects and likely arose after the split of protostomes from deuterostomes (Venter *et al.*, 2001). This split also led to an increase in the number of genes including generation of multiple orthologues (Wada *et al.*, 1998; Meinertzhagen *et al.*, 2004).

2.01.2.1 Principles of Comparative Neuroanatomy

Chordate brains are basically two-dimensional sheets of epithelial cells that have been folded during

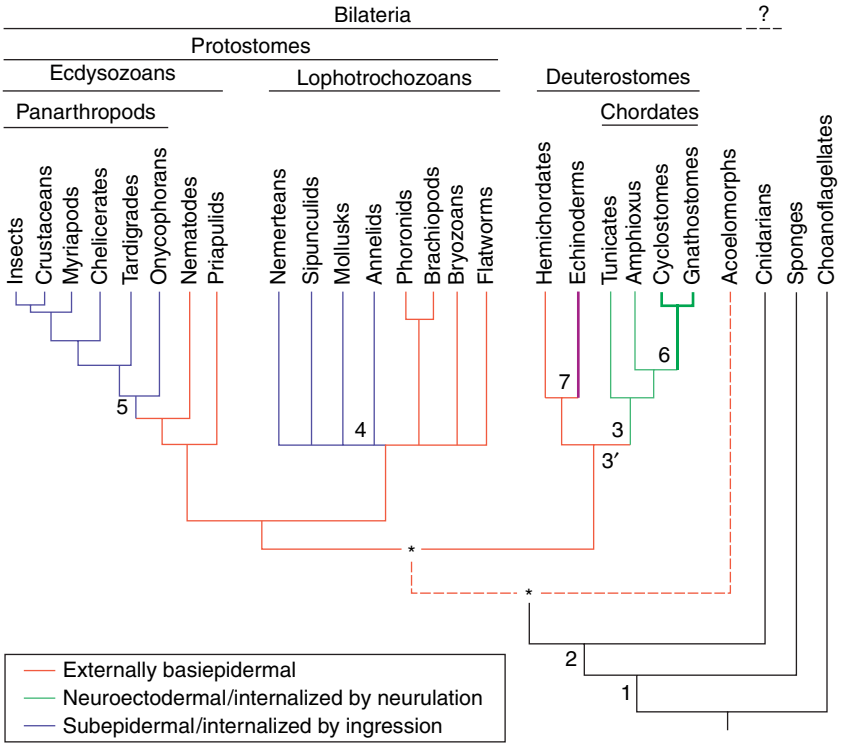

Figure 2 This cladogram of animal relationships shows major evolutionary steps in the generation of the nervous system. Outgroup comparison suggests that ancestral deuterostomes had an epidermal nerve plexus. Formation of a dorsal hollow nerve tube characterizes chordates (3), but a hollow epithelium with an epithelial nerve plexus is also found in hemichordates, suggesting that invagination of ectoderm may be primitive for deuterostomes (3′). Transforming the neural tube into a brain coincides with the formation of all craniate sensory systems, the eyes, ears, olfaction, taste, and lateral line (6). Clearly, under any scenario, the pentameric nervous system of echinoderms is considered as secondarily derived (7). Other major steps in evolution of the brain were the formation of interneurons (1) and the formation of a basiepithelial nerve net (2). Internalized nervous systems evolved independently in lophotrochozoans (4) and ecdysozoans (5). Asterix indicates uncertainty for the position of the common ancestor of deuterostomes and protostomes. Note that some data question the coherence of the taxon chordates (Winchell *et al.*, 2002). Adapted from Holland, N. D. 2003. Early central nervous system evolution: An era of skin brains? *Nat. Rev. Neurosci.* 4, 617–627.

development in various ways, have translocated below the epidermis and have differentially increased in thickness. Within this tissue sheet, connections are established that provide highways for information flow from sensory inputs, such as the eye, to motor outputs, such as spinal motoneurons. Comparative neuroanatomy tries to unravel the basic organizational principles of neuron populations and fiber tracts, whereas comparative embryology tries to relate species differences in this organization to developmental modifications, and ultimately to alterations in genes or gene expression (Fritzsch, 1998; Nieuwenhuys *et al.*, 1998; Nieuwenhuys, 2002). Essential for this approach is that the sameness of a given structure has to be established to verify homology. Two principal criteria have been used to identify homologous structures in the brain: topology and hodology. Topological analysis compares the relative positions of structures and neuron groups, preferably tracing them back to their origins in the proliferative centers at the ventricles (Bayer *et al.*, 1993). This approach can lead directly to the definition of a blueprint of clonal origin of neurons that can be related to transcription factor expression at the start of brain development and can therefore be integrated with known alterations in the sequences (both coding and regulatory sequences) and expression patterns of genes (Shubin *et al.*, 1997; Puelles and Rubenstein, 2003). Once completed, this approach will allow for establishing homology of a given neural structure across phyla using both topological and genetic criteria (Holland *et al.*, 1992; Takahashi and Holland, 2004). Such a phylogenetic definition of homology for the nervous system will have to face issues of homoplasy, deep homology, and serial homology, all problems already raised for the evolution of other systems (Figure 3).

A second approach to generating a blueprint of vertebrate and invertebrate brains is through hodological analysis of connections (Herrick, 1948; Butler and Hodos, 1996). Ideally, connections

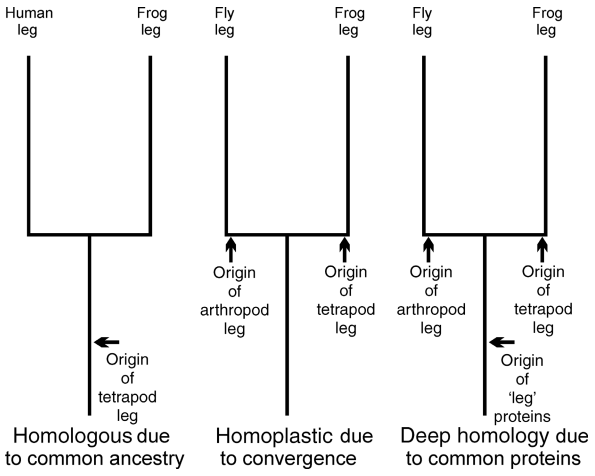

Figure 3 The basic problem of evolutionary homology is depicted. Homologous structures, such as various tetrapod appendages, are considered homologous because they all derive from the ancestral tetrapod limb. In contrast, homoplastic characters are independently derived from an ancestor that had no legs. Deep homology is rooted in molecular mechanisms as well as morphological similarity. For example, recent data shows that the proteins governing leg development are conserved across phyla, indicating that the molecular evolution predated the morphological evolution. Cases for which deep homology (also known as homocracy; Nielsen and Martinez, 2003) has been argued include the legs of arthropods and vertebrates, and the eyes and ears of multiple phyla. Whether this is also the case for brains remains more controversial (Arendt and Nubler-Jung, 1999; Lowe *et al.*, 2003; Meinhardt, 2004). Modified from Arthur (1997), Fritzsch and Beisel (2004), Kozmik *et al.* (2003), and Shubin *et al.* (1997).

between topologically identifiable neuron populations should exhibit a substantial degree of conservation through evolution, because the molecular basis for the homology of neuron populations is also likely to be involved in guiding axon pathway selection by the neuron populations that are specified. Indeed, numerous pathway selection genes appear to be conserved across phyla, suggesting that pathway selection molecules arose early in metazoan evolution (Ghysen, 2003). Nevertheless, the only existing direct analysis that compares cytoarchitectonic and hodologic approaches shows limited congruence between the two blueprints (Diaz *et al.*, 2003). In addition, despite the absence in animals such as salamanders of recognizable neuronal condensations that can be identified cytoarchitectonically, these brains nevertheless exhibit distinct fiber projections that allow for hodological comparisons across phyla in adults (Herrick, 1948; Nieuwenhuys *et al.*, 1998) and during development (Rettig *et al.*, 1981). Hodology may therefore provide a stronger basis for comparison than cytoarchitectonics, even if it does not derive from cytoarchectonically distinct groups of neurons, the so-called nuclei.

The analysis provided below will follow both of these approaches (topological and hodological), keeping in mind that topological relationships can be complicated through postnatal migration, modifications of input and output relationships, and alterations in absolute positions owing to intercalation of different cellular masses (Glover, 2001).

2.01.2.2 Comparative Appearances of Brains, Spinal Cords, and Nerves

2.01.2.2.1 Craniates (cyclostomes and gnathostomes)

The jawless cyclostomes, lampreys and hagfish, constitute a basic clade of craniates that shows the major features of brain organization also found in gnathostomes (jawed vertebrates). Externally, the brain of extant cyclostomes consists of a bilaterally symmetric rostral enlargement, the forebrain (telencephalon). This is composed of an olfactory bulb and a cerebral hemisphere (Figure 4). Each hemisphere is connected to the single, bipartite diencephalon. The diencephalon gives rise to a number of neural appendages. Ventrolaterally are located the paired optic nerves leading to the lateral eyes. Dorsally lies a single enlargement, the habenula, that in lampreys, but not hagfish, has an attached pineal organ (Pombal *et al.*, 1999). Ventrally is located the pituitary gland. The central ventricle of the diencephalon is continuous with the ventricle in the midbrain, the hindbrain, and the central canal of the spinal cord, all of which are greatly reduced in hagfish (Nieuwenhuys *et al.*, 1998). Lampreys have a dorsal central opening in the midbrain that is covered by a choroid plexus, a uniquely derived feature of lampreys not shared by other craniates. Lampreys have an oculomotor nerve leaving the midbrain ventrally, which hagfish do not possess. Likewise, the next more caudal nerve found in lampreys, the trochlear nerve, exiting lateral to the cerebellum, is not found in hagfish. Caudal to the small cerebellum in lampreys is the rhombencephalon (hindbrain) with a large choroid plexus covering the IVth ventricle. A small ventricle is also found in hagfish but there is no trace of a choroid plexus and the presence of a cerebellum has been questioned (Nieuwenhuys *et al.*, 1998). Whether the absence of a choroid plexus in hagfish is primitive and related to the unusual isotonicity of hagfish to seawater (Griffith, 1987) remains unclear.

As discussed previously (Fritzsch and Northcutt, 1993), lampreys have all the hindbrain nerves found in gnathostomes (trigeminal, abducens, facial, otic or statoacoustic, glossopharyngeus, and vagus), with the possible exception of the hypoglossal

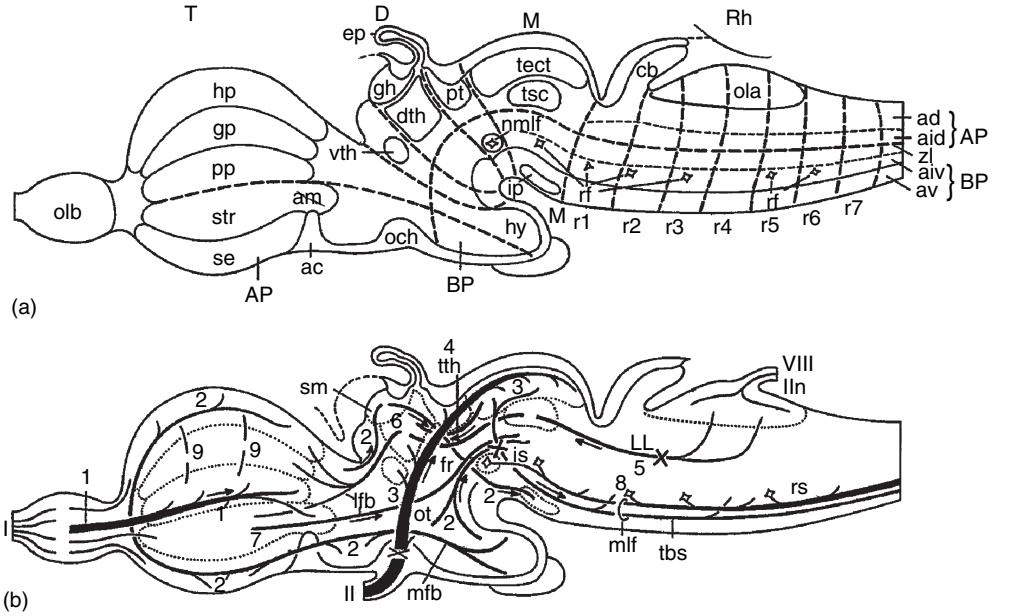

Figure 4 Basic organization (a) of a craniate brain (T, telencephalon; D, diencephalons; M, mesencephalon; Rh, rhombencephalon) and fiber tracts (b) are shown. Longitudinal divisions are the alar plate (AP) and basal plate (BP), which are separated by the sulcus limitans (zl). Each of these plates is subdivided into two areas (ad, area dorsalis; aid, area intermediodorsalis; aiv, area intermedioventralis; av, area ventralis). The rhombencephalon has approximately seven rhombomeres (r1–r7) with motoneurons and the reticular formation (rf) deriving from the basal plate and the octavolateral area (ola) and cerebellum (cb) from the alar plate. The midbrain has the tectum (tect), torus semicircularis (tsc), and interpeduncular (ip). Several prosomeres are shown in the diencephalon that extend from the hypothalamus (hy) to the pallium (hp, hippocampal pallium; gp, general pallium; pp, piriform pallium) and basal telencephalon (str, striatum; am, amygdale; se, septum). Several neuronal masses differentiate in the diencephalon (gh, habenula; pt, pretectum; nmlf, nucleus of the medial longitudinal fascicle; vth, ventral thalamus). The olfactory tract (1) originates from the olfactory bulb (olb) that receives the olfactory input (I). Secondary olfactory fibers reach various areas of the telencephalon, including the dorsal (sm, stria medullaris) and ventral thalamus (2). The fasciculus retroflexus (fr) relays information to the ip. Retinal axons (II) project through the optic chiasme (och) to the dorsal thalamus and optic tectum (3). The midbrain receives fibers from the octavolateral area via the lateral lemniscus (LL, 5), which projects (4) to the dorsal thalamus (6), which in turn projects to various parts of the telencephalon. Fibers descend via the lateral forebrain bundle (lfb) to the midbrain. Fibers from the interstitial nucleus of the medial longitudinal fascicle (mlf) (is), the tectum (tbs) and the reticular formation form together the mlf (mlf, 8) as well as the reticulospinal (rs) and tectobulbospinal (tbs) tracts. Association fibers (9) interconnect pallial areas. Modified from Nieuwenhuys, R. 2002. Deuterostome brains: Synopsis and commentary. *Brain Res. Bull.* 57, 257–270.

nerve (but see Kuratani *et al.*, 2002), the relative positions and fiber compositions of these nerves are very similar but not identical in cyclostomes and gnathostomes. For example, the abducent nerve root is almost integrated into the trigeminal nerve root in lampreys, whereas it is always a separate ventral nerve root at a more caudal level in gnathostomes. Moreover, gnathostomes have three distinct motoneuron populations in the brainstem, each innervating a different type of peripheral target: the somatic motoneurons that innervate somitomere-derived musculature, the branchial motoneurons that innervate branchial arch-derived musculature, and the visceral motoneurons that innervate neural crest-derived parasympathetic ganglia of the head and body. In contrast, cyclostomes as a group lack the somatic motoneurons of the hypoglossal nucleus and have no visceral motoneurons as no cranial parasympathetic ganglia are known to exist (Fritzsch and Northcutt, 1993; Nieuwenhuys *et al.*, 1998). To emphasize differences between gnathostomes and cyclostomes, the hagfish hindbrain, while recognizable as such, is unusually shaped, which relates to differences in its internal organization (Nieuwenhuys *et al.*, 1998). The organization of cranial nerves in hagfish, other than the apparent absence of the entire extraocular muscle-related nerves, shows a number of deviations from gnathostome vertebrates. Hagfish have three completely segregated parts of the trigeminal nerve, two otic (statoacoustic) nerves, no recognizable vagal ganglion, and a facial nerve that exits dorsal to the otic nerves (Figure 1). The composition and evolution of cranial nerves will be discussed below and compared with other deuterostomes (see Section 2.01.2.3).

The spinal cord in craniates is a continuous extension of the neural tissue of the hindbrain. Adult

cyclostomes have unusually shaped spinal cords that are dorsolaterally flattened and have ventral and dorsal nerve roots. Lampreys have separated dorsal and ventral roots that do not form mixed spinal nerves and are asymmetric between the left and right side. Similar organizations of spinal nerves are noted for cephalochordates (Bone, 1960) and have been suggested to be primitive for chordates as this pattern is also found in hagfish and some gnathostomes (Fritzsch and Northcutt, 1993). Hagfish have, like gnathostomes, fused dorsal and ventral nerve roots except in the tail. It is believed that having fused dorsal and ventral roots is a derived feature of gnathostomes and that the superficial similarity of this feature in hagfish is independently derived (Bone, 1963; Nieuwenhuys *et al.*, 1998). As we will see below, the overall fiber composition of spinal nerves is highly variable among cyclostomes and fits neither the cephalochordate nor the basal gnathostome composition. Consequently, the analysis of character polarity of such basic issues as cranial and spinal nerves, their composition and their relationship to nerves of cephalochordates and urochordates is problematic (Fritzsch and Northcutt, 1993). We will revisit this issue after the internal organization of deuterostome nervous systems and nerves has been described (see Section 2.01.2.3).

2.01.2.2.2 Cephalochordates The central nervous system of cephalochordates is a simple tube that does not show any obvious enlargement at the rostral pole that can be compared with the brain of craniates (Figure 5), hence the alternate name acrania for this taxon. The hollow tube has a central canal that shows a vesicular enlargement at the anterior pole. The open neural canal of this vesicle has processes of the frontal eye sensory cells extending into it (Lacalli, 2004). Like craniates, cephalochordates and urochordates have a unique structure that extends throughout the central canal, Reissner's fiber (Figure 5). However, cephalochordates show a number of obvious topological differences from craniates with respect to apparently similar structures. For example, in craniates the notochord ends at the level of the hindbrain, whereas the notochord extends in cephalochordates beyond the rostral aspect of the neural tube. The notochord is now known to have a major inductive influence on brain formation through diffusible proteins such as sonic hedgehog (Shubin *et al.*, 1997; Litingtung and Chiang, 2000) and thus this influence would likely be different in cephalochordates and craniates.

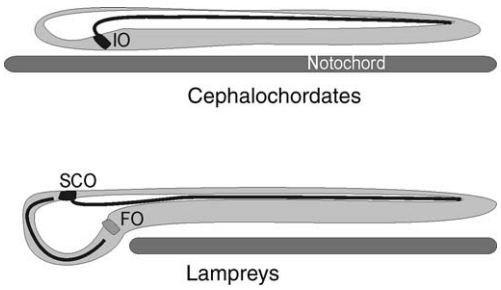

Figure 5 The different position of the origin of Reissner's fiber can be partially reconciled through developmental switches, as described in a single species of bony fish (Olsson, 1993). In principle there are three solutions: (1) Reissner's fiber is not homologous. (2) Despite the topographical differences of the organs that produce it, Reissner's fiber is homologous and is related to the apparently homoplastic structures through a deep homology for which no molecular evidence exists at the moment. (3) The infundibular (IO) and the subcommisural organ (SCO) represent a split of a single original cell population, intercalating a novel population (black line). If this is accepted, than all the area between the subcommisural and flexural organ (FO) of craniates and the infundibular organ of cephalochordates could be viewed as a craniate neomorph, that is the entire telencephalon and large areas of the thalamus of craniates have no equivalent in cephalochordates, as previously suggested (Takacs *et al.*, 2002). Modified from Fritzsch and Northcutt (1993) and Olsson (1993).

Turning now to the cranial and spinal nerves of cephalochordates, it is obvious that they are difficult to compare with those of craniates. To begin with, they are distributed too far rostral (Northcutt, 2001), leaving virtually no space for what is typically considered the brain. There are no true ventral roots in the sense of bundles of motoneuron axons leaving the spinal cord (Bone, 1960) and the notochord is composed of muscle fibers that form a unique synaptic contact with the ventral part of the spinal cord (Holland, 1996). Moreover, there are no dorsal root ganglia anywhere along the neuraxis (Fritzsch and Northcutt, 1993; Lacalli, 2004). Lastly, there is only one true ventral nerve, the first nerve. Starting with the second nerve, all dorsal nerves are more and more caudal on the right than on the left side, an asymmetry that by far exceeds anything found in other chordates (Fritzsch and Northcutt, 1993; Nieuwenhuys *et al.*, 1998). Other features of the cephalochordate anterior neuraxis are the conspicuous lamellar body (present only in larvae) and the Joseph cells. More caudally, one can find individual ocelli that extend throughout the spinal cord (Nieuwenhuys *et al.*, 1998; Lacalli, 2004). How these features relate to hindbrain and spinal cord divisions is unclear, leaving the questions of the caudal boundary of the brain and of the existence of brain subdivisions open (Northcutt, 2003). Paired sensory organs are

conspicuously absent in cephalochordates. However, certain primordia of mechanosensors and chemosensors may be present among the numerous single or multicellular organs (Fritzsch, 1996; Lacalli, 2004; Mazet *et al.*, 2004; Holland, 2005).

2.01.2.2.3 Urochordates In contrast to the rather uniform appearance of the neuraxis of cephalochordates, the developing urochordate CNS can be easily divided into a tripartite structure (Figure 6) that has long been recognized (Bone and Mackie, 1982). These divisions are (1) a rostral ganglion (totaling roughly 215 cells in *Ciona* and approximately 75 cells in *Oikopleura*), which contains sensory receptor structures, (an ocellus and/or an otolith), followed by (2) a caudal ganglion (containing approximately 45 cells in *Ciona* and approximately 25 neurons in *Oikopleura*), from which extends a caudal nerve cord (roughly 65 cells, mostly ependymal, in *Ciona*, and roughly 30 neurons and 25 support cells in *Oikopleura* (Meinertzhagen *et al.*, 2004; Søviknes *et al.*, 2005)). In addition, a slender neck region containing six cells lies between the two ganglia in *Ciona*. Each of these anteroposterior subdivisions of the ascidian CNS is itself patterned along the dorsoventral axis, a patterning that is revealed by the expression of specific marker genes. Other urochordate taxa seem to have primarily variations in size, not in structure (Meinertzhagen *et al.*, 2004). The ganglia of adult urochordates have the organization of an invertebrate ganglion, with cell bodies at the periphery and the neuropil in the center (Bullock and Horridge, 1965). Several nerves that vary considerably between species have been traced from adult ganglia, reaching up to 75 nerves in certain salps. These nerves appear to be mixed sensory and motor nerves and are asymmetric in several species, potentially related to the overall body asymmetry. The organization of motor projections varies among urochordates. In the *Ciona* larva, all the motoneurons are located in the caudal (visceral) ganglion and project along the aneural nerve cord and on to the peripheral muscle (Katz, 1983). In *Oikopleura*, the nerve cord contains motoneurons, which project directly laterally to the peripheral muscle (Bone, 1992). It is noteworthy here that comparisons between urochordates and cephalochordates claim similarities in the internal sensory organs, in particular the infundibular sensory cells and otolith (Nieuwenhuys, 2002; Lacalli, 2004). However, the interpretation of various sensory cells and organs outside the CNS is controversial with respect to vertebrate homology (Burighel *et al.*, 2003; Lacalli, 2004; Mackie and Singla, 2004; Holland, 2005). Some adult urochordates have fairly complex eyes attached to the cerebral ganglion. Modern tracing studies to unravel the details of neuronal connections within urochordate ganglia have yet to be conducted (Bullock and Horridge, 1965; Meinertzhagen *et al.*, 2004), but some are on their way.

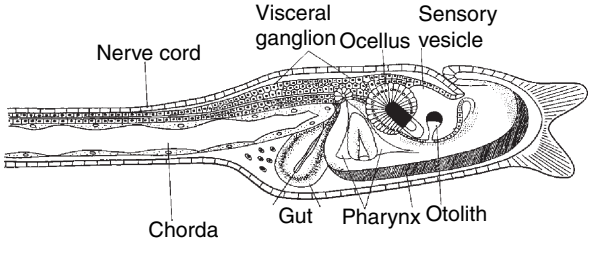

Figure 6 Simplified scheme of the urochordate *Ciona* larva. The nerve cord of *Ciona* consists only of epithelial cells (whereas in the appendicularian *Oikopleura* it contains neurons). In *Ciona*, the visceral (caudal) ganglion contains the motoneurons and is separated by a small neck from the sensory vesicle (rostral ganglion) that contains a rostral otolith and a caudal ocellus. The pharynx has gill slits.

2.01.2.2.4 Hemichordates Whether the simple nervous system of hemichordates should be referred to as a CNS is unclear (Bullock and Horridge, 1965). The overall organization is that of a basiepithelial plexus that shows regional concentrations in the three parts of the body, the protosome (the preoral proboscis), the mesosome (the postoral collar), and the metasome (or trunk, with rostral gill slits). The intraepithelial nerve plexus is well developed on the basement membrane but remains epithelial even in the invaginated collar region, which is hollow and opens through two neuropores (Figure 1). Concentrations of longitudinal strands of cells and fibers exist also on the trunk, where they form a dorsal and a larger ventral cord. The proboscis has a well-developed nerve plexus and numerous sense cells. A gut diverticle, the stomochord (which shares anatomic features with the notochord; (Welsch and Storch, 1970) but is different in its molecular organization (Shubin *et al.*, 1997)), extends into the proboscis. Except for the preoral ciliary organ with its abundance of sensory cells, there are no specialized sense organs. Concerning nerves, it appears that muscles are supplied by nerve fibers that cross the basement membrane singly and diffusely without forming obvious peripheral nerves.

2.01.2.2.5 Echinoderms The nervous system of echinoderms is interesting in its own right (Bullock and Horridge, 1965), but is likely of limited significance for chordates, as this would require the

transformation of a pentameric organization into the dorsal hollow nerve chord. While this cannot be ruled out, we assume for the sake of simplicity that echinoderms are derived and not directly related to the chordate ancestor. It needs to be stressed, however, that tremendous progress has been made in the study of sea urchin embryogenesis and these insights have recently been extended to nervous system development (Poustka *et al.*, 2004). However, the most detailed analysis seems to be concentrated on endoderm, ectoderm, and mesoderm formation (Davidson *et al.*, 2002). This analysis has revealed a complex network of gene interactions, many of which are conserved across phyla and are thus likely to be at least equally complex in other deuterostomes. It is important to understand that these data show that patterning genes function in networks and their function needs to be understood in the context of other genes with which they are co-expressed.

In summary, the CNS of deuterostomes shows a variety of forms, from a hardly specialized basiepithelial nerve plexus (hemichordates), to a few small ganglia with a tail nerve cord (urochordates; or a tailless head in adult sessile urochordates), to a swimming spinal cord with a hardly recognizable cerebral vesicle (cephalochordates), to a fully developed brain and spinal cord (craniates). Despite these overall differences, similarities related to a certain degree of rostrocaudal and dorsoventral patterning are present. These may be directly related to the overall rostrocaudal patterning of the body and to the fact that the neural tube evolved only once in ancestral chordates (Meinhardt, 2004) and has maintained a molecularly identical dorsoventral patterning scheme (Wada and Satoh, 2001). These issues will be revisited in a later section once we have introduced the transcription regulating genes that are involved in such patterning.

2.01.2.3 Organization of Identified Neuron Populations and Projections

Cytoarchitectonic specializations akin to cortical layers and aggregations of neurons into nuclei have not been observed in any deuterostome animal below the level of craniates. However, neuron populations can be identified throughout the animal kingdom on the basis of unusual size or through the use of specific markers.

2.01.2.3.1 Large neurons Particularly large neurons are a common feature of both invertebrate and vertebrate nervous systems. In many chordates, large neurons with descending axons have been identified in the rostral region of the neuraxis. Using cytological criteria, one can identify certain large reticulospinal neurons in the rhombencephalon of lampreys (the Muller and Mauthner cells) that have long descending axons that extend the length of the spinal cord (Nieuwenhuys *et al.*, 1998). Similar neurons are found in hagfish, but Mauthner cells cannot be identified among them and whether the other large cells are homologous to Muller cells is unclear. In the rostral neuraxis of juvenile cephalochordates, two pairs of larger interneurons with descending axons have been identified and termed ventral giant cells of the primary motor center. Similar large neurons have been serially reconstructed from electron micrographs in larval cephalochordates (Lacalli, 1996). These are potentially homologous to the reticulospinal neurons of cyclostomes. The axons of cephalochordate motoneurons also descend along the cord for unknown distance, however, and thus could be mistaken for reticulospinal neurons. Assessment of neurotransmitter phenotype could resolve this question, as motoneurons are expected to be cholinergic (see Section 2.01.2.3.2).

Cephalochordates are well known for another system of large neurons, the Rhode cells (Bone, 1960; Nieuwenhuys *et al.*, 1998). These cells are situated dorsally in the spinal cord starting at nerve VI in juveniles and somewhat more rostrally in adults (Ekhart *et al.*, 2003). Neurons with somewhat similar characteristics have been described in various craniates (Harper and Roberts, 1993) and may be a common feature of cephalochordates and craniates (Fritzsch, 1996).

No large neuronal elements have been described in urochordates (Bullock and Horridge, 1965), but large neurons have been described in hemichordates, clustered in the caudal part of the collar cord and also scattered in more rostral and caudal areas. Some of these neurons have uncrossed or crossed axons that extend toward the ventrolateral longitudinal muscles and therefore may be motoneurons (see below). Others have been compared to the Mauthner and Muller cells of craniates (Bullock and Horridge, 1965). However, independent confirmation of similarities with other deuterostome neurons needs to be established using immunocytochemistry or *in situ* hybridization for molecular markers, as in recent analysis of the urochordate ocellus (Sun *et al.*, 2003). At the moment, all the above anatomical similarities are tentative and more work combining tract tracing with assessment of gene expression is needed in more deuterostomes to substantiate potential homologies.

2.01.2.3.2 Motoneurons Motoneurons can be compared easily across deuterostomes for the following reasons. First, motoneurons are cholinergic in echinoderms, urochordates, cephalochordates, and craniates, and this may constitute a conserved feature of deuterostomes (Holland, 1996). Second, all motoneurons constitute efferent populations that target structures outside the CNS. However, these targets may be mesoderm-derived muscle fibers, neural crest-derived autonomic ganglia, or placode-derived hair cells (Fritzsch, 1999).

In hemichordates, motoneurons may be among the identified giant neurons, but details are unclear and no data on the cholinergic nature of these neurons is available (Bullock and Horridge, 1965). In some urochordates (such as *Ciona*), motoneurons are found only in the visceral ganglion where three to five pairs of cells have been recognized. The axons of these cells extend down the nerve cord and then exit to innervate adjacent muscle fibers (Katz, 1983). In other urochordates, such as *Oikopleura*, motoneurons are additionally found in the nerve cord itself (Bone *et al.*, 1996).

Cephalochordates have three different types of contacts with muscle fibers (Bone, 1960; Bone *et al.*, 1996; Holland, 1996). Two of these contacts are from muscle fibers to the spinal cord forming ventral roots and medioventral roots with the axial musculature and the muscles of the notochord. The motoneurons supplying these muscle fibers have only been tentatively identified for axial musculature and appear to project axons for at least three segments rostral or caudal before exiting (Figure 7). Motoneurons are found throughout the spinal cord and as far rostral as the primary motor center between the second and third dorsal nerves. An additional set of motoneurons encompasses the so-called visceral motoneurons. These are the most ventral neurons in the nervous system, situated virtually at the floor plate (Bone, 1960). The axons of these cells exit through the dorsal roots and supply the pterygeal muscle with cholinergic endings (Bone, 1960; Bone *et al.*, 1996).

In cyclostomes, somatic motoneurons are present only in the spinal cord and in the oculomotor nuclei near the isthmus, whereas branchial motoneurons are only present in the brainstem (Fritzsch and Northcutt, 1993; Fritzsch, 1998). Absence of extraocular motoneurons in hagfish may represent a primitive condition and, at least for oculomotor and trochlear motoneurons, may be related to a different organization of the isthmus and the apparent absence of a cerebellum. We will discuss this issue below when we consider the evolution of the midbrain/hindbrain boundary (MHB). It is important to note that cyclostomes do not have autonomic ganglia and lack visceral motoneurons that innervate such ganglia. It appears that autonomic ganglia arose with gnathostomes and in association with the formation of preganglionic parasympathetic motoneurons in the head and the caudal part of spinal cord (the craniosacral parasympathetic preganglionic motoneurons) and preganglionic sympathetic motoneurons in the thoracic and lumbar spinal cord (Fritzsch, 1998).

In summary, the motoneurons of deuterostomes show certain basic similarities across taxa but also exhibit unique taxon-specific features that do not appear to follow a progressive evolutionary transformation but rather indicate independently derived transformations that may have a common root in the basiepithelial nerve plexus of other deuterostome taxa (Figures 7 and 8).

2.01.2.3.3 Sensory afferents We next turn to the distribution of afferents, a feature that is highly indicative of specific sensory modalities in craniates (Fritzsch and Northcutt, 1993). As outlined above, sensory input through cranial nerves in craniates can be simply summarized: all sensory systems, except for olfaction and the visual system, reach the brain through nerves that terminate in the rhombencephalon (Nieuwenhuys *et al.*, 1998). More precisely, all sensory input is into the alar plate of the rhombencephalon or the alar plate of the midbrain (vision) or the forebrain (olfaction). The only noncraniate deuterostomes for which a reasonably detailed knowledge of central sensory projections exists are the cephalochordates. In these animals, the central projections of rostral nerves and the first pair of dorsal nerves have been described using tract tracing (Fritzsch, 1996) and serial electron microscopic reconstruction (Lacalli, 1996, 2004). Both approaches show that the first pair of dorsal nerves passes ventrally along the lamellar body and derives from the organs of de Quatrefages as well as some of the numerous sensory cells of the rostrum. However, interpretations of the likely homology of this region differ substantially. The serial reconstruction data have been interpreted to indicate that cephalochordate larvae have a midbrain that receives not only the fibers from the rostral sensory cells and organs, but also from the frontal eye that is a likely homologue of the lateral eyes of craniates (Lacalli, 1996, 2004). In contrast, the tracing studies have been interpreted to show that the termination area near the lamellar body is homologous to the alar plate of the hindbrain (Fritzsch,

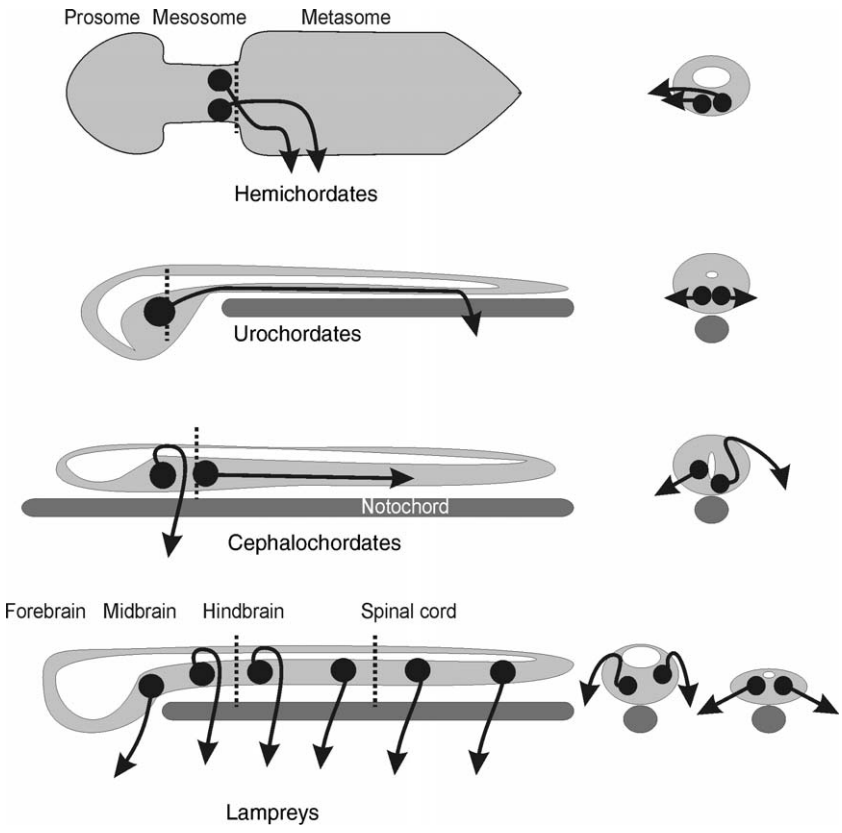

Figure 7 This scheme shows the distribution of motoneurons viewed from the dorsal aspect (Hemichordates) or lateral aspect (others) as well as in coronal sections at the levels indicated by the dotted lines. Motoneurons in hemichordates are predominantly located in the caudal half of the collar cord and their axons typically cross the basal membrane individually to reach muscle fibers. In some urochordate larvae, all motoneurons are concentrated in the caudal ganglion and project through the nerve cord to reach muscle fibers of the tail. In other urochordates, motoneurons are additionally present in the nerve cord and project directly to adjacent muscles (not shown). Cephalochordates have three types of motoneurons (only two are shown). One type is the somatic motoneuron that extends an axon along the spinal cord to innervate muscular processes that form synapses abutting the cord. The second type is the visceral motoneuron that projects through the dorsal root to innervate the pterygial muscle. Lampreys have in their spinal cord only somatic motoneurons whose axons project out through ventral roots, and in their hindbrain only dorsal exiting motoneurons, referred to as branchiomotoneurons because they innervate muscle derived from branchial arches. Lampreys have three populations of ocular motoneurons in the midbrain–hindbrain region, some of which have the appearance of somatic motoneurons. Modified from Bullock and Horridge (1965), Fritzsch and Northcutt (1993), and Fritzsch (1998).

1996). Under the second interpretation, cephalochordates lack a midbrain and hindbrain features start as far rostral as the first and second dorsal nerve. This interpretation was recently supported by an analysis of gene expression (Takahashi and Holland, 2004) and will be discussed below (see Section 2.01.3). Further studies as well as the clarification of the relationships of the various sensory organs of chordates across taxa is needed (Fritzsch and Beisel, 2004; Lacalli, 2004; Mackie and Singla, 2004; Mazet *et al.*, 2004; Holland, 2005). More molecular markers, in particular various transcription factors, need to be investigated to provide more plausibility to the various scenarios proposed.

Urochordates also have sensory input to their ganglia that is derived from cells that share certain developmental steps with the neural crest (Meinertzhagen *et al.*, 2004), but much more analysis is required in these species before arguments about homology can be made.

2.01.2.3.4 Immunocytochemically identified neuron populations We turn next to certain populations of immunocytochemically identified neurons in chordates. We will here focus only on three neuron types, those expressing the oligopeptide neurotransmitter FMRFamide, those expressing tyrosin hydroxylase (TH), the rate-limiting enzyme for synthesis of catecholamine neurotransmitters, and those expressing GABA, the principal inhibitory neurotransmitter. These examples are selected because the data are most revealing for the overall question of similarities and uniqueness of features across deuterostomes. We should emphasize, however, that

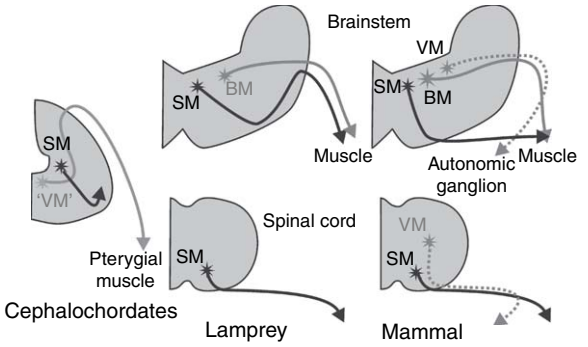

Figure 8 Motoneurons of craniates and cephalochordates are compared. Two motoneurons have been characterized in cephalochordates, the visceral motoneurons (VM) that innervate the pterygial muscle and exit through dorsal roots and the somatic motoneurons that do not exit the neural tube but rather form synapses with muscle processes at the lateral wall of the spinal cord. Lampreys have only somatic motoneurons that exit through the ventral root in the spinal cord. Mammals have evolved visceral motoneurons in the spinal cord that migrate into a distinct position and project to autonomic ganglia. The brainstem of lamprey has mainly branchiomotoneurons (BM) that project through the dorsal root. Whether the abducens motoneurons are somatic motoneurons (SM) in lampreys is unclear as they also project through the dorsal root. In mammals there are additionally visceral motoneurons in several cranial nerves that project to the parasympathetic ganglia. These visceral motoneurons can be regarded as special branchiomotoneurons. Modified from Fritzsch and Northcutt (1993) and Fritzsch (1998).

immunohistochemical identification of proteins (such as TH) in lower deuterostomes using antibodies raised against the craniate proteins should be regarded as tentative without independent confirmation using other molecular techniques.

2.01.2.3.4.(i) FMRFamide FMRFamide is widely distributed in the nervous system of invertebrates, including coelenterates (Katsukura *et al.*, 2003) and may be among the oldest neurotransmitters (Cazzamali and Grimmelikhuijzen, 2002; Seipel *et al.*, 2004). In deuterostomes, this small peptide has been demonstrated in echinoderms (Garcia-Arraras *et al.*, 1991) and immunoreactivity is abundantly present in the central and peripheral nervous system of cephalochordates (Uemura *et al.*, 1994; Bone *et al.*, 1996). In contrast, craniates seem to have only a few RFamide peptides (Hinuma *et al.*, 2000; Yano *et al.*, 2004), and the almost complete absence of FMRFamide in the peripheral nervous system and the gastrointestinal system of craniates stands in stark contrast to the abundant presence of this peptide in protostomes, coelenterates, echinoderms, and cephalochordates. This difference supports the notion that the enteric nervous system of the cephalochordate atrium is not related to the enteric system of craniates as has been previously suggested (Bone, 1961; Fritzsch and Northcutt, 1993). This may be related to a more recent evolution of the vertebrate enteric nervous system from the neural crest (Fritzsch and Northcutt, 1993).

In cephalochordates, the visceral motoneurons and possibly some of the somatic motoneurons are FMRFamide-immunoreactive (Pestarino and Lucaroni, 1996). No FMRFamide immunoreactivity has been reported for craniate motoneurons, indicating that the visceral motoneurons and some somatic motoneurons of cephalochordates may resemble a more primitive condition characteristic of coelenterates and protostomes. In this regard, it will be important to assess the expression of FMRFamide in urochordates and hemichordates.

2.01.2.3.4.(ii) Catecholaminergic neurons The TH gene has been sequenced in several deuterostomes and shown to be a single orthologue with high sequence similarity between cephalochordates and vertebrates and lower sequence similarity between either of these and the urochordates. Immunocytochemistry and *in situ* hybridization in cephalochordates shows a rostral and dorsal distribution near the anterior end of the neural tube and the first two dorsal nerves. This has been interpreted as indicating similarities with the di-, mes-, and rhombencephalic catecholaminergic neuron groups known in craniates. In this context, it is important to note that a genetic basis of the development of catecholaminergic and serotonergic neurons in mammals has been studied extensively and upstream regulators have been identified (Qian *et al.*, 2001; Brunet and Pattyn, 2002; Pattyn *et al.*, 2003a). Serotonergic neurons have been found to form through a positionally and temporally regulated fate switch of visceral motoneuron progenitors (Pattyn *et al.*, 2003b). If such developmental linkage is conserved, one would expect serotonergic neurons to form only near motoneurons, which closely fits the currently known distribution of serotonergic neurons near the first motor center in cephalochordates (Lacalli, 1996; Moret *et al.*, 2005). Most interesting is the case of catecholaminergic neurons related to the solitary tract in vertebrates (Qian *et al.*, 2001). These neurons form a longitudinal column in the hindbrain and depend on several transcription factors that are longitudinally expressed (Brunet and Pattyn, 2002). Interestingly, in cephalochordates a longitudinal column of putative catecholaminergic neurons is located adjacent to the fibers of sensory cells on

the rostrum that enter through the first nerves (Fritzsch, 1996; Lacalli, 1996). This molecular and topographic relationship could indicate that at least some of these fibers are chemosensory and that they terminate in the equivalent of the solitary tract. Given the paucity of data, other interpretations are possible.

In urochordates, a small population of dopamine+ and TH+ cells is found in the ventral region of the rostral ganglion in both *Ciona* (Moret *et al.*, 2005) and *Oikopleura* (Søviknes and Glover, unpublished data). Moret *et al.* (2005) surmise on the basis of this location that the ventral part of the rostral ganglion is homologous to the vertebrate hypothalamus, which also contains catecholaminergic neurons. As far as we know, catecholaminergic neuron populations have not yet been described in hemichordates.

2.01.2.3.4.(iii) GABA GABA is the principal inhibitory transmitter in vertebrates and is also a major inhibitory transmitter in invertebrates. It therefore appears to be a common currency for inhibition throughout the animal kingdom. In invertebrates, GABA-immunopositive neurons typically occupy specific locations within ganglia either as distinct single neurons or clusters of neurons (reviewed in Søviknes *et al.*, 2005). In vertebrates, GABA-immunopositive neurons have widespread distributions and nearly all regions of the brain are replete with GABA-immunopositive terminals (Anadon *et al.*, 1998a; Melendez-Ferro *et al.*, 2000, 2002, 2003). However, the developmental origins of mammalian GABA-immunopositive neurons are much more discrete, with subsequent migration giving rise to their far-flung positions (Stuhmer *et al.*, 2002a, 2002b).

In the lamprey, the earliest GABA-immunopositive neurons appear in late embryos in the basal plate of the isthmus, in the caudal rhombencephalon, and in the rostral spinal cord (Melendez-Ferro *et al.*, 2002, 2003). Somewhat later, GABA appears in the prosencephalon, first in the diencephalon and later in the cortex. GABA neurons then appear elsewhere, but with distinct regional differences in distribution.

In the urochordate *Oikopleura*, GABA-immunoreactive neurons also originate at discrete sites within the CNS, both in the rostral ganglion and in the caudal ganglion (Søviknes *et al.*, 2005). GABA-immunoreactive neurons are not found, however, in the nerve cord, in contrast to the extensive population of GABA neurons in the spinal cord of vertebrates. Thus, GABA neurons appear to be regionally patterned in both urochordates and vertebrates, but there seems to be an increasingly broader distribution of GABA neurons in higher taxa, perhaps in conjunction with an increasing demand for local inhibitory inputs to provide finer regulation of sensory and motor information traffic.

In summary, comparative analysis of common neuronal cell types indicates a variety of patterns of topology and homology. Enough similarities exist to suggest that most of the apparent differences can probably be interpreted as variations on a theme that may already have been set up in the last common ancestor of all deuterostomes.

2.01.3 Making and Placing Neurons: The Evolution of Cell Fate and Regional Patterning

The expression of multiple genes in a nested anteroposterior pattern in the basiepithelial nerve plexus of hemichordates (Holland, 2003; Lowe *et al.*, 2003), combined with the presence of neuron-specific patterning genes and transcription factors in coelenterates (Seipel *et al.*, 2004) make it likely that ancestral deuterostomes had a skin brain that was regionally patterned by virtue of specific gene expression. Obviously, this basiepithelial nervous system had no dorsoventral patterning, since this would first arise in chordates in conjunction with the process of invagination of the neural plate into the neural tube. It has been noticed in both protostomes and deuterostomes that many genes governing the processes of invagination and of dorsoventral patterning are different (Arendt and Nubler-Jung, 1999). It seems therefore plausible that both processes were derived independently from an organism that already had evolved anteroposterior patterning. This would explain the utilization of two different sets of similar genes to regulate the two different processes (Meinhardt, 2004). It is fair to say that the relationship of most of the patterning genes to cell fate-determining genes and, ultimately, to neuronal organization is largely unknown. Thus, understanding how these genes relate within the context of the ancestral basiepithelial nerve net may provide insight into their functional relationships in the context of the brain of higher taxa. Examples such as otx, which helps specify the forebrain as an element of anteroposterior patterning in gnathostomes but is also expressed regionally in the brainless coelenterates (Ghysen, 2003), illustrate the possibility of an ancestral role that presumably was co-opted along with the neurogenic genes into the process of forming and patterning the brain *per se*.

In recent years, a number of cell fate determining genes have been identified in the nervous system and their functions experimentally determined (Lee, 1997; Anderson, 1999; Bermingham *et al.*, 1999; Brunet and Pattyn, 2002). Many of these patterning genes code for basic helix-loop-helix (bHLH) transcription factors. Much recent work has shown that the bHLH genes are ancient and are found not only in bilaterians, but also in coelenterates (Muller *et al.*, 2003; Seipel *et al.*, 2004). These findings begin to shed light on a major question in neurobiology: what is the origin of the neuron, the basic building block of any brain or nerve net? We propose here that the evolution of cellular diversification is closely associated with the evolutionary divergence of the bHLH genes and their restricted expression in the peripheral and central nervous system (Figure 9). The evolutionary expansion of this gene family as well as the currently known expression patterns of its members is consistent with a previously proposed hypothesis of cellular diversification as the basis for brain evolution (Mackie, 1990).

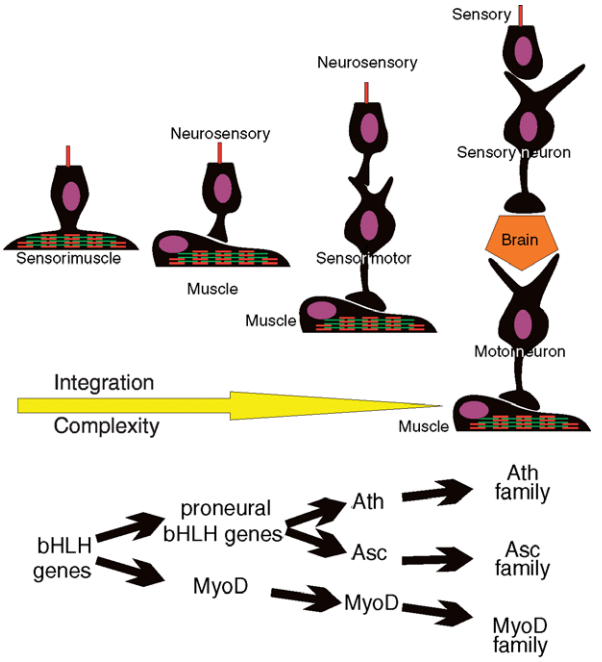

Figure 9 The proposed co-evolution of cellular complexity and expansion of the bHLH gene family that guides development of cellular diversity. It is hypothesized that the original neuronal cell type was a sensorimuscle cell that had both sensory capacity and contractile capacity. As the *bHLH* gene family expanded by generation of additional paralogous family members, the repertoire of neuronal cell types increased. Eventually, specialized sensorimotor cells that interconnected pure neurosensory cells and pure muscle cells evolved, thus providing the basis for integration of input from multiple sensory cells to govern the activity of multiple muscle fibers. These sensorimotor neurons ultimately diverged to give rise to the entire interneuronal compartment of the brain, an evolution that occurred in parallel with the increased specialization and diversification of sensory organs to govern more complex motor output. Modified from Mackie (1990) and Seipel *et al.* (2004).

Essential for the formation of any CNS is the specification of a neural cell fate in the developing ectoderm: cells have to be diverted from an epidermal fate to a neural fate, which is to say into neurons instead of skin cells. This fate switch is likely to be triggered by activating the bHLH genes that specify neuronal fate. We therefore turn our attention now to the question of how neuronal induction might have evolved.

Over the last 10 years, the predominant view has been that Spemann's organizer generates signals that can change cell fate from epidermal to neuronal through the generation of bone morphogenic proteins (BMP) antagonists (De Robertis *et al.*, 2000; Munoz-Sanjuan *et al.*, 2002). Based on several transplantation and *in vitro* experiments, it has been proposed that neuronal induction is a direct consequence of BMP inhibition in the ectoderm and that the neural fate can be considered to be a ground state that is revealed in the absence of the instructive (negative) BMP signals and the ectopic expression of proneural bHLH genes (Ma *et al.*, 1996; Lee, 1997). One possibility is that in the ancestral deuterostome, BMP expression was suppressed, or at least downregulated, in a scattered pattern, thus triggering neurogenesis at the scattered locations characteristic of the basiepithelial nerve net. bHLH genes evolved already in coelenterates, where they are involved in specifying neuronal precursors in the ectoderm and endoderm (Seipel *et al.*, 2004).

In the chicken, Spemann's organizer, but not previously defined BMP inhibitors, leads to neurogenesis, and it has been claimed that it is FGF signaling that is elicited by the organizer and that antagonizes BMP signaling (Munoz-Sanjuan *et al.*, 2002). However, recent data from ascidian embryos suggest that FGF signaling alone, without acting through BMP inhibition, is a direct inducer of early neurogenic genes (Bertrand *et al.*, 2003). More recent work seems to generate a unified position and suggests that FGF is not only inhibiting BMP signaling, but also has an independent and direct neural inductive capacity possibly conserved among chordates (Delaune *et al.*, 2004; Kuroda *et al.*, 2005). Specifically, it now appears that FGFs can directly activate neuralization through the MAPK pathway rather than by overriding the BMP-mediated inhibition (Kuroda *et al.*, 2005). Unfortunately, the function of FGFs in the context of neural induction in other deuterostomes has not been fully analyzed

(Davidson *et al.*, 2002). It is important to note that a cooperative FGF/BMP signaling system also exists in insects, but not in connection with CNS development. The FGF pathway is used for branching morphogenesis of trachea (Sutherland *et al.*, 1996) and null mutants of the single known *Drosophila* FGF ligand (branchless) do not show overt brain development deficits (Hirth *et al.*, 2003). This difference is significant, as in the past it appeared that both insect and vertebrate neural induction relied on BMP/dpp suppression (Urbach and Technau, 2004). Now it appears that either chordates have evolved the new feature of FGF involvement, or, conversely, that FGF involvement in neural induction was lost in insects. Clearly, the emerging issues regarding phylogenetic differences in the signaling that underlies neural induction support the model that neurulation in protostomes and deuterostomes may have evolved independently (Lowe *et al.*, 2003; Meinhardt, 2004). These divergent views need to be reconciled by more detailed analysis of nonchordate deuterostomes. Such data could help to resolve the basic question of conservation of neural induction across phyla, which as of this writing is controversial.

Upon invagination, the vertebrate neural tube becomes patterned in the transverse plane according to an intricate process involving multiple transcription factors (Figure 10). In a simplified version, the dorsoventral expression domains of these transcription factors are established by a bipolar gradient of diffusible signaling molecules that act in concert (Sander *et al.*, 2000; Vallstedt *et al.*, 2001; Maklad and Fritzsch, 2003; Pattyn *et al.*, 2003b), a fact that can be unmasked if one pole of the gradient is experimentally abolished (Litingtung and Chiang, 2000). Several of these patterning genes have been identified across deuterostome phyla (Wada and Satoh, 2001). However, it is also clear that factors such as the Wnts are expressed differently in cephalochordates and craniates (Holland *et al.*, 2000; Schubert *et al.*, 2001), suggesting that certain aspects of dorsoventral patterning may not be fully conserved across deuterostomes. Clearly, more work on other relevant dorsoventral patterning genes is needed in more deuterostomes before any firm conclusion can be drawn.

One specific brain region that has attracted intensive studies and has generated a large set of comparative gene expression data is the MHB (Figure 11). The formation of the MHB has been shown to be critically dependent on several transcription factors that interact with each other to stabilize the boundary (Wang and Zoghbi, 2001). If any of these genes is mutated, the boundary does not form and nearby neuron populations, including certain extraocular motoneurons, fail to differentiate (Fritzsch *et al.*, 1995). Certain genes expressed in this region, such as Pax2/5/8, can be used as markers to delineate the MHB (Wada *et al.*, 1998). It is interesting that no midbrain seems to exist in noncraniate deuterostomes in the sense of the specific overlapping gene expression patterns that are found in gnathostomes. Likewise, it is unclear whether a true isthmus region with the organizer capacity of gnathostomes exists in any nonvertebrate deuterostome (Figure 11). Given that the MHB is so important for cerebellar, oculomotor motoneuron, and trochlear motoneuron development, it is conceivable that the absence of a cerebellum as well as of all extraocular motoneurons in hagfish may reflect a primitive absence of a true MHB. Likewise, the absence of a mesencephalic root of the trigeminus nerve in cyclostomes may be related to a different pattern of gene expression in this region.

Another long-standing comparative issue is the number and significance of neuromeres and how they relate to gene expression domains and neuronal differentiation. Based on gnathostome data, it appeared that most cranial motoneuron nuclei showed a simple relationship to specific rhombomeres in a pattern that was reasonably well conserved across gnathostome phyla (Fritzsch,

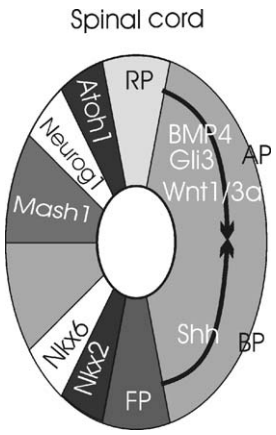

Figure 10 Genes involved in the dorsoventral patterning of the neural tube. The floor plate produces sonic hedgehog (*Shh*), which diffuses through the basal plate (BP) to interact with GLI-Kruppel family member 3 (*Gli3*). Opposing diffusion gradients are set up by bone morphogenic factor 4 (*BMP*4) and wingless-type MMTV integration site family member 1/3a (*Wnt1/3a*). The interaction of these gradients sets up domains of transcription factor expression that govern more directly the cell fate of neurons developing in their expression area. For example, the expression of NK6 transcription factor related, locus 1 (*Nkx6.1*) is essential for the formation of somatic motoneurons. Modified from Sander *et al.* (2000), Vallstedt *et al.* (2001), Maklad and Fritzsch (2003), and Pattyn *et al.* (2003b).

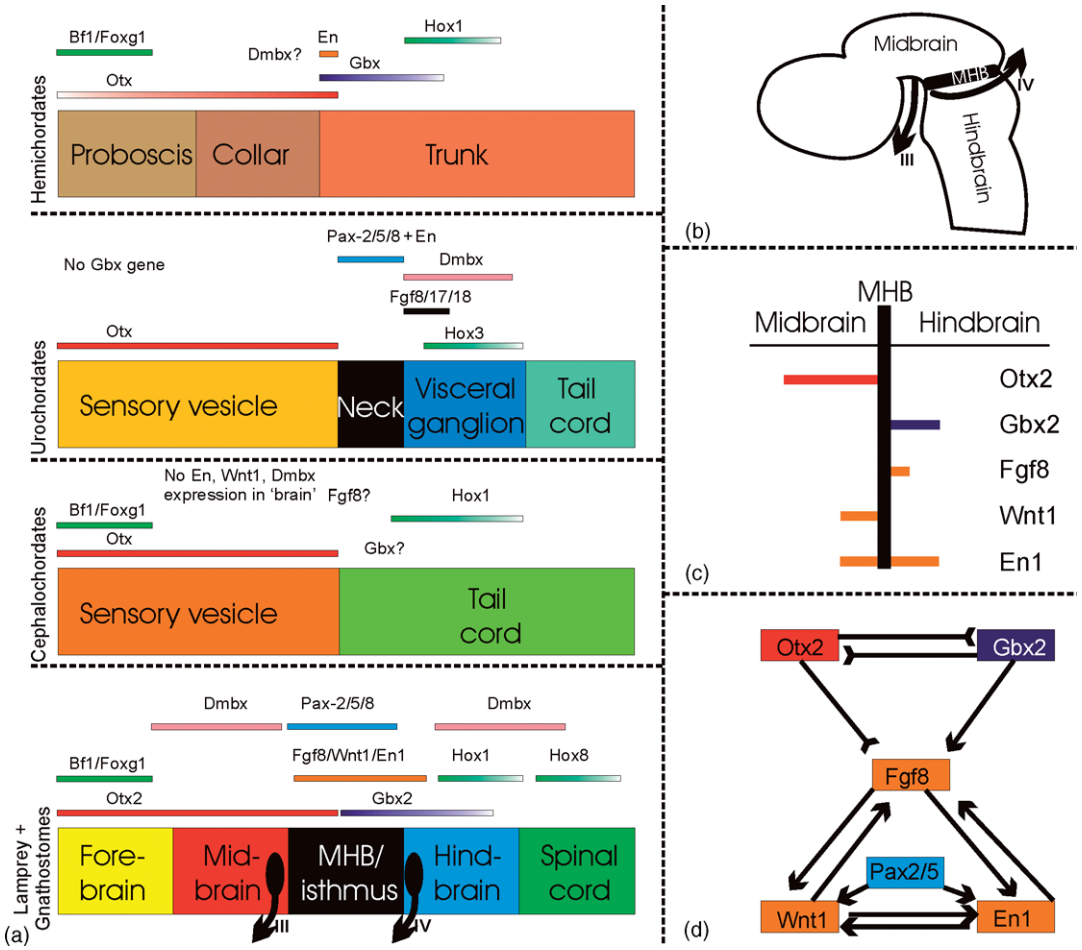

Figure 11 The evolution of gene expression at the MHB is shown for deuterostomes. The MHB forms in gnathostomes where *Otx2* and *Gbx2* expression is abutting. This stabilizes the expression of *Fgf8*, which in turn stabilizes the expression of *Wnt1* and *En1*. Mutation of *Otx2*, *Gbx2*, *Fgf8*, or *Wnt1* eliminates the MHB. *Pax2/5/8* are also expressed at the MHB, whereas the expression of *Dmbx* occurs immediately rostral to the MHB and later in the hindbrain and spinal cord. Cephalochordates have *Dmbx*, *Wnt1*, and *Engrailed* genes, but these are not expressed in any close relation to the *Otx* expression domain. The expression of *Fgf*s and *Gbx* has not yet been characterized in cephalochordates. Urochordates lack a *Gbx* gene and have nonoverlapping *Pax* and *Fgf* expression domains meeting between the visceral ganglion and the neck, with *Dmbx* expression caudal to *Pax* expression as opposed to rostral to *Pax* expression as is seen in gnathostomes. Hemichordates have overlapping expression of *Gbx*, *Otx*, and *En* in the rostral trunk. Outgroup data suggest that coelenterates have a *Dbmx* orthologue, thus raising the possibility that hemichordates also have a *Dmbx* gene, but neither this gene nor the *Fgf* and *Pax* genes have been characterized in terms of expression pattern in hemichordates. Together these data show that certain gene expression domains are topographically conserved (*Hox*, *Otx*), whereas others show varying degrees of overlap. It is conceivable that the evolution of nested expression domains of transcription factors is causally related to the evolution of specific neuronal features such as the evolution of oculomotor and trochlear motoneurons around the MHB. Modified from Fritzsch (1996), Wang and Zoghbi (2001), Lowe *et al.* (2003), and Takahashi and Holland (2004).

1998; Glover, 2001; Murakami *et al.*, 2004; Kiecker and Lumsden, 2005). However, a number of exceptions have been noted among cyclostomes in the organization of hindbrain motor nuclei compared to mammals (Figure 12). These data suggest that while the formation of rhombomeres might be a constant feature of craniate hindbrains (with the possible exception of hagfish) the content of rhombomeres is not stably conserved. The distribution of motoneurons in the adult hindbrain is complicated by longitudinal and radial migrations that make comparison among craniates somewhat tentative. For example, facial motoneurons migrate in hagfish and mammals, whereas they remain near their ventricular origin in lampreys. Rhombomeric differences in the distribution of craniate motoneurons have recently been analyzed during development in cyclostomes and correlated with gene expression patterns (Murakami *et al.*, 2004). Beyond the branchiomotor neurons analyzed in this study, the abducens nucleus has also been found not to have boundaries coinciding with rhombomere

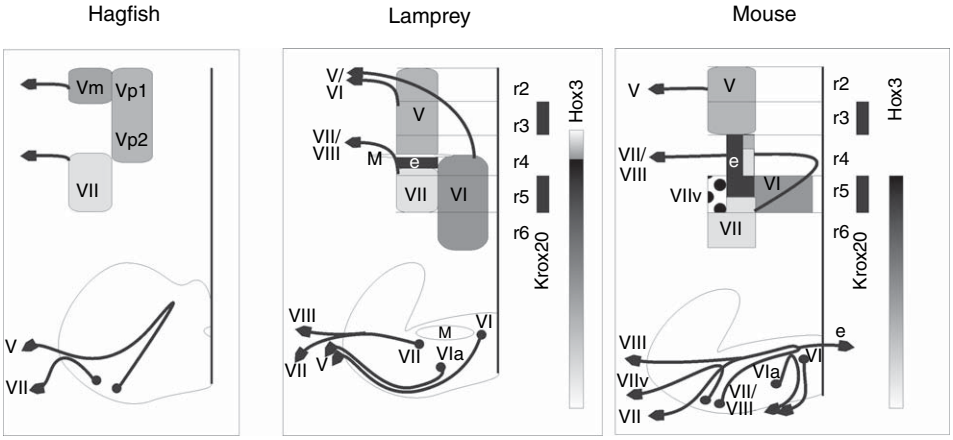

Figure 12 The distribution of motoneurons relative to rhombomeres is shown for three adult craniates in combination with gene expression in a dorsal view (top) and a coronal section (bottom) of the left half of a hindbrain. Hagfish and lampreys have a much larger contingent of trigeminal (V) than facial motoneurons (VII). Hagfish are unusual in the trajectories taken by their motoneuron axons, a feature that is related to the overall unusual organization of the hindbrain. In lampreys, the abducens (VI) and facial motoneurons (VII) originate from rhombomeres 2.5 and 1.5, respectively, and trigeminal and facial motoneurons approximate each other in the middle of rhombomere 4 near the Mauthner cell (M) and the inner ear efferents (e). Lampreys have two adjacent populations of motoneurons that each innervates distinct ocular muscles. Fibers of the abducens exit through the trigeminal nerve (V). In mice, the facial branchial (VII) and visceral (VIIv) motoneurons originate in rhombomeres 4 and 6, respectively. The facial branchiomotor neurons migrate during development from rhombomere 4 to 6, trailing their axons behind them. Each of the three motoneuron types generated in rhombomere 4/5 has a distinct exit either bilaterally through the octaval nerve (VIII) for efferents (e) or through the intermediate nerve for facial visceral motoneurons (VIIv) or through the facial nerve for facial branchiomotoer neurons (VII). Note that abducens fibers exit in mammals as a ventral root (VI). Gene expression studies show that rhombomeres 3 and 5 are characterized across phyla by the expression of *Krox20*, whereas *Hox* expression changes from a midrhombomere 4 expression in lampreys to a rhombomere 4/5 boundary expression in mice. No expression data exist on hagfish. Modified from Fritzsch (1998), Glover (2001), and Murakami *et al.* (2004).

boundaries (Fritzsch, 1998). This detailed analysis has confirmed earlier assessments of motoneuron distribution and shows a correlation of motoneuron populations not with rhombomeric boundaries *per se* but with Hox gene expression domains, as had previously been hypothesized for craniates in general (Glover, 2001). Indeed, the details of distribution of motoneurons generated in a given rhombomere are not stable across craniate evolution, as illustrated by the formation of a visceral motor component in rhombomere 5 of the mammalian hindbrain, a motoneuron population that is entirely missing in cyclostomes. Experimental manipulation of gene expression is now needed in lampreys to show that the boundaries of motoneuron domains are specified by *Hox* gene expression.

Overall, these data show that rhombomeres are not fully invariant with respect to motoneuron composition, and similar conclusions have been reached with respect to reticulospinal and vestibulospinal neurons (Auclair *et al.*, 1999; Diaz and Glover, 2002; Diaz *et al.*, 2003; Maklad and Fritzsch, 2003). This instability in detail relates to the unresolved problem of the origin of neuromeres. While past research assumed that this might have happened early in deuterostome evolution and may have been related to segmentation from a hypothetical common urbilaterian ancestor (Arendt and Nubler-Jung, 1999), more recent data based on the study of the *Krox20* gene suggests that neuromeres evolved first among craniates. This conclusion is warranted, as in null mutants of that gene there is selective disappearance of rhombomere 3 and 5 (Voiculescu *et al.*, 2001). Genetic analysis has shown that in the absence of *Krox20* the remaining cells of rhombomeres 3 and 5 acquire rhombomere 2 or 4 identities, demonstrating that *Krox20* is essential for odd- and even-numbered territories in the hindbrain. *Krox20* is not expressed in the brain of cephalochordates, thus indicating that rhombomere formation probably evolved after *Krox20* was co-opted into hindbrain patterning (Knight *et al.*, 2000).

In summary, these data indicate a promising beginning but also show, despite their current paucity, that gene expression domains do not constitute a magic bullet that reveals the basic homology of neuromeres across phyla. Evolutionary alterations of gene expression and/or neuronal population differentiation patterns within any given neuromere will make the road ahead as difficult as the road was until here. Still, experimental manipulation of the emerging framework of nested gene expression through gain and

loss of function studies, combined with more sophisticated tracer studies, should reveal the origins of region-specific neuronal phenotypes and their relationships to specific gene expression domains.

2.01.4 Multiplying and Diversifying Sensory Systems for More Detailed Sensory Analysis and More Appropriate Motor Responses: The Conundrum of the Co-Evolution of Ever More Sophisticated Input, Processing, and Output Processes as a Basis for a Runaway Selection

The chicken and egg question to be addressed next is the co-evolution of sophisticated sensory systems and the brains necessary to process the information they provide. As with the evolution of brain regionalization outlined above, it is likely that the evolution of genes predated the evolution of sensory systems, which, in turn, predated the evolution of sophisticated brains. We base this assertion on the simple fact that even animals with a basiepithelial nerve plexus, like some jellyfish, may have both eyes and ears (Kozmik *et al.*, 2003). Conversely, to date, no animal is known that has a sophisticated brain but lacks sensory inputs entirely. Indeed, in animals in which a specific sensory system becomes the leading sensory input, the brain areas dedicated to this input tend to increase in absolute size and complexity (Nieuwenhuys *et al.*, 1998). Clearly, among deuterostomes the degree of development of sensory systems and brain go rather well with each other. For example, no specialized sensory system is known for hemichordates or echinoderms (Bullock and Horridge, 1965). Urochordates have some specialized systems associated with water intake (Mackie and Singla, 2004) and have two simple receptors in the rostral ganglion (Meinertzhagen *et al.*, 2004). The most sophisticated sensory system of cephalochordates encompasses the organs of de Quatrefages and their function is still unknown (Lacalli, 2004). This raises the problem of ancestral craniate senses that are causally linked to the progressive evolution of the brain. Judging from the central representation size and abundance of receptors, it appears that chemical sense and tactile sense are the dominant sensory inputs to the hagfish brain (Braun, 1996, 1998), but it remains unclear whether this is a primitive or a derived condition. Overall, craniate evolution is clearly correlated with greater sophistication of eyes and ears. We therefore provide below a brief comparison of these two organs in craniates and gnathostomes.

2.01.4.1 Eyes

Vertebrate eyes have long been regarded as uniquely derived features that are homoplastic to arthropod eyes. This is related to the unusual development as an evagination of the forebrain not found in any other phylum. However, recent years have highlighted a number of transcription factors and, more recently, opsin proteins that are related across phyla (Kozmik *et al.*, 2003; Arendt *et al.*, 2004). Indeed, a molecular link between the photoreceptors and opsins of invertebrates and vertebrates was recently proposed (Arendt *et al.*, 2004) and may be extendable to deeper evolutionary connections between eyes and mechanoreceptors (O'Brien and Degnan, 2003; Fritzsch and Beisel, 2004; Niwa *et al.*, 2004; Piatigorsky and Kozmik, 2004). Minimally, this raises the possibility that at least the molecular building blocks of eyes are homologous across phyla and arose already in coelenterates, thus indicating a deep homology of all eyes. While it cannot be excluded that hemichordates and echinoderm ancestors had more sophisticated eyes, it seems more plausible to assume that lack of eyes in these two taxa is primitive. If so, eye evolution would begin in deuterostomes with the chordates. It has been proposed that the frontal organ of cephalochordates is homologous to the lateral eyes of craniates and this suggestion is backed by some connectional data as well as by the expression of Pax6 (Lacalli, 2004). Furthermore, some data suggest that all retinal neurons may be directly related to the sensory receptors (Arendt, 2003) and past differences perceived in opsins among metazoans have recently been reconciled (Arendt *et al.*, 2004).

This may be so, but we want to explore here the basic organization of cyclostome retinas that appears to be primitively different from gnathostome retinas in several respects (Figure 13). First, like the submeningeal layer of the brain, the vitreal part of the lamprey and hagfish retina is devoid of nerve fibers, which run instead at the level of the inner nuclear layer. Second, most of the ganglion cells are located within the inner nuclear layer and do not form a distinct ganglion cell layer. Last, but not least, cyclostome eyes receive a proportionally large GABAergic retinofugal input from apparently homologous efferent nuclear centers in the midbrain (Fritzsch and Collin, 1990; Fritzsch, 1991; Nieuwenhuys *et al.*, 1998) and this input develops before receptor cells mature (Fritzsch, 1991; Anadon *et al.*, 1998b). These data suggest that cyclostome retinas are only partially transformed from their original neuroectoderm-like cell and fiber layering. The organization of the gnathostome

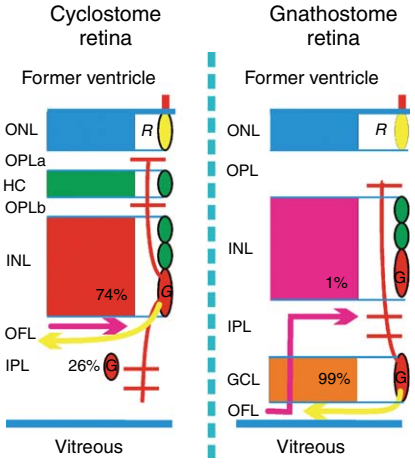

Figure 13 Differences in the fiber and cell layers of the cyclostome and gnathostome retinas. Note that the receptor processes of the outer nuclear layer (ONL) protrude into the former ventricle. In lampreys, horizontal cells are separated from the ONL and inner nuclear layer (INL) by two sublayers of the outer plexiform layer. Lampreys have about 74% of their ganglion cells (G) located in the INL, whereas only 1% of ganglion cells of jawed vertebrates are found in this layer, with 99% being in the ganglion cell layer (GCL). Afferent (yellow) and efferent (lilac) fibers project in lamprey along the INL. In jawed vertebrates, such fibers run in the outer fiber layer (OFL) at the vitreous. It is conceivable that the formation of myelin necessitated a different distribution of myelinated fibers near the vitreous and farther away from the receptors in jawed vertebrates. Reorganization of ganglion cells into the IPL to eventually form the ganglion cell layer may have been a consequence of this fiber reorganization. HC, horizontal cell; IPL, inner plexiform layer; OPLa, outer plexiform layer a; OPLb, outer plexiform layer b. Modified from Fritzsch (1991) and Anadon *et al.* (1998b).

retina seems to be a transformation of this ancestral pattern (Figure 13). It is important here to realize that the eye of cyclostomes is moved by a different organization of eye muscles and that the pattern of innervation of these eye muscles is also different from gnathostomes (Fritzsch, 1998). In addition, lampreys lack a ciliary muscle and accommodation must happen by other means, potentially related to the triangular shape of the lens. Overall, these data on the eye support the monophyly of cyclostomes and show a radical reorganization of retina organization and eye muscles in gnathostome vertebrates.

2.01.4.2 Ears

Like the eye, the ear has been proposed as an example of deep homology with several transcription factors shared between deuterostomes and coelenterates (Kozmik *et al.*, 2003; Fritzsch and Beisel, 2004; Seipel *et al.*, 2004; Fritzsch and Piatigorsky, 2005). Comparable to the retina, the ear of cyclostomes also shows an apparently primitive organization different from gnathostomes: cyclostome ears have only two canal cristae, lacking the horizontal canal and crista, and they have only a single otoconia-bearing epithelium (Fritzsch and Beisel, 2004). It has been suggested that a gene otherwise related to the forebrain is causally linked to the evolution of the horizontal canal (Cantos *et al.*, 2000; Fritzsch *et al.*, 2001). *Otx1* appears in the gnathostome lineage through duplication and has acquired a novel expression and function in the ear that is not shared by *Otx2* (Morsli *et al.*, 1999). Moreover, several other genes discussed above in the MHB context (see Section 2.01.3) all appear in the ear, again emphasizing that evolutionary change in expression, multiplication of an ancestral gene, and acquisition of a novel function need to be considered when gene expression is to be related to evolutionary alterations. In this context, the evolutionary relation with structures in other deuterostomes that show expression of *Pax2* also have a phenotype in *Pax2*-null mutants (Burton *et al.*, 2004). *Pax 2* has been discussed as a major factor relevant for ear evolution (Wada *et al.*, 1998; Mazet *et al.*, 2003) and has been used to suggest homologies across phyla. However, this idea should be regarded at the moment as tentative and requires support through the expression of other genes (Fritzsch and Beisel, 2004; Holland, 2005). It is fair to say that the ear can be viewed as a miniature problem of craniate head evolution and unraveling the molecule basis of its evolution might pave the way for furthering our understanding of head evolution in general.

Overall, the sensory systems reviewed here show a remarkable progression between cyclostomes and gnathostomes and indicate that these sensory systems present unique components of cyclostomes that set them apart in a likely primitive way from gnathostomes. More detailed understandings of the molecular alterations underlying jaw formation (Shigetani *et al.*, 2002) need to be revealed and related to the sensory and motor reorganizations of the cyclostomes. At the moment, it is safe to say that we do not understand the molecular basis of these changes in enough detail. More experimental work is needed to support the current notion of nested gene expression patterns and their potential evolutionary significance.

2.01.5 Summary and Conclusion

Attempts at linking the evolution of organisms and organs to the evolution of transcription factors that direct developmental processes are greatly complicated by the multitude of poorly understood transcriptional regulatory networks. Emerging issues are the apparent pleiotropic effects of many

transcription factors, the modularity of development, and the evolution of *cis*-acting regulation of transcription factors (Carroll *et al.*, 2005). Conflicts between morphology and genetics, such as the many examples of morphological divergence arising from apparently identical transcription factor expression across taxa, are bound to leave conclusions on molecular and developmental homologies controversial for the near future. For example, recent progress in the long-standing issue of the molecular basis of organ formation has shown that certain *Pax* genes are relevant for the formation of eyes (*Pax6*) and ears (*Pax2*) across many phyla and have evidently arisen from an ancestral *Pax2/6* gene, *PaxB*, that is found in cnidarians (Sun *et al.*, 1997). Moreover, *PaxB* is expressed in the eye and statocyst of cubomedusan jellyfish, cnidarians that already contain these sophisticated organs for vision and mechanoreception (Piatigorsky and Kozmik, 2004). This suggests that evolution may have used paralogues of a single ancestral *Pax* gene to organize the development of both of these peripheral organs, no matter what shape, cell types, and transducer molecules are involved (Kozmik *et al.*, 2003; Piatigorsky and Kozmik, 2004). Since *PaxB* is also expressed in sponges (Hoshiyama *et al.*, 1998), its involvement in sensory development may even have predated the formation of a central nervous system (Lowe *et al.*, 2003; O'Brien and Degnan, 2003; Piatigorsky and Kozmik, 2004; Fritzsch and Piatigorsky, 2005). Such ideas imply that *Pax2/6* expression in the brain and the use of *Pax2/5/8* in the MHB is a secondary co-option of these genes from their original involvement in sensory organ development. As an extension of the principles governing the determination of organs, the fates of individual cells within organs have to be similarly directed. Much as with *Pax* genes, a number of common cell fate-determining transcription factors are co-utilized in the eyes and ears of different species. Examples include the *bHLH* gene *atonal/Atoh1/Atoh5* (Ben-Arie *et al.*, 2000; Niwa *et al.*, 2004), and the Pou domain factor *Pou4f3* (Liu *et al.*, 2001; Wang *et al.*, 2002). It is noteworthy that the selective co-expression pattern of these genes, which appear to be situated downstream of the *Pax2* gene regulating development of the optic nerve and ear (Torres *et al.*, 1996), suggests their specialization for the specification of cells within these two sensory organs. Another example is the *Eya1/Six1* signaling system, which is essential for development of the vertebrate and insect eye and the vertebrate ear (Zou *et al.*, 2004) as well as being used in other aspects of development (Piatigorsky and Kozmik, 2004). Extending this line of inquiry into the CNS, several examples exist of transcription factors controlling the same neuronal phenotype across taxa, such as the *Islet/LIM* genes, which are involved in specifying the motoneuron phenotype in urochordates as well as vertebrates (Price and Briscoe, 2004; Katsuyama *et al.*, 2005), and the *Unc30/Pitx2* gene, which has been shown to control the determination of the GABAergic phenotype in vertebrates as well as the nematode *C. elegans* (Westmoreland *et al.*, 2001). Clearly, a number of the transcription factors and developmental cascades that determine cell fates are shared across phyla and across sensory organs and the CNS. Assuming that these similarities are more than coincidental and in fact indicative of a co-evolutionary history of eyes, ears, and brains, the present challenge is to trace the common origins of these structures to conserved developmental programs. In keeping with the recently emerging concept of a second code for gene regulation (Pennisi, 2004), namely the *cis*-acting regulatory elements, it is possible that enhancers orchestrating the development of specific structures in different species share motifs and properties that have led to the modular use of common transcription factors during the evolution of those structures. One of the challenges ahead is therefore the careful mapping of regulatory elements associated with the transcription factor genes that play pivotal roles in such modules. Obtaining an overarching conceptual framework of the molecular characterization of brain development is likely to provide more than unifying insight into the evolution of the brain and senses. Evolutionary molecular neurobiology is likely also to provide novel insights into human diseases common to such apparently dissimilar organs as the eye and ear, such as Usher syndrome (Fritzsch and Beisel, 2004), myosin IIIa-associated nonsyndromic hearing loss, and retinal degeneration (Walsh *et al.*, 2002), choroideremia (Starr *et al.*, 2004), and Norrie disease and exudative vitreoretinopathy (Xu *et al.*, 2004). Understanding the regulation of common transcription factor modules, and linking this to their expression in topographically distinct contexts, may ultimately lead to a better understanding of the causes and differential penetrance of such inherited diseases.

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2.02 Origins of the Chordate Central Nervous System: Insights from Hemichordates

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Glossary

amphioxus	Belongs to the cephalochordates, and is the most basally branching node of the chordates.
direct developer	Animals that form an adult body plan from the embryo without an intervening larval stages.
hox *genes*	Homeodomain transcription factors arranged in clusters in the genome that have conserved roles in patterning a range of axial elements in animals.
indirect developer	Animals that employ a discrete feeding larval stage before metamorphosis into an adult.
larvacean	A nonvertebrate chordate closely related to ascidians.
life history	The variety of developmental and behavioral strategies utilized by animals and plants to maximize reproductive success.

2.02.1 Introduction

The origin of the chordate body plan and its unique nervous system has been debated for over a century. The early part of the twentieth century was a particularly active period of speculation, when many of the most influential hypotheses were proposed, but in the latter part of the century the issue stagnated through a lack of new data. The early evolution of deuterostomes has proven to be a particularly problematic node to reconstruct, and remains largely unresolved (Gee, 1996; Lowe *et al.*, 2003; see Evolution of the Deuterostome Central Nervous System: An Intercalation of Developmental Patterning Processes with Cellular Specification Processes, Gene Expression in the Honeybee Mushroom Body and Its Gene Orthologues). There are a variety of factors that contribute to difficulties in addressing this issue, but probably the two most challenging obstacles have been, and continue to be, the uncertainty of deuterostome relationships and the large morphological disparity between extant deuterostome phyla. This has been compounded by a poor fossil record and, by the end of the 1970s, the issue of chordate origins had essentially reached an impasse. The last 20 years have seen a surge in new data with the advent of molecular systematics and molecular developmental genetics. These new data are radically beginning to reshape both our understanding of comparative body plan specification and development, but also, just as importantly, the phylogenetic relationships between the deuterstome phyla (Field *et al.*, 1988; Lake, 1990; Adoutte *et al.*, 2000). Deuterostome phylogeny is currently a very active area of research and will likely be largely resolved at the level of the relationships between the major phyla, as more basal deuterostome genomic data sets become available for more comprehensive molecular phylogenetic analysis. The application of comparative molecular genetics to characterize the development of poorly described groups at critical phylogenetic positions, within the deuterostomes, is beginning to enhance our understanding of the early steps in deuterostome evolutionary history (Holland, 2002; Lowe *et al.*, 2003). We are currently amidst a new wave of discovery in deuterostome evolution as many of the phylogenetically key genomes are now either sequenced or are currently being sequenced. A recent, radical revision to the phylogenetic relationships based on a large genomic data set suggests that there are still plenty of surprises ahead (Delsuc *et al.*, 2006).

In this article, I focus on the insights gained into the origins and evolution of the vertebrate nervous system by the characterization of early development of the nervous system of a bilateral phylum closely related to vertebrates, the hemichordates. The nervous system has been characterized as barely more complicated than that of cnidarians (Bullock and Horridge, 1965) yet the expression of a large suite of transcription factors with critical and conserved roles in anteroposterior patterning and brain regionalization in vertebrates shows unprecedented similarities in relative expression topology in the ectoderm of the developing embryos of hemichordates (Lowe *et al.*, 2003). I discuss the implications of this data for the understanding of early deuterostome evolution and the evolution of vertebrate brains.

2.02.2 Deuterostome Phylogeny and Hemichordate Biology

The phylum Hemichordata is a bilateral phylum of animals, the sister group of echinoderms, and closely related to the chordates. Along with the newly reclassified marine worm, *Xenoturbella bockii*, these four groups constitute the deuterostomes (Figure 1).

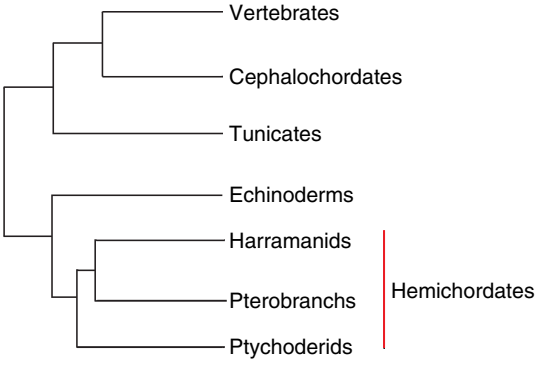

Figure 1 Deuterostome relationships based on 18S RNA. After Cameron, C. B., Garey, J. R., Swalla, B. J. 2000. Evolution of the chordate body plan: New insights from phylogenetic analyses of deuterostome phyla. *Proc. Natl. Acad. Sci. USA* 97, 4469–4474.

Deuterostome phylogeny has been revised many times over the past few years, which has played a major role in refining hypotheses on early deuterostome evolution and the origin of chordates. The first major reorganization occurred with the advent of molecular systematics and resulted in the reclassification of five phyla into the protostomes, leaving only three deuterostome phyla (Field *et al.*, 1988; Lake, 1990) in a topology that has generally been well supported by subsequent studies (Adoutte *et al.*, 2000; Cameron *et al.*, 2000; Bromham and Degnan, 1999). Recently, an obscure, rare, and morphologically unremarkable marine worm, *Xenoturbella,* was reclassified into the deuterostomes with an as yet uncertain phylogenetic position within the lineage. The phylogenetic relationships are continuing to be refined, with some potential for yet more dramatic reorganization as new genomic data sets are generated and phylogenetic methods continue to improve. A new study utilizing new, large genomic data sets from amphioxus, tunicates, and sea urchin challenges the topology established from 18S RNA studies (Delsuc *et al.*, 2006). These results support the sister taxon status of tunicates and vertebrates and are statistically robust. Even more surprising is the grouping of amphioxus with echinoderms (Figure 2b). This node has weaker support, and the analysis lacks sequence data from hemichordates and *Xenoturbella*. If this topology holds up to future analysis, it has sweeping consequences for our understanding of chordate origins and nervous system evolution as it implies the ancestral deuterostome already possessed the fundamental features of the chordate body plan such as somites, notochord, gill slits, and the dorsal centralized nervous system.

Of all the nonchordate deuterostome phyla, the hemichordates are the most promising for addressing issues of chordate origins and the early evolution of the deuterostomes (Lowe *et al.*, 2003).

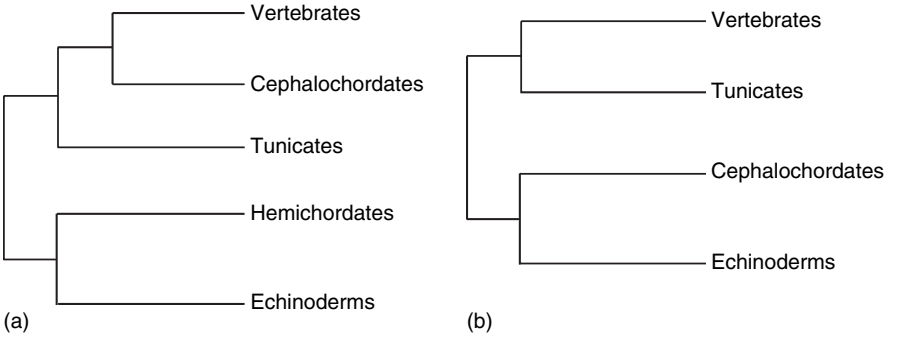

Figure 2 Deuterostome relationships from different molecular data sets. a, 18S RNA (Cameron *et al.*, 2000). b, 146 genes gathered from the genome of a range of deuterostome organisms (Delsuc *et al.*, 2006).

While basal chordates such as amphioxus and ascidians have been key for understanding the diversification of the basic chordate body plan, understanding the origins of the chordate body plan can only be addressed by using outgroups. There are limited options within the deuterostomes for informative outgroups, and the issue of divergent morphologies plays a large role in guiding appropriate choices. The most familiar deuterostome outside chordates is the echinoderms. While a fascinating group, their adult body plan is highly derived (Lowe and Wray, 1997) and difficult to compare directly with the chordate body plan. *Xenoturbella* is rare and poorly characterized so far, which leaves the hemichordates as the only other bilaterian phylum closely related to chordates. Zoological descriptions of hemichordates by Morgan (1894) and Bateson (1885, 1886) recognized their promise for addressing issues of chordate origins and the origin of the chordate central nervous system. As a result of proposed morphological affinities with the chordates, they were originally classified as a subphylum of the chordates and it was not until the 1940s that they were reclassified into their own phylum (Hyman, 1940).

The phylum is divided into two major classes: the pterobranchs and the enteropneusts. The pterobranchs are poorly characterized, in part due to their sparse distribution (having only been described in a few specific locations) and their small size. Their life history is largely colonial, with a few exceptions, and they are all direct developers, each zooid producing only small numbers of oocytes. The enteropneusts are all solitary marine worms, with a broad biogeographic range and habitats from the intertidal down to deep waters and hydrothermal vents. They range in size from a few centimeters up to 2 m and form U-shaped burrows, feeding by ingesting particles and filtering phytoplankton from the seawater. The phylogenetic relationships of the various groups within the hemichordates are currently uncertain, but are beginning to be resolved by molecular analyses (Cameron *et al.*, 2000; Winchell *et al.*, 2002). Currently, phylogenies based on 18S and 28S RNA phylogenies conflict in their placement of the pterobranchs (Winchell *et al.*, 2002). The topology shown in Figure 1 is based on 18S data.

Of the two lineages of enteropneust worms, there is a clear division in life history strategy: in the Harramanid lineage, all species are characterized by direct development, whereas in the other lineage that includes the Ptychoderids, all species have complex life cycles with a prolonged larval period (Cameron *et al.*, 2000; Lowe *et al.*, 2004).

The continued interest in this group is largely a result of proposed morphological affinities of these worms to the chordates. Figure 3b shows the characteristics of the external and internal hemichordate body plan, and Figure 3a shows a photomicrograph of an early juvenile of the Harramanid *Saccoglossus kowalevskii*. The animal has a classic deuterostome tripartite body plan with an anterior proboscis used for burrowing and feeding, a collar or mesosome, and trunk or metasome.

The mouth opens on the ventral side between the collar and proboscis. The metasome or trunk is very long in the adult. Gill slits are another key feature and are located in the anterodorsal region of this body region and occur paired, in large numbers in adults. From a morphological perspective, the gill slits resemble those of amphioxus quite closely and both morphological analysis and molecular analyses suggest that they may represent true homologues (Ogasawara *et al.*, 1999; Okai *et al.*, 2000; Lowe *et al.*, 2003; Rychel *et al.*, 2006). An anterior projection from the gut called the stomochord supports the heart–kidney complex and has been classically compared to the notochord. However, more recently, both morphological and molecular studies have failed to find support for this homology (Ogasawara *et al.*, 1999; Peterson *et al.*, 1999; Okai *et al.*, 2000; Lowe *et al.*, 2003; Ruppert, 2005).

2.02.3 Morphological Characteristics of the Hemichordate Nervous System

Our understanding of hemichordate nervous system organization and structure is based heavily on studies that all date back to before the 1960s. The first person to describe the nervous system of the hemichordates was Spengel (1877). His description of the major elements and organization of the enteropneust nervous system has been borne out by further detailed analysis in the 1940s and 1950s, notably by Bullock (1945), whose reinvestigated the general organization of balanoglossids in his classic paper. In addition, he investigated the giant axon system in 1944 (Bullock, 1944) and the general function of the nervous system in 1940 (Bullock, 1940). The last comprehensive study of the nervous system of this group was by Knight-Jones (1952), further developing the work of Bullock. Silen (1950) also contributed to the body of work, but some of his data and interpretations have been questioned by both Knight-Jones and Bullock. While the basic descriptions are clearly established, there remain

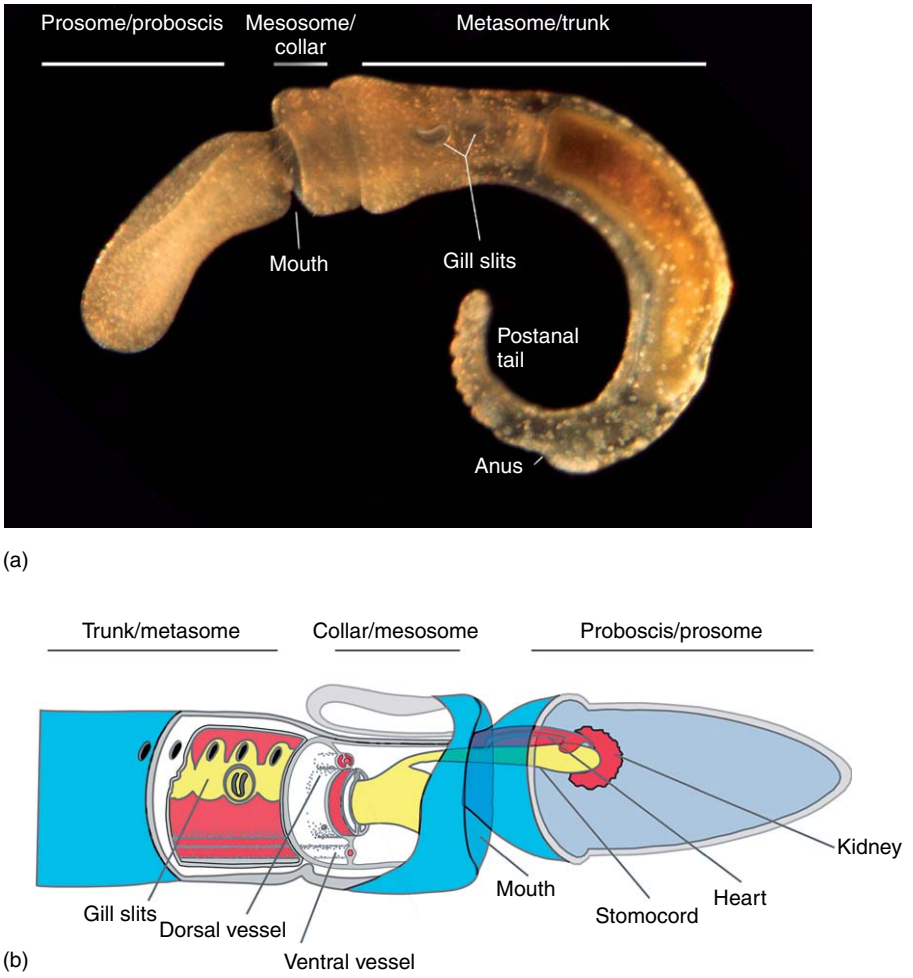

Figure 3 Structure and organization of the enteropneust hemichordate body plan. a, A 13-day-old *Saccoglossus kowalevskii* juvenile. b, Model of the anterior-most region of an enteropneust. Blue, ectodermal derivatives; yellow, endodermal derivatives; and red, mesodermal derivatives.

many unresolved issues in the structure and function of the nervous systems that would benefit greatly from a molecular characterization.

The major organizational feature of the nervous system is a basiepithelial plexus: there is no central nervous system. Cell bodies are scattered throughout the epithelia of the body, with a few exceptions, such as the intestine and gill slits. A mat of axons is spread out along the basement membrane of the epithelia, which is thickened in certain areas of the ectoderm, such as at the base of the proboscis, along the anterodorsal region of the body in the mesosome, and in both the dorsal and ventral midlines of the metasome (Figure 4).

In the proboscis ectoderm, there is a particularly dense concentration of nerve cells that have been proposed to be primarily sensory (Figure 4b; Bullock, 1945; Knight-Jones, 1952). Around the base of the proboscis, there is a particularly dense aggregation of cell bodies. There do not generally seem to be any true sensory organs, but rather individual sensory neurons. One exception is possibly the ciliary organ at the base of the ventral proboscis (Brambell and Cole, 1939). The nerve plexus in the proboscis is particularly thick and axons are organized into lateral and longitudinal tracts that are thought to funnel back to the dorsal peduncle that connects to the collar, passing axons down to the rest of the body (Knight-Jones, 1952). The plexus of axons is particularly thick in this region around the base of the proboscis and forms the so-called anterior nerve ring. Figure 4a shows an updated version of the classic picture from Knight-Jones of the organization of the nerve plexus in hemichordates. A low-magnification confocal Z-series of a 13-day-old juvenile of *S. kowalevskii* following

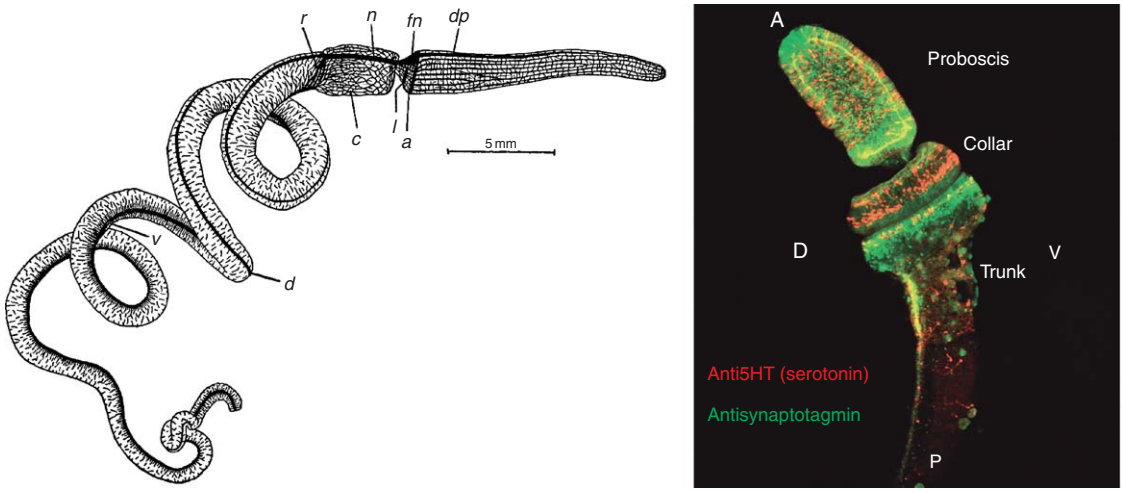

Figure 4 General organization of the nervous system of enteropneusts. a, Drawing from Knight-Jones (1952) of the nerve plexus of *Saccoglossus cambriensis*. d, dorsal cord; v, ventral cord; r, prebranchial nerve ring; c, collar; n, neurocord; fn, fan-shaped thickening of nerve fiber layer; l, ciliary organ; a, anterior nerve ring; dp, dorsal concentration of fibers on the proboscis. b, Confocal micrograph of 13-day-old juvenile of *S. kowalevskii*. Immunocytochemistry of antibodies against serotonin and synaptotagmin (monoclonal antibody courtesy of Bob Burke, University of Victoria). A, anterior; P, posterior; D, dorsal; V, ventral. a, Reproduced from Knight-Jones, E. 1952. On the nervous system of *Saccoglossus cambriensis* (Enteropneusta). *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* 236, 315–354, with permission from The Royal Society. b, Lowe (unpublished).

immunocytochemistry against serotonin and synaptotagmin shows the broad distribution of cell bodies in the ectoderm, particularly densely packed in the anterior ectoderm, collar, and a dense mat of axons, particularly prominent in the base of the proboscis and throughout the proboscis ectoderm, lining the basement membrane (unpublished data).

Probably the most well-known aspect of hemichordate anatomy is the mid-dorsal region of the dorsal cord, or collar cord, which is internalized into a hollow tube of epithelium in some species within the Ptychoderidae and in one species of the Spengeliidae. However, in the other major enteropneust lineage, the Harramanids, there is no contiguous hollow tube, but scattered blind lacunae (Bullock and Horridge, 1965; Nieuwenhuys, 2002; Ruppert, 2005; Figure 5a). This structure has been widely compared to the dorsal cord of chordates due to the superficial similarities to the hollow ciliated nerve cord, and similarities in its morphogenesis with neurulation in vertebrates (Morgan, 1891). However, the similarities have generally been overemphasized as it seems to be more of a conducting tract rather than processing center (Ruppert, 2005), as evidenced by both ultrastructural (Dilly *et al.*, 1970) and physiological data (Cameron and Mackie, 1996). Another striking feature of the dorsal cord is the presence of giant axons (Figure 5b). The number is quite variable between different species, but they are always associated with the dorsal cord and are unipolar cells from 15 to 40 μm in diameter. The cell bodies project their axons across the midline and continue posteriorly within the collar cord. It is not known where the axons finally project: Bullock (1945) proposed that they innervate the ventrolateral muscles of the trunk and suspected that their primary function is to elicit a rapid contraction of the ventrolateral musculature.

In the metasome, the third body region, the most prominent features are the ventral and dorsal nerve cords, which are both thickenings of the nerve plexus. The dorsal cord is contiguous with the collar cord and projects down the entire length of the metasome. The ventral cord is comparatively much thicker, and more cell bodies are associated with the ventral cord, but both cords are interpreted as being through axon tracts. They seem to play a role in the rapid retreat of the animals following anterior stimulation (Knight-Jones, 1952; Bullock and Horridge, 1965). The collar cord seems to play a more minor role than the ventral cord in this response. Ruppert has proposed that the collar cord may be more associated with innervation of the collar musculature that is involved in retraction of the proboscis into the collar, thus sealing the mouth (Ruppert, 2005).

From these studies, there remain some quite fundamental and important questions about the organization of the nervous system. There are still discrepancies between the major texts in terms of the extent of neural plexus and the density of the cell bodies at various regions of the body (Bullock and

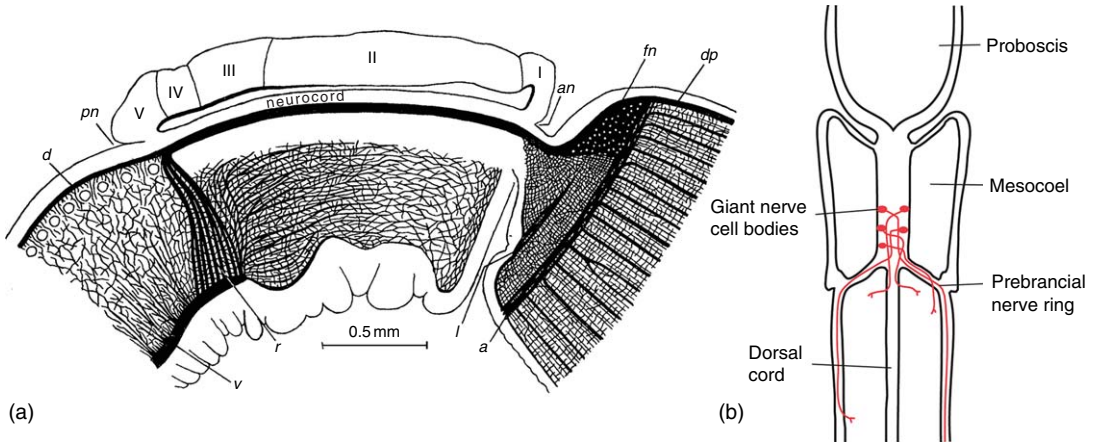

Figure 5 Nervous system of the enteropneust collar/mesosome. a, Drawing of a sagittal section of the enteropneust collar. a, anterior nerve ring; d, dorsal cord; pn, posterior neuropore; an, anterior neuropore; fn, fan-shaped thickening of the nerve fiber layer; v, ventral cord; r, prebranchial nerve ring; dp, dorsal concentration of fibers on the proboscis. b, Dorsal view of collar showing the arrangement of the giant axons. a, Reproduced from Knight-Jones, E. 1952. On the nervous system of *Saccoglossus cambriensis* (Enteropneusta). *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* 236, 315–354, with permission from The Royal Society. b, Redrawn from Bullock, T. H. and Horridge, G. A. 1965. Structure and Function in the Nervous Systems of Invertebrates. W. H. Freeman.

Horridge, 1965). Of fundamental importance is to what extent the nerve plexus and cell bodies show functional differentiation. The classical papers have been unable to distinguish between motor neurons and interneurons, and there is disagreement over which of the ectodermal cells represent neurons, and which ones are glandular or more characteristic of epidermis. The extent to which the plexus shows differentiation along the anteroposterior and dorsoventral axis is also still an open question. Perhaps the most interesting question is whether a greater characterization of the development and differentiation of the nervous system of this group will shed light on the early evolution of the deuterostome nervous system and the evolutionary origins of the chordate central nervous system. Our understanding of the function and differentiation of this nervous system will greatly benefit from the application of basic molecular biological techniques, such as neuron back-filling to map the distribution of cell bodies and where they project their axons; and immunocytochemistry with antibodies raised against conserved neural markers and neurotransmitters to determine the extent of neural differentiation in the nerve plexus.

2.02.4 Proposed Morphological Homology between Vertebrate Central Nervous System and Hemichordate Nervous System

As discussed in the previous section, the classic papers on hemichordate development and evolution have emphasized the critical phylogenetic position of the hemichordates and their relevance for understanding the early evolution of the chordate body plan. Morphological studies have proposed hypotheses on the possible homologies of the enteropneust cords to the dorsal nervous system of chordates. The majority of the papers focus on the possible homology of the dorsal nervous system of chordates with the dorsal collar cord of the enteropneusts. However, more recently an alternative proposal was made that the ventral cord is homologous to the arthropod ventral cord, and the dorsal cord and proboscis plexus homologous to the dorsoanterior brain of arthropods (Nubler-Jung and Arendt, 1996). This would imply that the ventral side of hemichordates is homologous to the dorsal side of chordates. In all of these proposals, both molecular and morphological, the focus of comparisons has been on the axon cords. However, these cords are not the information-processing centers of animals with central nervous systems, but conduction tracts. Both Bullock (1945) and Ruppert (2005) favor homoplasy over homology when comparing the dorsal cord directly with chordates, and argue that the mere presence of a hollow tube is an unreliable character, having evolved independently in several lineages, such as in the cerebral ganglion of some ectoproct bryozoans and in ophiuroids. Other authors have reached the same conclusion (Nieuwenhuys, 2002). Knight-Jones (1952) supports cord homology and argues that the dorsal cord represents a degenerate structure, disagreeing with Bullock. In the next section, I will argue that the fundamental element of relevant comparison between chordates and hemichordates is not the two cords, but the entire basiepithelial nervous system.

2.02.5 Molecular Patterning Events in the Anteroposterior Patterning of the Hemichordate Nervous System

Molecular genetic studies carried out over the past 25 years have established some remarkable similarities in the suite of developmental genes involved in patterning both the dorsoventral and anteroposterior axis of chordates and arthropods and likely all bilaterian phyla (Krumlauf *et al.*, 1993; Arendt and Nubler-Jung, 1994; Finkelstein and Boncinelli, 1994; De Robertis and Sasai, 1996; Lowe *et al.*, 2003; Lichtneckert and Reichert, 2005). Many of the most striking similarities revealed have been during the patterning of the central nervous systems of these two groups (Figure 6). So similar are the relative expression domains of the orthologues of a range of transcription factors along the anteroposterior axis in the developing nervous system of flies and vertebrates that some authors have concluded that the ancestor of bilaterians must have already possessed a complex central nervous system, with an anterior brain already organized into a tripartite structure (Lichtneckert and Reichert, 2005). The plausibility of the arguments depends on this suite of genes being correlated with the development of central nervous systems. However, there has been little comprehensive study of neural specification of any phyla without a highly developed and centralized nervous system. Without comparative data sets in other phyla with more diffuse nervous systems, the hypothesis is difficult to test. The interpretation of this molecular genetic data set is clearly at odds with the classical zoological view of how nervous system evolution has occurred during the evolution of the bilaterian nervous system (Holland, 2003). The current molecular phylogeny of bilaterians most parsimoniously supports a reconstruction of a bilaterian ancestor with a modest nervous system organized as a basiepithlial nervous net, and not the complex, highly regionalized and centralized nervous system, as suggested by the molecular genetic data set.

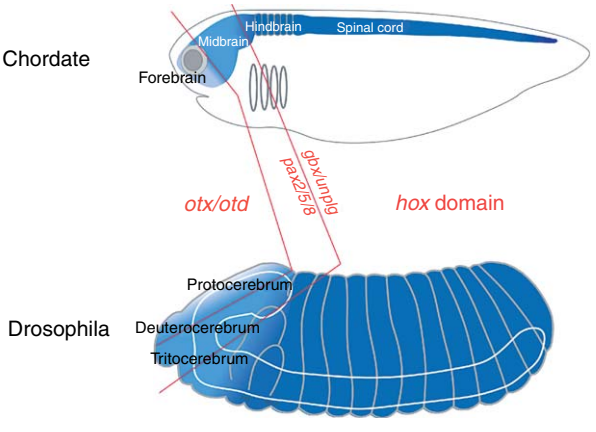

Figure 6 Conserved expression domains of transcription factors between vertebrates and chordates in the developing central nervous system.

We undertook a large comparative study to investigate the role of the transcription factors with conserved roles in anteroposterior patterning of arthropods and chordates during the development of the hemichordate *S. kowalevskii*. The study had two aims: (1) to test the hypothesis of a bilaterian nervous system evolution and (2) to investigate the relationship between the chordate and hemichordate nervous systems. Clearly these suites of genes are very useful axial markers in bilaterian phyla, and their relative expression domains can act as a basic means of comparing body plans of quite distantly related phyla with divergent morphologies (Lowe *et al.*, 2003). *S. kowalevskii* belongs to the Harramanids, which are all characterized by direct development (Figure 1): the adult body plan is formed directly from the egg without any intervening larval stages. We first investigated the observations of Bullock that the entire ectoderm of this animal is neurogeneic without contiguous areas of non-neurogenic ectoderm as in arthropods and chordates. The first group of genes described are all markers of neural differentiation, such as *sox1/2/3*, *musashi,* and *elv*. All of these genes are early markers of neural plate in vertebrates: the first two are early markers of proliferating neural precursors while *elv* is a marker of differentiating neurons (Kim and Baker, 1993; Kaneko *et al.*, 2000; Sasai, 2001). All three orthologues of these genes are expressed throughout the ectoderm of the developing embryos of *S. kowalevskii* from very early stages of gastrulation through to all stages examined. The expression of these genes begins broadly throughout the ectoderm, with expression uniform at early developmental stages. At later stages, expression in the prosome and mesosome is stronger than in the metasome. This observation generally correlates with the distribution of cell bodies described by Bullock (1945).

The remaining characterization of the expression of developmental transcriptional regulators describes the expression of genes involved in the anteroposterio patterning of the vertebrate neural plate. Twenty-two transcription factors with conserved roles in patterning the anteroposterior neuraxis of vertebrates were examined. The surprising result from the study was that the relative expression patterns of these genes in developing embryos of *S. kowalevskii* are highly correlated

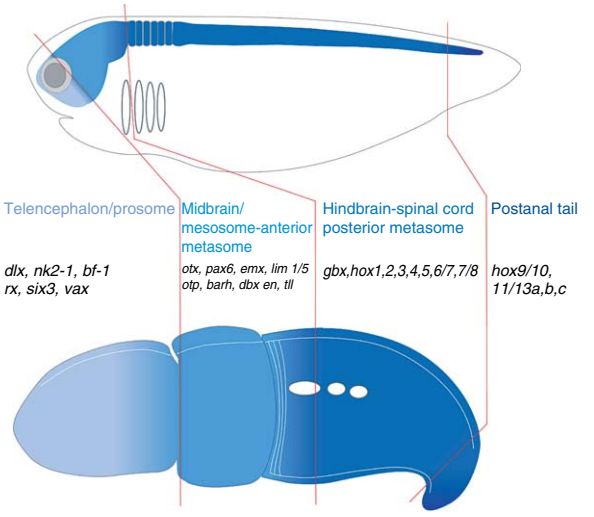

Figure 7 Model showing the similarities of expression domains between *S. kowalevskii* and vertebrates for conserved transcription factors with critical roles in patterning the brain and central nervous system of chordates.

when compared to those in vertebrates (Figure 7). The expression domains, however, were not restricted to either the dorsal or ventral side of the developing embryo, but were expressed in concentric rings in the ectoderm, reflecting the organization of the basiepithelial nerve net. The general domains of expression can be divided into three main fields. In the first, genes that are generally markers of vertebrate forebrain development (*retinal homeobox* (*rx*), *six3*, *nk2-1*, *brain factor-1* (*bf-1*), *distalless* (*dlx*), and *ventral anterior homeobox* (*vax*)) are expressed uniquely in the developing proboscis ectoderm.

In the second region, genes that are predominantly markers of the forebrain and midbrain show correspondingly further posterior domains of expression in the hemichordate embryo; their expression is detected in the more caudal region of the proboscis ectoderm and into the collar/anterior mesosome (*pax6*, *tailless* (*tll*), *emx*, *barH*, *dorsal brain homeobox* (*dbx*), *lim1/5*, *iroquois* (*irx*), *orthodenticle* (*otx*), and *engrailed* (*en*)). Again, their expression is detected in concentric rings in the ectoderm in patterns that approximate their relative expression domains in the midbrain and into the anterior hindbrain of vertebrates. Moving even further posterior in the vertebrate brain, into the hindbrain and spinal cord, in the most anterior domain at the midbrain–hindbrain boundary (MHB), *gbx* defines the limit of the midbrain and antagonizes the expression of *otx,* which is expressed rostrally. The mutual antagonism of these two transcription factors is involved in placing the MHB (Li and Joyner, 2001; Rhinn *et al.*, 2005). In hemichordates, there is a large degree of overlap in *gbx* and *otx* expression, suggesting that these genes may not have similar regulatory interactions in this group. Posterior to *gbx* is the expression of *hox* genes in the hindbrain and spinal cord. As has been well characterized, *hox* cluster members in the 3′ region of the cluster are expressed more anteriorly during embryonic development than cluster members located more 5′. A similar expression relationship is found in hemichordates, with *hox1* expressed just below the first gill slit and the remaining cluster members expressed increasingly more caudally in the ectoderm. This is a consistent pattern for *hox3, 4, 6/7, 7/8*, and *11/13c*. All the remaining *hox* members have been characterized (*hox 2, 5, 9/10*, and *hox11/13a, b*) and all members exhibit colinearity in the same way that other bilaterian groups do (unpublished data). The most posterior members of the hox cluster, *hox11/13, a, b,* and *c* are all expressed in the ventral postanal tail of the late juvenile (Figure 7).

The extent to which vertebrates and hemichordates share patterning similarities is work in progress and is clearly critical for establishing the early patterning systems of the deuterostome ancestor. My lab is investigating the role of signaling molecules with conserved roles in early neural patterning in vertebrates during the early development of hemichordates. The expression of the transcription factors described in the previous section is downstream of early patterning from signaling factors immediately following neural induction in vertebrate development. By the end of gastrulation, the neural plate has already become subdivided into the precursors of the fore-, mid-, and hindbrain (Rhinn *et al.*, 2006). Immediately following neural induction, the entire neural plate expresses *otx2* that in later stages becomes restricted to the fore- and midbrain. This suggests that, at neural induction, the entire neural plate is initially fated as anterior (Gamse and Sive, 2000). Posteriorization by transformation of anterior to posterior fates has been postulated to be the primary mechanism to achieve neural partitioning with candidate signaling from *wnt* (McGrew *et al.*, 1995; Fekany-Lee *et al.*, 2000; Kiecker and Niehrs, 2001), *fgf* (Kengaku and Okamoto, 1993; Lamb and Harland, 1995), and retinoic acid (RA) (Durston *et al.*, 1989; Sive *et al.*, 1990; Conlon, 1995), which are all present in the posterior region of the embryo. Following gastrulation, after the initial global action of *wnts*, *fgfs,* and RA, local signaling centers are set up in the neural plate that further refine the anteroposterior patterning established from earlier signals. These

centers are the anterior neural ridge at the far rostral tip of the neural plate (Shimamura and Rubenstein, 1997; Houart *et al.*, 2002), and the zona limitans intrathalamica at the boundary of prosomeres 2 and 3 (Kiecker and Lumsden, 2004). Further caudally is the MHB or isthmus, which maintains distinct cell fates between the midbrain and hindbrain (Wurst and Bally-Cuif, 2001; Raible and Brand, 2004; Nakamura *et al.*, 2005; Rhinn *et al.*, 2005), and at the far posterior is the tail organizer (Agathon *et al.*, 2003). Is there any evidence of either early action of *wnts*, *fgfs*, and RA in posteriorization of hemichordate ectoderm and nervous system? Is there evidence of signaling centers homologous to those found in vertebrate neural plate?

The evidence for a role of *fgf*, *wnt*, and RA in global posteriorization is mixed outside vertebrates. RA shows the strongest evidence for a conserved role in posteriorization by experiments carried out in both ascidians and amphioxus (Hinman and Degnan, 2000; Matsumoto *et al.*, 2004; Schubert *et al.*, 2004, 2005). There is strong evidence of a posterior organizing center in amphioxus, and weaker evidence in ascidians, but there is ample evidence to propose an ancestral posterior signaling center homologous to the tail organizer in vertebrates (Holland, 2002). However, the role of *fgf* in posteriorization is not well established outside the vertebrates. There are currently no *fgf* data from amphioxus, and data from ascidians showing some localized expression of *fgf8/17/18* in a region that loosely resembles an MHB (Imai *et al.*, 2002) but no functional data have yet been described. Beyond chordates, the evolutionary origins of these signaling factors in ectodermal patterning are not well characterized. RA metabolism has no published account outside chordates, and the role of *wnts* and *fgfs* in *Drosophila* does not support a conserved role in neural patterning homologous to that of vertebrates. The origins of the MHB are equally uncertain: outside vertebrates there is weak evidence for the presence of an MHB-like patterning cassette based on the profile of gene expression (Tallafuss and Bally-Cuif, 2002). Amphioxus perhaps shows the least amount of similarities to vertebrates and lacks many critical markers of this boundary (Takahashi, 2005). Within the urochordates, *Ciona intestinalis* exhibits the most likely candidate for an MHB. However, between species of ascidians, the domains shift quite significantly, and in the larvacean there is no good evidence for such a region (Cañestro *et al.*, 2005). Hemichordates, as an outgroup of chordates, offer an opportunity to investigate the origins of vertebrate neural patterning centers. We have some preliminary molecular evidence that the evolutionary origins of *wnts*, *fgfs*, and RA may be far more ancient than previously anticipated (Gerhart *et al.*, 2005, unpublished data). The localization of the expression of certain *fgfs*, *wnts*, and *wnt* antagonists in the ectoderm of the developing embryo of *S. kowalevskii*, when mapped onto the existing transcriptional map of conserved anteroposterior genes, shows remarkable topological similarities with the localization of their vertebrate orthologues during neural plate regionalization (unpublished data). From our expressed sequence tag (EST) data we have also cloned many members of RA metabolism, such as the receptors RXR and RAR, the enzyme Raldh2 that metabolizes retinol to produce RA (Conlon, 1995), and the enzyme cyp26 that breaks down RA (Ray *et al.*, 1997). Functional data will be required to test whether the early patterning events, following the induction of neural plate, have more ancient deuterostome origins than previously anticipated, but the data so far are very promising.

2.02.6 Evolutionary Interpretations of the Molecular Data from Hemichordates

The expression of the correlated suite of anteroposterior patterning genes with such conserved domains in the hemichordate ectoderm has many implications for our understanding of the evolution of deuterostome nervous systems, but also for how reliable body-patterning genes can be used for reconstructing ancestral neural anatomies of distantly related groups of animals. Do these data allow us to test hypotheses of morphological homology between hemichordates and other bilaterians? For example, a subset of the study genes from Lowe *et al.* (2003) has similar relative expression domains between arthropods and chordates. These similarities have been used to support the hypothesis that the ancestor of protostomes and deuterostomes must have already possessed a complicated and central nervous system (Lichtneckert and Reichert, 2005), as was discussed in an earlier section. However, the correlated expression domains of this suite of genes broadly in the ectoderm of hemichordates raises the possibility that this group of genes acts as a conserved regulatory suite for patterning all bilaterian nervous systems. The study establishes that these genes are not uniquely associated with central nervous system development. It is, of course, also possible that hemichordates lost centralization from a deuterostome ancestor with a central nervous system. However, even if this proves to be the case, correlated suites of developmental

genes expressed in similar topologies between distantly related groups are clearly deployed to pattern diverse types of neural architectures. Caution should therefore be used about using this suite of genes to test hypotheses of morphological homology of specific neuroanatomies.

Clearly the similarities in gene expression between vertebrates and hemichordates does not indicate any sort of morphological homology between regions of the vertebrate brain and the basiepithelial plexus of the hemichordates, so what exactly do these data allow us to compare between groups, and what do they tell us about the early evolution of the chordate nervous system? These data provide, for the first time, a transcriptional rationale or map with which to compare the body plans of vertebrates and hemichordates, and give some critical insights into the deterostome ancestor that gave rise to the chordates. While the details of the morphology of the nervous system (centralized, or diffuse) are difficult to reconstruct from this kind of data, the ancestor clearly used this group of genes to pattern its anteroposterior axis, and likely its nervous system. The various suites of genes involved in patterning the three regions of the vertebrate brain were already present in the deuterostome ancestor. This is a surprising finding based on the studies of similar genes from cephalochordates and urochordates (Takahashi and Holland, 2004; Cañestro *et al.*, 2005; Takahashi, 2005). Neither of these two groups exhibits the degree of similarity in the relative expression domains between vertebrates and hemichordates, suggesting that they have lost some of the complexity of the ancestral transcriptional network. Work from larvaceans, ascidians, and amphioxus has reached sometimes conflicting conclusions regarding the evolution and early chordate origins of the vertebrate brain. These issues have been extensively reviewed elsewhere (Wada *et al.*, 1998; Wada and Satoh, 2001; Mazet and Shimeld, 2002; Tallafuss and Bally-Cuif, 2002; Takahashi and Holland, 2004; Takahashi, 2005). Given the uncertainties of the neural organization of the deuterostome ancestor, searching for morphological homologues of vertebrate brain regions in the early chordates using gene expression as the primary data could lead to misleading conclusions.

2.02.7 Life History Considerations

A discussion of the origins of the chordate nervous system would not be complete without a discussion of life history issues and particularly the potential role of larval forms in the evolution of the chordate dorsal nervous system. As described earlier in this article, enteropeust hemichordates are divided into two major lineages characterized by divergent developmental strategies (Cameron *et al.*, 2000). Developmental studies have been carried out from species from both lineages (Lowe *et al.*, 2004). While *S. kowalevskii* is a direct developer, *Ptychodera flava* is an indirect developer and its early development is characterized by a prolonged larval period in the plankton before metamorphosis into a juvenile many months after fertilization (Tagawa *et al.*, 2001). The early larval development of hemichordates resembles the early larva of many echinoderm species and has been termed dipleurula-type. Study of *P. flava* allows for the investigation of the development of both the larval and the adult body plan of enteropneusts.

One of the most influential and enduring hypotheses on the origin of the chordate nervous system is from Garstang (1894, 1928). He proposed the auricularian hypothesis, which drew directly on the larval similarities between the echinoderms and hemichordates. While the adult body plans of hemichordates and echinoderms are highly divergent, the larval body plans are very similar. The key component of the hypothesis is that Garstang derived the chordate dorsal central nervous system by a dorsal migration of the lateral ciliated bands of an auricularian-type (dipleurula) larva, similar to the early larva of echinoderms and hemichordates. Garstang's hypothesis has been extremely influential on subsequent work in this area (Gee, 1996). There have been many modifications to the original hypothesis, but the essential elements remain the same: the adult body plan of chordates is derived by transformation of a larval life history stage. There are a range of reviews on the features of Garstang's original hypothesis (Lacalli, 1995; Nielsen, 1999), and I will only focus on the molecular genetic studies that are relevant to this hypothesis. There have been a series of papers over the past 10 years on the role of body-patterning genes in larval development relevant for testing the auricularian hypothesis (Lowe and Wray, 1997; Harada *et al.*, 2000, 2002; Shoguchi *et al.*, 2000b; Tagawa *et al.*, 2000; Lowe *et al.*, 2002; Taguchi *et al.*, 2002; Poustka *et al.*, 2004). Some of these studies propose molecular support for the auricularian hypothesis and show expression domains for developmental genes with conserved roles in the development of the central nervous system in chordates, as described in an earlier section. In order to argue compellingly for a molecular case to support this hypothesis, there should be evidence that the anteroposterior patterning domains, which show nested in the nervous system of chordates, have

similarly correlated domains during larval development. Not finding such domains does not disprove the auricularian hypothesis, but finding a correspondingly complex set of domains during the adult life history stage would argue quite strongly against Garstang's theory (Lowe *et al.*, 2003; Haag, 2005, 2006).

Of the genes so far studied, there is the most comparative information on *otx*, which has an anterior localization during the development of the vertebrate nervous system and likely plays a conserved role in the determination of the anterior region of all bilaterians (Finkelstein and Boncinelli, 1994). The expression of *otx* during the development of larvae shows a general association with the development of ciliated bands. There is not, however, any particular anterior bias, with quite a variation in detailed expression domains between larval types of hemichordate (Harada *et al.*, 2000) and holothuroid development (Lowe and Wray, 1997; Shoguchi *et al.*, 2000b; Lowe *et al.*, 2002). *t-brain* is a T-box gene with a largely conserved anterior domain of expression in the development of the forebrain of vertebrates (Bulfone *et al.*, 1995; Mione *et al.*, 2001). Tagawa *et al.* (2000) investigated the expression of the hemichordate orthologue of *t-brain* during early larval development in *P. flava* and detected its expression in the apical organ, at the very anterior tip of the larval ectoderm. They discussed the evolutionary implications of the data and raised the possibility of homology between the apical organ and vertebrate forebrain. However, in a similar study on asteroid larval development, whose early development resembles that of *P. flava* quite closely, there is no such expression detected in the apical organ, and it is confined to the blastopore and endoderm (Shoguchi *et al.*, 2000a). How does one resolve the variability in the expression domains of conserved developmental genes in similar larval morphologies? It is hard to draw firm conclusions when there is such variability in gene expression domains. The same observation is made when *Dlx* expression is examined across echinoderm larval types (Lowe *et al.*, 2002). There is quite a range of expression domains of *Dlx* throughout three different classes of echinoderms, and between different developmental modes during development. Expression in a range of holothuroid larval types is associated with cilia and ciliated bands. However, expression of *Dlx* is not detected in the ciliated bands of asteroid or echinoid larvae, and, unlike vertebrates, is not expressed anteriorly. Of particular note in this study was that, while larval domains of *Dlx* expression varied extensively, the expression domains during early adult body patterning is conservative, and is initially expressed at the onset of the adult body plan as the hydrocoel first begins to form five pouches – the first sign of the radial symmetry of the adult body plan. Perhaps the most telling study is of the expression of *hox* genes during the development of echinoid larvae (Arenas-Mena *et al.*, 2000). Very few *hox* genes are expressed during early development of the larva, and those that are, are expressed in a lineage-specific fashion and not in nested domains, as found in most other bilaterian groups. The first evidence of the expression of *hox* genes in a co-linear manner is late in larval development during the formation of the adult body plan, and expression is detected in the mesoderm rather than the ectoderm.

While a full characterization of body-patterning genes during the development of larval body plans is still lacking, the current evidence supporting larval origins of the chordate body plan is weak . Evidence from Lowe *et al.* (2003) in hemichordate adult patterning, and from limited data during adult body patterning of echinoderms (Morris and Byrne, 2005), support the alternative view that the most fruitful approach for understanding the early origins of the chordate body plan will come from the adult life history stage and not larvae (Lowe *et al.*, 2003; Haag, 2005, 2006). However, it is important to reiterate here that the search for morphological homologies between chordate central nervous system and either life history stage using molecular markers is unlikely to be informative, and even misleading, if gene expression is the only criterion to assess hypotheses of homology. Even similarities in suites of correlated expression domains, as found in Lowe *et al.* (2003), only allow for a reconstruction of the regulatory map that an ancestor must have utilized in early development. Additional data in the form of morphological or fossil data are also required to make strong conclusions about homology.

2.02.8 Future Directions

It seems likely that the story of the origins of chordates and the murky history of early deuterostome evolution is going to remain a topic of hot debate in the near future. The most significant advances will likely occur in the next few years, and most of the progress will be directly due to the completion in sequencing of several key genomes. The sea urchin and amphioxus genomes are close to completion and the genome for *S. kowalevskii* is also timetabled for completion this year. The phylogenetic issues raised in Delsuc *et al.* (2006) will begin to be

resolved one way or another with increased phylogenetic sampling to include additional hemichordates, echinoderms, and *Xenoturbella*. This study clearly shows how interpretation of the molecular genetic data is only as good as the phylogeny used to map the characters. One of the largest gaps in our understanding of body patterning in the deuterostomes is that of the echinoderms. There have only been a handful of studies investigating body patterning of the adult echinoderms, and mention of echinoderms in discussion of deuterostome body plans generally dismisses them as being too derived to give informative data for reconstructing early deuterostome evolution. It is still possible that the highly divergent symmetry system of echinoderms, and their odd morphologies, are still regulated by the conserved ectodermal transcriptional network as described in this review. Further work will be required to test this hypothesis. Ongoing research in my lab is investigating the similarities in nervous system patterning between vertebrates and hemichordates, and this work will help establish the extent to which transcriptional and signaling elements of vertebrate brain patterning were established early in deuterostome evolutionary history, and which ones evolved much later during chordate diversification.

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2.03 Evolution of the Nervous System in Fishes

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Glossary

actinopterygians	Sistergroup of sarcopterygians, include all ray-finned fishes, that is, bichirs (*Polypterus*) and the reedfish (*Calamoichthys*), together forming the cladistians, the sturgeons (chondrosteans), the gars (*Lepisosteus*; ginglymodes), and the bowfin (*Amia*; halecomorphs), as well as the manifold modern ray-finned fishes, the teleosts.
agnathan(s)	Descriptor for all jawless fishes; the two extant groups, lampreys (petromyzontids) and hagfishes (myxinoids) are not considered monophyletic here, since petromyzontids are more closely related to gnathostomes.
Bauplan	Set of ancestral characters shared by all organisms (or one of their organs; e.g., the brain) forming a given taxon.
chondrichthyans	Outgroup of remaining gnathostomes, including all cartilaginous fishes, that is, elasmobranchs (sharks, skates, and rays) and holocephalans (chimaeras).
cladogram	Branching diagram of taxa exclusively based on shared derived characters (synapomorphies).
gnathostomes	Vertebrates with true jaws (chondrichthyans, actinopterygians, sarcopterygians).
monophyletic group	Taxon that includes all descendants of a last common ancestor.
neuromeres	Transverse units (segments) of developing neural tube (rhombomeres, prosomeres).
neuromeric model	Assumes transverse (neuromeres) as well as longitudinal units (roof, alar, basal, floor plates) along the entire anteroposterior neural tube axis, and that their arrangement is guided by selective regulatory gene expression that allows for regionalized developmental processes.
organizing centers and patterning	Restricted regions of the embryo that secrete specific signalling molecules, responsible for specifying distinct domains (molecularly, anatomically, functionally distinct) in competent neighbouring tissues. This process is called patterning.
phyletic method	Uses cladistic methodology (cladograms, outgroup comparison) for establishing evolutionary polarity (i.e., ancestrality vs. derivedness) of characters.
sarcopterygians	Sistergroup of actinopterygians, including lobe-finned fishes, that is, *Latimeria* (actinistians) and the lungfishes (dipnoi), and all land-vertebrates (tetrapods).

2.03.1 Introduction: *Scala Naturae* Concept is Hard to Kill

The designation of fishes and amphibians as 'lower' vertebrates and of amniotes – or even mammals only – as 'higher' vertebrates expresses the pervasive

ladder concept of linear progress along the vertebrate phylogenetic tree (*Scala naturae*). Instead of considering the mammalian condition as the peak of vertebrate Bauplan perfection – a very idealistic viewpoint of the ladder of progress concept – Charles Darwin and his followers have argued that one should rather view the evolution of species in general, and the evolution of vertebrates in particular, as having occurred in a bush-like fashion (see Butler and Hodos, 2005 for recent review). Viewing evolution as a bush and not as a ladder implicates that each living species is neither 'higher' nor 'lower' than the others. Species simply diverged from each other at different time points during phylogenesis. This separation always included changes in early development, resulting in different animal forms and functions. Thus, many modern, derived features evolved independently in various vertebrate lineages, at the same time as many ancestral traits were shared by all vertebrates, representing the inheritance of their common ancestor. Most animal forms became extinct at some point or another, the extant species representing merely a relatively small selection of those forms that survived for reasons of adaptation or other factors, such as geographic isolation or escaping mass extinctions.

To reconstruct the evolution of the vertebrate brain, modern neurobiologists use the comparative (cladistic) method for establishing the evolutionary polarity of brain traits. This method analyzes single-organism characters instead of entire organisms or their brains, and allows to determine whether a neural character is ancestral (plesiomorphic) or derived (apomorphic) using well-supported cladograms (Figure 1). Cladograms are exclusively based on shared derived characters (synapomorphies). Sistergroups (e.g., sarcopterygians–actinopterygians in Figure 1a) are characterized by synapomorphies inherited from their last common ancestor, separating them from the outgroup taxa (e.g., cartilaginous fishes in Figure 1a). The outgroup comparison determines the evolutionary polarity of particular neural characters. If two conditions occur in sistergroups (evagination of telencephalic hemispheres in sarcopterygians; eversion in actinopterygians), the condition in the outgroups is investigated (evagination in cartilaginous fishes) which, for reasons of parsimony (i.e., principle of choosing the simplest explanation), is considered to represent the ancestral condition.

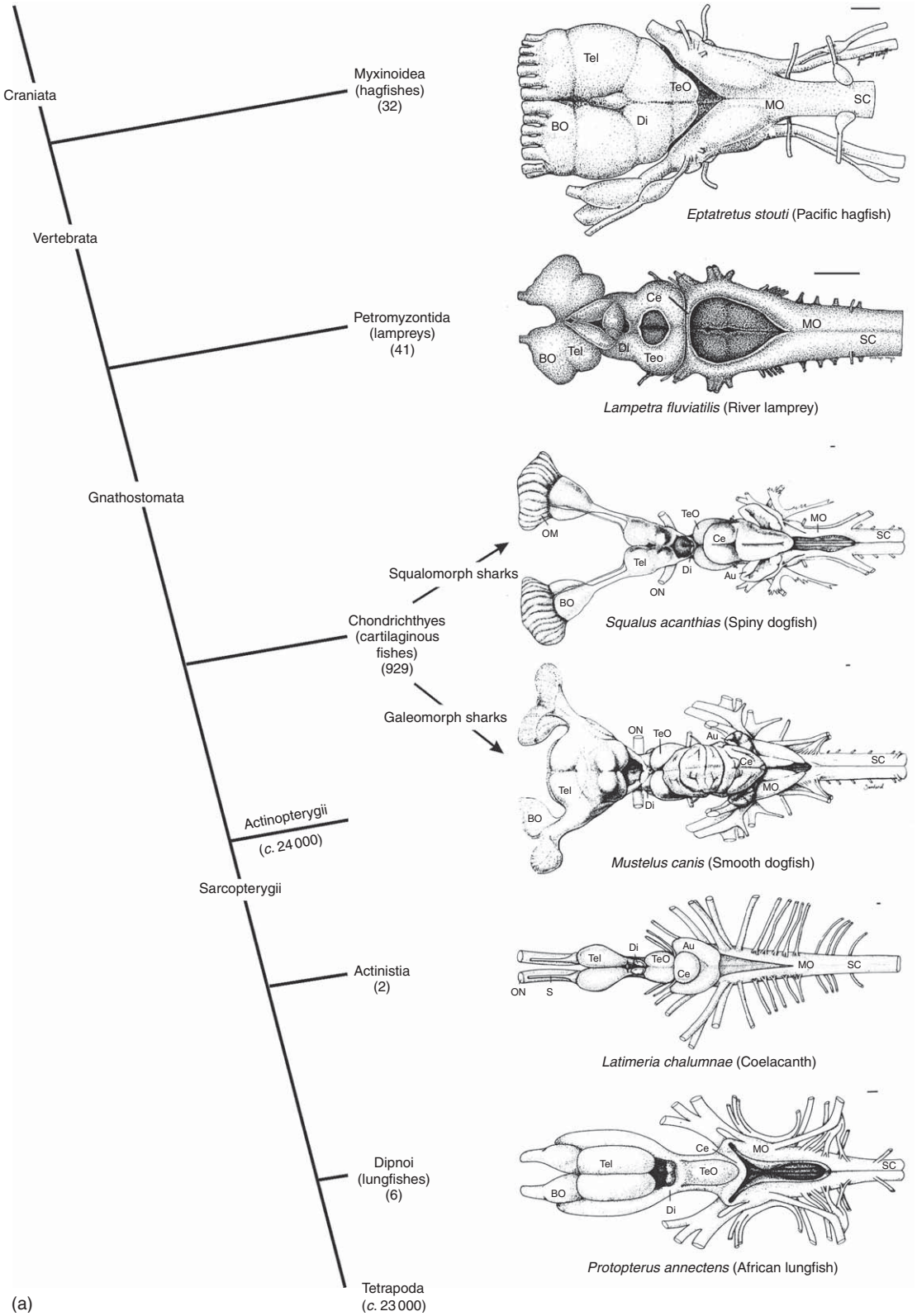
Despite this meanwhile well-accepted change of perspective brought about especially by the cladistic school in evolutionary theory, the metaphoric value of the *Scala naturae* concept is hard to kill. Especially the vertebrate brain evolution is still often viewed as proceeding from an assumed simple brain in various fish groups or amphibians to increasingly larger and more complex brains with greater capacity in turtles, lepidosaurs (lizards and snakes) and birds, with mammals being at the summit of the ladder, and this phenomenon is believed to be notable in the brain diversity of extant vertebrates. However, this view is highly biased by anthropocentric, teleological thinking. In the following, we discuss the notion of progress during vertebrate brain evolution, with special emphasis on fishes, and ask in particular whether *Scala naturae* thinking is supported by brain weight/body weight data or degree of histological and morphological complexity (see Section 2.03.2), by functional neuroanatomy (see Section 2.03.3), and by neurochemical brain organization (see Section 2.03.4).

2.03.2 Diversity and Bauplan of Fish Brains from Agnathans to Lungfishes

What are fishes? Fishes represent a way of life rather than a monophyletic craniate group (Figures 1a–1c). Extant fish groups include species such as the Comoran and Indonesian coelacanths (Actinistia; two species) and the lungfishes (Dipnoi; six species), both of which are sarcopterygians (lobe-finned fishes), the taxon that includes tetrapods (Figure 1a). Within the tetrapod radiation of approximately 23 000 species, amphibians amount to almost 4000 (18%), reptiles to more than 6000 (26%), birds to almost 9000 (38%), and mammals to slightly more than 4000 (19%) species.

The sistergroup of the sarcopterygians is comprised of the actinopterygians (ray-finned fishes, often regarded as the 'true fishes'). Actinopterygians are the most successful vertebrate radiation, since they represent half of all living vertebrate species (almost 24 000), forming a symmetrical dichotomy in vertebrate evolution. Thus, it is hard to argue objectively that there is a linear increase in evolutionary success going from fishes to mammals. The outgroup to sarco- and actinopterygians are the chondrichthyans or cartilaginous fishes (holocephalans, sharks, and batoids, i.e., rays and skates), a rather successful ancient gnathostome radation (around 1000 extant species). Two more outgroups to gnathostomes exist, namely the extant agnathan petromyzontids (lampreys; around 41 species), which constitute the vertebrates together with the gnathostomes and myxinoids (hagfishes; 32 species), which complement the vertebrates in the formation of the craniates (Figure 1a).

The craniate taxon indeed entails a large increase in anatomical and physiological complexity, as



(a)

Figure 1 (*Continued*)

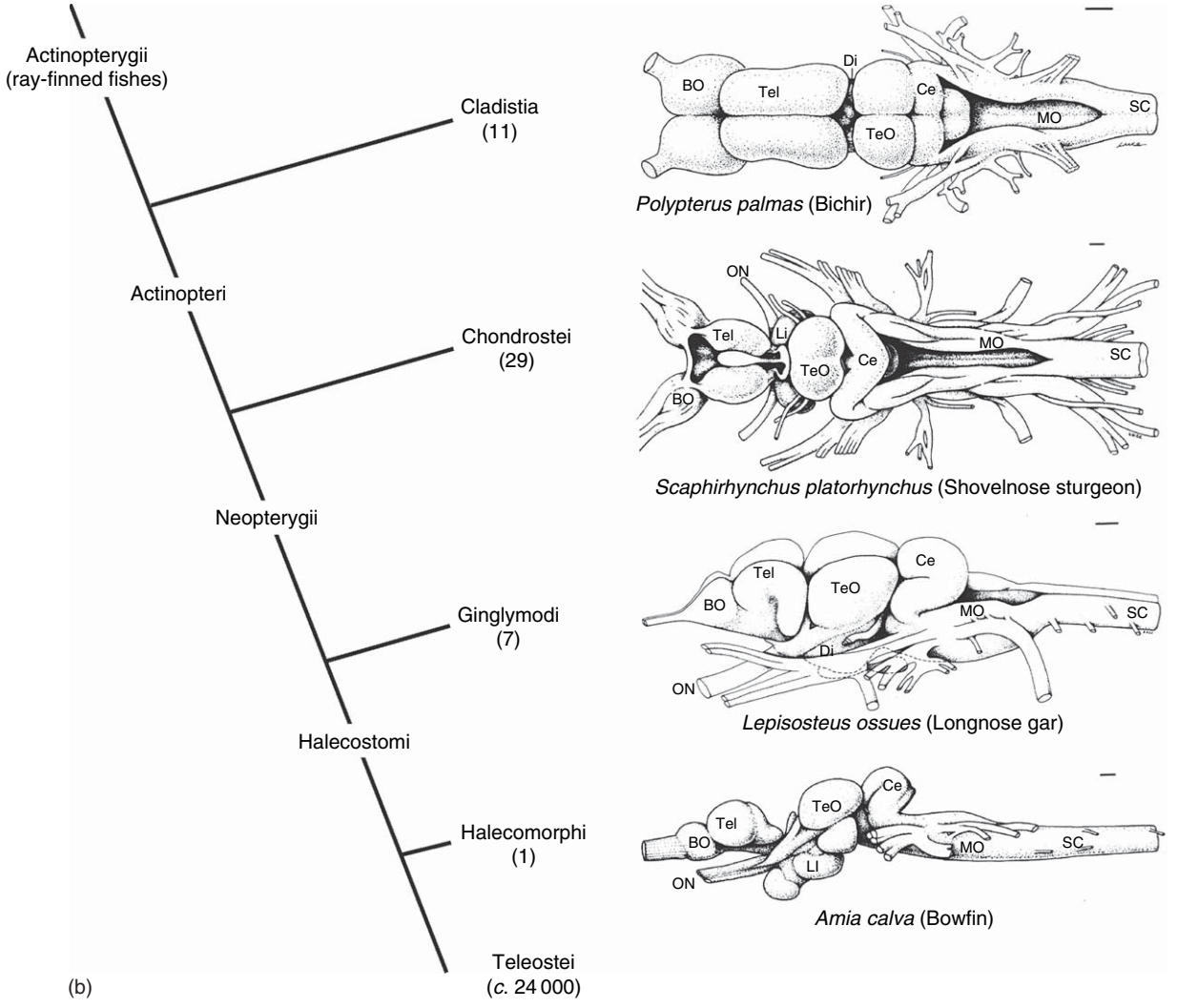


Figure 1 (*Continued*)

compared to its protochordate outgroups, that is, cephalochordates (amphioxus) and urochordates (ascidians). The recent advances of genome sequencing have rejuvenated the idea put forward originally by S. Ohno that the generation of craniate morpho-functional complexity relies upon the generation of a genetic complexity (Ohno, 1970). Indeed, craniates have on average 2 times more genes than protochordates, mainly resulting from the duplication of already existing genes (Furlong and Holland, 2002). Although no agreement has been reached on the mechanisms at the origin of this increased genetic complexity (Hughes and Friedman, 2003), a double duplication of the whole genome of a chordate ancestor (the 2R hypothesis) may have been instrumental in the emergence of craniates, more than 500 Mya (Lynch and Conery, 2000; Levine and Tjian, 2003).

Considering actinopterygians (Figure 1b), modern teleosts are as remote from their Paleozoic ray-finned fish ancestors as modern mammals differ from their Early Mesozoic sauropsid ancestors. Moreover, ray-finned fishes (actinopterygians) flourished several times in evolution (Carroll, 1988), first as chondrosteans (especially the palaeoniscoids) in the Paleozoic, and continuing with the (historically called) holosteans in the Mesozoic. These two actinopterygian radations independently generated many forms apparently adapted to all sorts of enviroments, probably from deep sea and free water to coral reefs. In contrast, the living descendants (i.e., cladistians, chondrosteans, gynglimodes, halecomorphs; Figure 1b) of nonteleost actinopterygians are small in species number.

Interestingly enough, the analysis of the whole genome of several teleost fishes, and gene data from

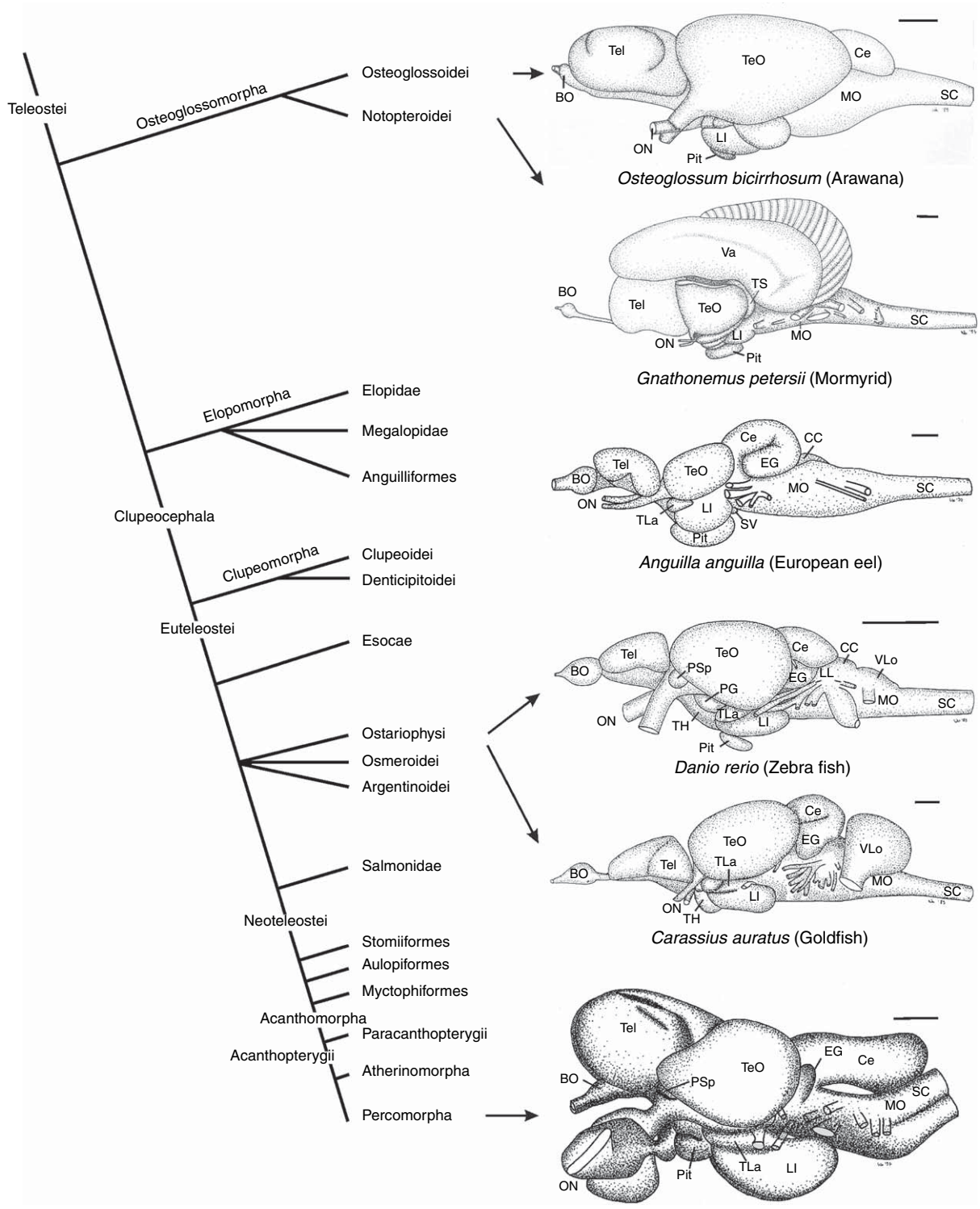


Figure 1 Cladograms depict systematics of extant a, craniate; b, actinopterygian; c, teleostean taxa. Lateral or dorsal views of representative brains are shown on the right side. Au, auricle; BO, olfactory bulb; CC, crista cerebellaris; Ce, cerebellum; Di, diencephalon; EG, eminentia granularis; LI, hypothalamic inferior lobe; LL, lateral line nerves; MO, medulla oblongata; OM, olfactory mucosa; ON, optic nerve; PSp, parvocellular superficial pretectal nucleus; Pit, pituitary; PG, preglomerular area; S, secondary olfactory peduncle; SC, spinal cord; SV, saccus vasculosus; Tel, telencephalon; TeO, optic tectum; TH, tuberal hypothalamus; TLa, torus lateralis; TS, torus semicircularis; Va, valvula cerebelli; VLo, vagal lobe. Scale bars: 1 mm. Adapted from Lauder, G. V. and Liem, K. F. 1983. The evolution and interrelationships of the actinopterygian fishes. *Bull. Mus. Comp. Zool.* 150, 95–197. Some drawings courtesy of Helmut Wicht (*Eptatretus*), Christoph Weigle (*Lampetra*) and R Glenn Northcutt (cartilaginous fishes, sarcopterygians, nonteleost actinopterygians). Species numbers according to Berra (1981), except cartilaginous fishes (Hamlett, 1999).

other groups of actinopterygians, has consistently shown that an additional round of genome duplication occurred between nonteleost actinopterygians and teleosts around 335–404 Mya (Hoegg *et al.*, 2004). Accordingly, teleost fishes have about 2 times more genes than the other craniate/vertebrate groups including mammals, a fact that also breaks the ladder of an assumed increasing complexity from fish to mammals. It is plausible that this genetic complexity of teleosts may have been critical for their tremendous species diversification, leading to the invasion of all aquatic environments covering 80% of the Earth's surface (Meyer and Van de Peer, 2005). The fossil origin of teleosts (Figure 1c) lies in the Early Mesozoic (Late Trias, as does that of mammals; Carroll, 1988), when teleosts started to form free water fish swarms (pholidophorids, leptolepids). But teleost speciation, in particular that of the acanthomorphs (which include the percomorphs), increased tremendously toward and after the cretaceous–tertiary boundary. Thus, acanthomorphs and placental mammals originate in the fossil record at around the same geological time.

2.03.2.1 Bauplan: The Shared Ancestral Brain Morphotype

Despite the apparent considerable diversity of general morphology (Figure 1) and internal organization of fish brains (see Sections 2.03.2 and 2.03.3), one can nevertheless establish a brain Bauplan or morphotype, that is, use the comparative method to demonstrate shared primitive characters that define the ancestral craniate or vertebrate brain (Northcutt, 1985; Wicht and Northcutt, 1992). Such a comparison makes clear that no stepwise addition of brain parts at the anterior pole of the neuraxis occurred during craniate evolution, as had been envisaged historically by E. Haeckel and followers (terminal addition and recapitulation; see Butler and Hodos, 2005, for review). Rather, it shows that most basic brain parts were initially present in craniate/vertebrate ancestors, with the possibility to develop novelties and peculiarities from a common morphotype, as originally proposed (in a different form) by von Baer (1928).

All gnathostome fishes exhibit the five conventionally recognized amniote brain parts (shown in Figure 2): from rostral to the caudal telencephalon, diencephalon (both together: forebrain), mesencephalon (midbrain), metencephalon (including the cerebellum), and myelencephalon (both together: hindbrain). The latter two, without the cerebellum, are often referred to as medulla oblongata. Classical embryology describes that the vertebrate brain develops from a three-vesicle stage (rhombencephalic vesicle including metencephalon and myelencephalon, mesencephalic vesicle, prosencephalic vesicle including diencephalon and telencephalon) into a five-vesicle stage (representing the primordia of the five adult brain parts mentioned). The rejuvenation of paradigms of neuromeric organization further suggests that the craniate rhombencephalon develops from seven to eight transitory neuromeres (rhombomeres), and that the prosencephalon does so from at least three more neuromeres and a less clearly segmented secondary prosencephalon (prosomeres; Puelles and Rubenstein, 1993, 2003; cf. the discussion of molecular data below). Neuromeres, originally described by von Baer (1928), are both morphologically defined entities, as well as territories of gene expression representing a useful framework to compare and interpret morphological observations from one species to another.

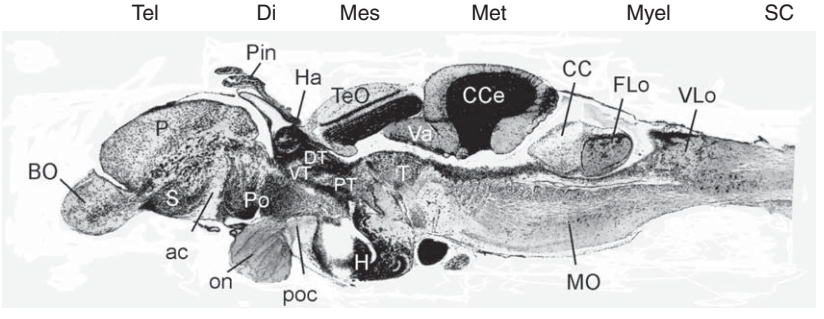


Figure 2 Sagittal section of adult zebra fish brain shows histology of major brain parts. ac, anterior commissure; BO, olfactory bulb; CC, crista cerebellaris; CCe, corpus cerebelli; Di, diencephalon; DT, dorsal thalamus; FLo, facial lobe; H, hypothalamus; Ha, habenula; Mes, mesencephalon; Met, metencephalon; MO, medulla oblongata; Myel, myelencephalon; on, optic nerve; P, pallium; Pin, pineal organ; Po, preoptic region; poc, postoptic commissure; PT, posterior tuberculum; S, subpallium; SC, spinal cord; T, tegmentum; Tel, telencephalon; TeO, optic tectum; Va, valvula cerebelli; VLo, vagal lobe; VT, ventral thalamus (prethalamus). Modified from Wullimann, M. F., Rupp, B., and Reichert, H. 1996. Neuroanatomy of the Zebrafish Brain. A Topological Atlas. Birkhäuser Verlag.

The vertebrate rhombencephalon is ancestrally characterized by its association with the majority of cranial nerves and their primary motor and sensory centers, that is, the trochlear (IV), trigeminal (V), abducens (VI), facial (VII), otic (VIII), glossopharyngeal (IX), and vagal (X) nerves, as well as the lateral line nerves (including a mechano- and an electroreceptive component).

The mesencephalon of vertebrate fishes includes dorsally an optic tectum (visual-multisensory; corresponding to mammalian superior colliculus) and a torus semicircularis (auditory-lateral line; corresponding to mammalian inferior colliculus) which may become somewhat ventrally displaced during ontogeny in certain taxa, as well as a ventral tegmentum which is dominated by motor structures, for example, oculomotor nerve (III) and nucleus.

Classically, the vertebrate diencephalon has been described in dorsoventral order to consist of epithalamus, dorsal thalamus, ventral thalamus, posterior tuberculum, and hypothalamus, with the pretectum intricately intermingled with diencephalic cell groups. The neuromeric model instead (Puelles and Rubenstein, 1993, 2003) proposes that pretectum, dorsal thalamus, and ventral thalamus (prethalamus) represent three transverse neural tube units or prosomeres along the longitudinal brain axis. The posterior tuberculum of fishes develops from the ventral portions of prosomeres 2 and 3, and the region of the nucleus of the medial longitudinal fascicle represents the ventral portion of prosomere 1. The optic nerve (II) enters the diencephalon at the ventral boundary region of preoptic region and hypothalamus.

The telencephalon of all fishes includes a pallium and a subpallium representing, together with the hypothalamus (as well as eminentia thalami and preoptic region), the most anterior, prechordal part of the neural tube (proposed to represent three prosomeres of the secondary prosencephalon; see the discussion above). Thus, the diencephalon gains a new meaning in the neuromeric model, since the classical dorsoventral order of diencephalic divisions transforms into a caudorostral sequence of the anterior neural tube. The olfactory nerve (I) enters the olfactory bulb at the anterior pallial pole of the telencephalon. The terminal nerve (0) is also associated with the telencephalon.

Most of the above-discussed vertebrate neural characters also apply to myxinoids (craniates). However, in contrast to gnathostomes, hagfishes have no recognizable cerebellum, and lampreys also only have a rudiment of it, lacking Purkinje cells or other major rhombic lip-derived elements. Interestingly, the lamprey rhombic lip region also lacks *Pax6* expression which is mandatory for the development of cerebellar structures in gnathostomes (Murakami *et al.*, 2005). Another novelty of gnathostomes is the presence of three semicircular canals in the vestibular inner ear, whereas hagfishes and lampreys have a simpler labyrinth. This is directly related to the absence of expression of one *Otx* gene in the otic placode of agnathans in contrast to gnathostomes (Germot *et al.*, 2001). Therefore, both agnathan groups offer no reasonable distinction between met- and myelencephalon.

Furthermore, myxinoids, but not lampreys, lack external eye muscles, as well as the associated cranial nerves and nuclei (e.g., III, IV, VI) and a terminal nerve, as well as the electroreceptive – but not the mechanoreceptive – component of the lateral line nerves (Braun, 1996; Wicht, 1996; Wicht and Northcutt, 1998). The absence of these and additional neural characters may be part of the ancestral condition for craniates, corroborating that myxinoids form the outgroup to vertebrates. Unfortunately, the lack of an appropriate outgroup to craniates prevents a test of an alternative explanation, namely that these myxinoid characteristics evolved as secondary reductions.

In any case, considerable evidence from modern developmental studies is in support of a basic craniate/vertebrate Bauplan just outlined.

2.03.2.2 Extension of Bauplan: Phylotypic Stage in Brain Development

Neuromeres are useful paradigms for comparing brain morphologies. They are also morphogenetic units resulting from the specification of the neural phenotype, which is an ongoing succession of cell-fate determination in spatially defined regions of the neuroepithelium. The conservation of neuromeres throughout the craniate taxon reflects major constraints on the development of the neural tube. During neurulation, the neural tube closes and becomes patterned along the anteroposterior axis (neuromeres) and along the dorsoventral axis (roof, alar, basal, floor plates). This patterning corresponds to the restriction of cell movements and to polyclonal cell divisions that become confined to a neuromeric unit.

A key feature of this cell patterning in the neural tube is that it also corresponds to gene patterning. In other words, morphogenetic units are also territories of defined gene expression. A probable proximal cause of this gene patterning is the existence of local sources of inductive signals located in the so-called organizing centers such as in the roof plate (members of secreted bone morphogenetic

proteins (BMPs) and wingless-related factors – WNTs) in the floor plate and zona limitans intrathalamica (ZLI) (secreted Hedgehog factors, Shh; induced after expression in notochord and prechordal plate), in the anterior neural ridge (ANR) or in the midbrain–hindbrain boundary (MHB), both secreting FGF8. The signals emitted by these centers coordinate the action of proliferation-related neurogenic or proneural genes at specific locations to maintain or inhibit proliferation of neural progenitors, leading to the formation of segments or neuromeres (Wurst and Bally-Cuif, 2001; Bertrand *et al*., 2002; Lekven *et al*., 2003; Buckles *et al*., 2004; Wilson and Houart, 2004; Figure 3). In addition to neurogenesis, local signals control the expression of other classes of genes, providing an identity, that is, a restricted fate of differentiation, to the precursor born in one of these neuromeres, and linking neurogenesis to neural differentiation.

Thus, there is a stage during neural development where the segmental or neuromeric organization of the neural tube is easy to recognize. During this stage, the acquisition of positional identity of neuroblasts under the control of signaling coordinates and specific genetic networks takes place. The important aspect of this paradigm is that neural determination and subsequent differentiation is acquired in a strict spatially defined manner, similar to the so-called phylotypic stage in the hourglass model of development by Duboule (1994) and Raff (1996). Before this phylotypic step, gastrulation and neurulation can significantly vary from one vertebrate species to another, provided they lead to a neural tube that is spatially organized into morphogenetic units, which may be neuromeres or finer units. The direct consequence of the phylotypic gene regulations is generation and differentiation of neural precursors in a time-and-space-defined manner within morphogenetic units. Neural precursors produced in a given morphogenetic unit will then proliferate, migrate, and establish connections with other brain parts, often losing their original spatial distribution. These events may differ from one species to another and render the neuromeric organization difficult to recognize at later stages of development or in adults.

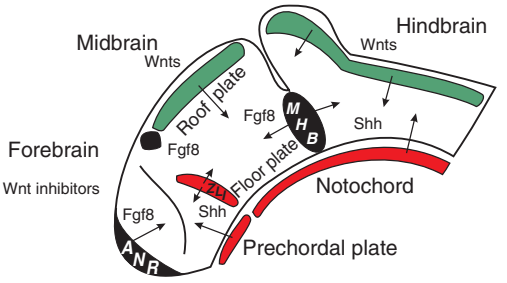


Figure 3 Schematic lateral view of early zebra fish brain with some major signaling centers indicated. Their activity underlies dorsoventral and anteroposterior regionalization leading to the formation of a Bauplan during the phylotypic brain development stage. ANR, anterior neural ridge; MHB, midbrain–hindbrain boundary; ZLI, zona limitans intrathalamica. Adapted from Buckles, G. R. Thorpe, C. J., Ramel, M.-C., and Lekven, A. C. 2004. Combinatorial Wnt control of zebrafish midbrain–hindbrain boundary formation. *Mech. Dev.* 121, 437–447 and Wilson, S. W. and Houart, C. 2004. Early steps in the development of the forebrain. *Dev. Cell* 6, 167–181.

Thus, this central nervous phylotypic stage results from tight constraints of neural differentiation, and certainly accounts for the striking conservation of the neuromeric organization of the neural tube in vertebrates, providing an easily recognizable framework for brain comparisons between species. From an evolutionary point of view, the phylotypic stage is also the developmental master theme on which many species-specific brain variations emerge depending on functional adaptation.

The best example of phylotypic structures is found in the neuromeric organization of the vertebrate rhombencephalon. Here, the combined action of proneural genes and positional cues, which depends on the spatially defined expression of *Hox* and *Nkx*-related genes (the so-called *Hox* code for acquiring positional identity), will, for example, specify the identity of motor neurons in the cranial nerve nuclei and of serotoninergic neurons in the reticular raphe nuclei (Cordes, 2001). In most vertebrates, the pattern of cranial nerves is highly similar, highlighting the power of segmental specification. Still, differences exist, such as the position of the trigeminal and facial nerve nuclei which are not in register with rhombomeres 2 through 4 in lampreys as they are in jawed vertebrates (Murakami *et al*., 2005). This is due to a rostral shift of *Hox3* expression in lampreys, revealing that positional information is retained, but in this case, independent of rhombomere segmentation.

Also in the forebrain, an astonishing degree of similarity of brain patterning has been described between zebra fish, *Xenopus*, and mouse at critical time point (zebra fish: 2–3 days postfertilization, *Xenopus*: stage 48, mouse: embryonic day 12.5/13.5). This morphogenetic pattern nicely corresponds to the differential presence of neurogenic and proneural gene expression, which later affects the differentiation of neurotransmitter phenotypes. For example, in the mammalian brain, γ-aminobutyric acid (GABA)-ergic cells are born and determined in the embryonic ventral subpallium (i.e., the medial ganglionic eminence), the determination of which depends on a genetic pathway that includes the regionalized expression of *Dlx1/2*, *Nkx21* and *Lhx6* and

of the proneural *Mash1* gene. A very similar situation is observed in *Xenopus* and zebra fish (Wullimann *et al.*, 2005; Mueller *et al.*, 2006). In the mouse, a large fraction of these ventrally born GABA neurons later migrate tangentially into the pallium, that is, the future cortex (reviewed in Wullimann and Mueller, 2004) where they become interneurons. In the zebra fish, migration of GABA neurons into the pallium likely also occurs (Mueller *et al.*, 2006), but the adult arrangement of interneurons in the zebra fish pallium remains to be described precisely. Interestingly, the absence of *Nkx2.1* expression in the lamprey subpallium correlates with the absence of GABA cells in the pallium (Murakami *et al.*, 2005). In contrast, the determination and differentiation of glutamatergic cells of the pallium (cortex) depends on the concerted activity of *Neurogenin1* and *NeuroD* in areas where *Pax6*, *Emx1/2*, *Tbr1*, and *Lhx9* are expressed in a regionalized manner (Wullimann and Mueller, 2004, and references therein).

These observations suggest that there are indeed strictly defined temporal and spatial requirements for a given neuronal phenotype to be differentiated (e.g., for GABAergic neurons), reflected in the strict spatiotemporal patterning of gene expression at the phylotypic stage. It is also the stage when proliferation of neural precursors is the most tightly regulated, affecting thereby relative final size of brain areas and total brain size as a consequence. Assuming comparable changes in cell cycle lengths during development of different species, the later the cell divisions stop the larger the brain will be. This requires that sufficient energy is produced by the organism to support brain metabolism, linking brain size to body size.

2.03.2.3 Brain Weight–Body Weight Data

How do vertebrate brain weights compare with a *Scala naturae* concept? All organs increase in size/weight with increase in body size. The brain does so at a coefficient of 0.66 of the body weight on average. In other words, the steepness of the regression line reveals a negative allometric growth of brain weight compared with body weight (van Dongen, 1998; Jerison, 2001). A common measure for relative brain size (degree of encephalization) is real brain weight over the expected brain weight. This value is 1.0 if a brain weight lies on the regression line. If the brain is twice the expected size, the value would be 2.0. In such a comparison, humans amount to 6.45, whereas the trout is at a value of 1.2. This may appear supportive of a linear increase in relative brain weight from fish to human and of *Scala naturae*. However, the goldfish amounts to 2.2 and the African electric fish *Gnathonemus petersii* reaches 5.5 (calculated based on brain weights/body weights given in Nilsson, 1996) Furthermore, within mammals, independent brain enlargement is seen in primates, carnivores, whales, and elephants (van Dongen, 1998). This clearly shows that there is independent increase in relative brain weight between and also within major vertebrate taxa and is in accord with a bush-like evolution of relative brain weight as discussed above.

Another way of looking at relative brain weight is to construct minimum convex polygons (Figure 4). It is true that, in such a comparison, the mammalian and bird polygons lie above agnathans, amphibians, and reptiles, that is, mammalian and avian brains are always much larger for a given body weight compared to these other three groups (Jerison, 2001) Interestingly, fossil information (endocasts) shows an increase in relative brain weight during geological times (i.e., tertiary) in mammals, but not in diapsid reptiles (van Dongen, 1998). A similar tendency of brain enlargement is seen in fossil versus extant birds. Thus, based on their evolutionary history of brain enlargement, mammals and birds might be considered 'higher' vertebrates. However, a large fraction of cartilaginous fish and certain teleost (i.e., mormyrid) brain weights overlap with the avian and mammalian polygons. The fact that the mormyrid data have only recently been added (Jerison, 2001) furthermore indicates that the values for ray-finned fishes may not be representative in the face of their large species number (see the discussion above). The fact that many extant cartilaginous fishes as well as at least the mormyrid fishes among ray-finned fishes lie in a range as to overlap with the mammalian or bird polygons also shows that there is no rule that keeps early diverging vertebrate groups intrinsically constrained by way of their systematic alliance to have small relative brain weights. Moreover, in contrast to the general belief that all brain parts increase to the same degree, relative brain enlargement in cartilaginous fishes and teleosts may largely be accounted for by disproportional growth of the cerebellum.

Consequently, these big-brained sharks, rays, and mormyrids would have to be considered as 'higher' vertebrates, together with mammals and birds, which is not useful. Clearly, brain weight–body weight data do not support the common ladder notion of *Scala naturae*, but rather show that independent cases of relative brain enlargement did occur in mammals, birds, cartilaginous fishes, and ray-finned fishes – several times in each –and, thus, represent cases of homoplastic (convergent) evolution of brain enlargement.

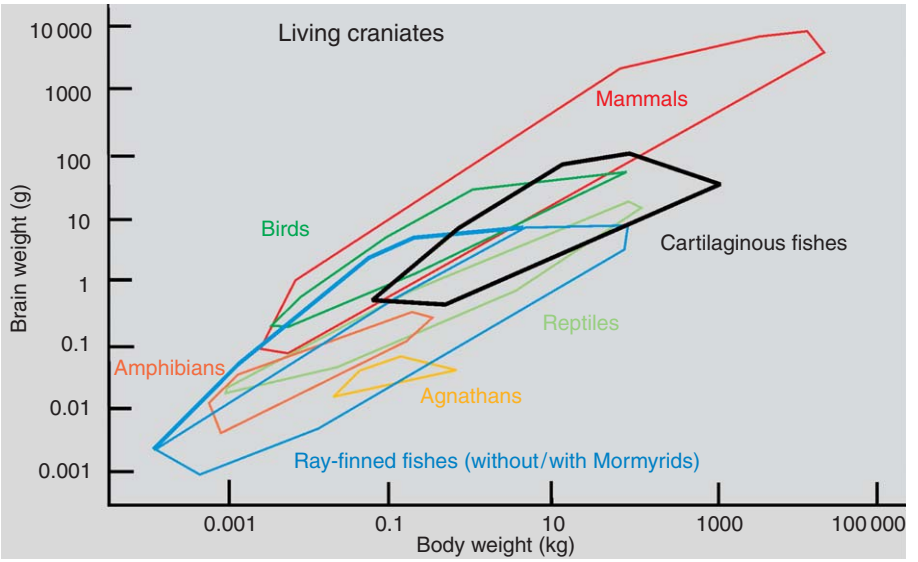


Figure 4 Brain weight/body weight relationships of living craniates. Note that both cartilaginous and ray-finned fish polygons overlap greatly with those of mammals and birds in comparable body size ranges. Adapted from Jerison, H. 2001. The evolution of neural and behavioral complexity. In: Brain Evolution and Cognition (*eds.* G. Roth and M. F. Wullimann), p. 523. Spektrum Akad Verlag/Wiley.

The analysis of environmental factors that may have guided the evolution of relative brain enlargement fits into this picture (van Dongen, 1998). The need for improved sensory and neural processing for finding and discriminating food has been suggested in primates and bats where fruit eaters have larger brains than herbivores/insectivores. Also, socially demanding environments for any given species generally seem to correlate with larger brain size (Striedter, 2005). Alternatively, the high-energy content of certain foods may allow for brain enlargement. Similarly, food-storing birds have a considerably larger hippocampus than closely related birds that do not store food. The degree of precociality at birth in birds – but interestingly not in mammals – is negatively correlated with brain size (van Dongen, 1998). The fact that apparently no general evolutionary factor does account for brain enlargement in mammals and birds, let alone in cartilaginous or ray-finned fishes, is in further support of the convergent nature of brain enlargement in various vertebrate taxa.

2.03.3 Functional Neuroanatomy of Fish Brains

2.03.3.1 How the World and Brain Interconnect: The Peripheral Nervous System

The peripheral nervous system of the head is represented by the cranial nerves. They connect the brain with the sensory and motor periphery and, thus, represent a natural starting point for understanding functional neuroanatomy. As already noted, myxinoids lack terminal, oculomotor, trochlear, and abducens nerves, as well as electrosensory (but not mechanosensory) lateral line nerves, while the situation in lampreys for sensory systems and cranial nerves is similar to gnathostomes in this respect. Therefore, here, we will describe only briefly the ancestral set of cranial nerves and sense organs that defines vertebrates (reviews: agnathans: Braun, 1996; teleosts: Wullimann, 1998; cartilaginous fish: Hofmann, 1999).

The relationship of cranial nerves and brain can only be understood in the larger context of how the vertebrate head is developmentally constructed (Northcutt and Gans, 1983). The interactions of the three embryonic germ layers during neurulation and their anatomical consequences are considerably more complex in the head than in the vertebrate body trunk (see Wilson and Houart, 2004; Butler and Hodos, 2005). For our purpose, it is important to keep in mind that in addition to neural tube (all somato- and visceromotor nerve components) and neural crest (sensory nerve components) – which are involved in spinal nerve development as well – a third set of neuroectodermal structures, namely the placodes, are involved in cranial nerve development. Placodes are embryonic epidermal thickenings representing neurogenic tissues that give rise to most special head sensory organs and – together with the head neural crest – to their innervating sensory ganglia and nerves.

Of the classical twelve cranial nerves recognized in human neuroanatomy, the hypoglossal (XII,

motor innervation of tongue) and spinal accessory nerve (XI, motor innervation of some neck and larynx muscles) are unique to tetrapods. The remaining ten nerves characterize all vertebrates and, thus, are present in lampreys and gnathostome fishes. The olfactory nerve (I) consists of primary sensory cells in the olfactory epithelium with an axon that projects to the olfactory bulb. In contrast, the terminal nerve (0) is formed by ganglion cells that often lie close to the ventral olfactory bulb and send a peripheral dendrite toward the olfactory epithelium and a central axon into the telencephalon beyond the olfactory bulb. The optic nerve originates from ganglion cells of the retina and is, thus, part of the central, not the peripheral, nervous system. The oculomotor (III), trochlear (IV), and abducens (VI) motor nerves innervate the extraocular eye muscles, with the oculomotor nerve including a parasympathetic component controlling pupillary light reflex. Branchiomeric nerves (V, VII, IX, X) are related to the innervation of one or more (only X) branchial arches or their derivatives (Butler and Hodos, 2005). The trigeminal (V) nerve is concerned with somatosensation of face and oral cavity. The facial (VII), glossopharyngeal (IX), and vagal (X) nerves all include a gustatory component innervating taste buds which may also lie outside the oral cavity on the body surface in fishes where they are always innervated by the facial nerve. Some teleosts have separate primary sensory facial and vagal lobes in the medulla oblongata (cf. Figure 2). All branchiomeric nerves in fishes have a motor contribution for innervating the jaw musculature (V), the hyoid arch (VII) or gill arch musculature (pharynx; IX, X), as well as a viscerosensory and parasympathetic component related to the innervation of head glands or viscera. Clearly, the ancestral vertebrate condition involves more than those ten cranial nerves just discussed. The lateral line nerves of vertebrate fishes – as many as six may be ancestral for gnathostomes (Northcutt, 1989) – innervate mechanosensory neuromasts (hair cell receptors) and electroreceptors on the body surface (Bullock *et al.*, 1983). Closely associated developmentally and functionally is the otic nerve (VIII) which innervates the mechanoreceptive hair cells of the labyrinth. Next to the vestibular sense, an auditory component is meanwhile assumed to be ancestral for vertebrates. Northcutt and various co-workers (summary in Northcutt and Bemis, 1993) furthered the comparative and embryological study of placodes, their developmental fate, and adult configuration of cranial nerves which resulted in a new understanding of the vertebrate head and its evolutionary history. This modern view gives a clear definition of sensory cranial nerves including a distinct placodal origin, the resulting peripheral ganglion and sensory receptor structures, and, most importantly, separate primary central nervous projection nuclei. This led to the falsification of the so-called octavolateralis hypothesis, which assumed that lateral line mechanoreceptors on the fish body surface were internalized in evolution into the labyrinth to serve tetrapod auditory function. The related concept of a primary sensory octavolateralis region in fishes where lateral line and otic nerve input forms an overlapping input is also factually false. The ancestral condition for vertebrate fishes is that they have, from dorsal to ventral, three separate sensory medullary columns dedicated to receive segregated lateral line electrosensory, mechanosensory, and otic nerve information (McCormick, 1992).

2.03.3.2 Sensory Systems from Primary Sensory to Higher Order Integrative Centers

There is a great general similarity in the synaptic relay from the primary sensory centers throughout the ascending neuraxis into the subpallium and/or pallium between amniotes and fishes.

2.03.3.2.1 Actinopterygians In teleosts, almost all sensory system pathways have been neuronally traced from primary sensory centers into the telencephalon (for detailed review of original literature, see Wullimann, 1998), which definitely receives largely nonoverlapping information from all sensory systems. Although the homology of sensory pathways between teleosts and tetrapods is not certain in each single case, the degree of similarity is nevertheless of great functional interest. Secondary olfactory input reaches a limited pallial territory in teleosts (in particular the posterior zone of the dorsal telencephalon, Dp, which is considered the homologue of the lateral pallium or olfactory cortex; Figure 5d) as well as most subpallial areas. The teleostean visual system has been described to display a direct retino-thalamofugal and an indirect retino-tecto-thalamofugal system with synaptic relays in the dorsal thalamus. In contrast to amniotes, both teleost visual pathways may be terminating in the subpallium and not in the pallium. However, tectofugal visual information reaches the pallium in certain teleosts via the preglomerular region, a complex of migrated nuclei lateral to the posterior tuberculum. The sensory systems which ascend multisynaptically in the lateral longitudinal fascicle via mesencephalic torus semicircularis and diencephalon to the pallium, that is, audition, lateral line mechanoreception, and electroreception,

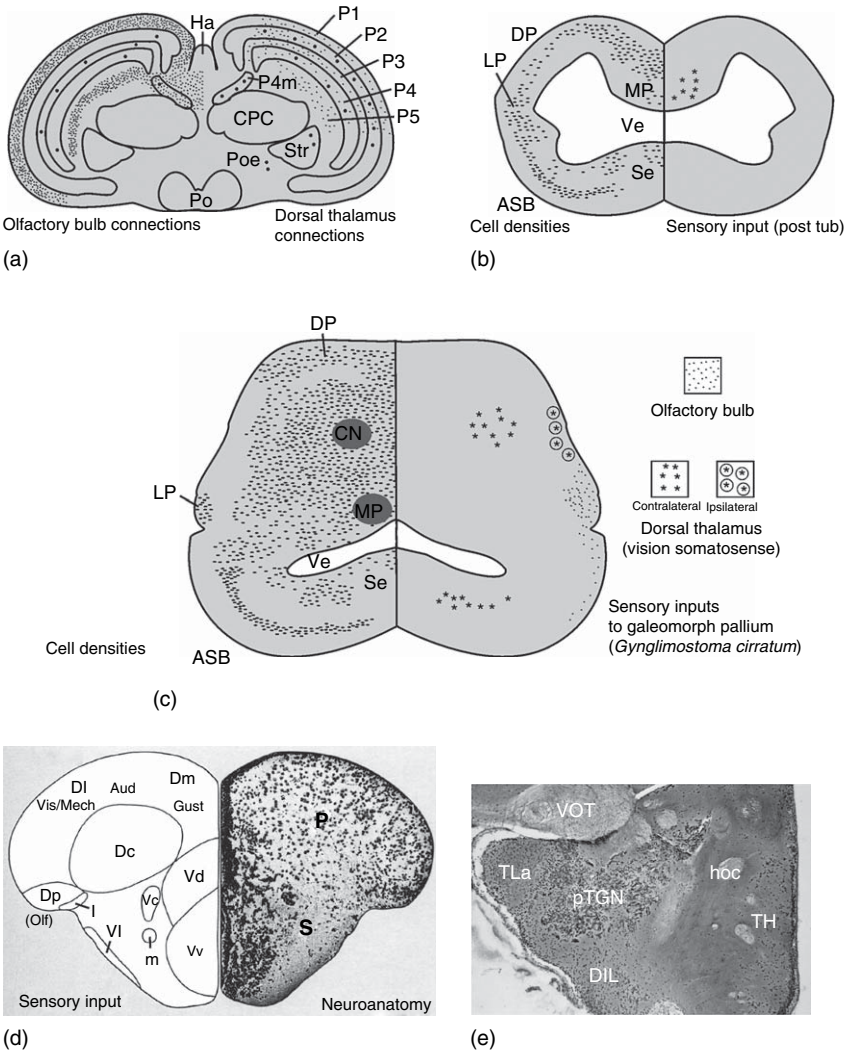


Figure 5 Telencephalic sensory input and output relationships in various fish taxa. a, *Eptatretus stouti* (Pacific hagfish). Left side: Olfactory bulb input to two pallial layers throughout most of their mediolateral extent. Right side: Dorsal thalamic input to all pallial layers. Note also reciprocity of connections with both sources of sensory input. b, *Squalus acanthias* (spiny dogfish, a squalomorph shark). Left side: Medial, dorsal, and lateral pallial divisions dorsal to the subpallium. Right side: Multimodal sensory input from posterior tuberculum to medial pallium. c, *Mustelus canis* (smooth dogfish, a galeomorph shark). Left side: A large pallial central nucleus is recognized in addition to three conventional pallial divisions. Right side: Telencephalic sensory input (established in another galeomorph, the nurse shark *Gynglimostoma cirratum*). d, *Danio rerio* (zebra fish). Right side: Histology of the teleostean pallial dorsal telencephalic area (P) and subpallial ventral telencephalic area (S). Left side: Pallial locations of olfactory bulb (Olf), visual (Vis), lateral line mechanosensory (Mech), auditory (Aud), and gustatory (Gust) inputs (originating in preglomerular nuclei lateral to the posterior tuberculum) as established in various other species (for details, see Wullimann and Mueller, 2004; Northcutt, 2006). e, Photomicrograph of the preglomerular gustatory projection nucleus to the telencephalon in percomorph teleosts (*Hemichromis lifalili*). ASB, area superficialis basalis; CN, central nucleus; CPC, central prosencephalic complex; Dc, Dl, Dm, and Dp, central, lateral, medial, posterior zones of area dorsalis telencephali (pallium); DIL, diffuse nucleus of inferior lobe; DP, dorsal pallium; Ha, habenula; hoc, horizontal commissure; l, lateral olfactory tract; LP, lateral pallium; m, medial olfactory tract; MP, medial pallium; P1–5, pallial layers; P4m, medial part of pallial layer; Po, preoptic region; Poe, external preoptic region; pTGN, preglomerular tertiary gustatory nucleus; Se, septum; Str, striatum; TH, tuberal hypothalamus; TLa, torus lateralis; Vc, Vd, Vl, and Vv, central, dorsal, lateral, ventral nuclei of area ventralis telencephali (subpallium); Ve, ventricle; VOT, ventrolateral optic tract. a, Adapted from Wicht, H. and Northcutt, R. G. 1998. Telencephalic connections in the Pacific hagfish (*Eptatretus stouti*), with special reference to the thalamopallial system. *J. Comp. Neurol.* 395, 245–260. b, Adapted from Northcutt, R. G. 1981. Evolution of the telencephalon in nonmammals. *Ann. Rev. Neurosci.* 4, 301–350; Smeets, W. J. A. J. and Northcutt, R. G. 1987. At least one thalamotelencephalic pathway in cartilaginous fishes projects to the medial pallium. *Neurosci. Lett.* 78, 277–282; Bodznick, D. A. 1991. Elasmobranch vision: Multimodal integration in the brain. *J. Exp. Zool.* Suppl., 108–116. c, Adapted from Ebbesson, S. O. E. 1980. On the organization of the telencephalon in elasmobranchs. In: Comparative Neurology of the Telencephalon (*ed.* S. O. E. Ebbesson), pp. 1–16. Plenum; Luiten, P. G. M. 1981. Two visual pathways to the telencephalon in the nurse shark (*Gynglimostoma cirratum*). II: Ascending thalamo-telencephalic connections. *J. Comp. Neurol.* 196, 539–548; and Bodznick, D. A. 1991. Elasmobranch vision: Multimodal integration in the brain. *J. Exp. Zool.* Suppl., 108–116. e, Adapted from Ahrens, K. and Wullimann, M. F. 2002. Hypothalamic inferior lobe and lateral torus connections in a percomorph teleost, the red cichlid (*Hemichromis lifalili*). *J. Comp. Neurol.* 449, 43–64.

are very comparable to the lateral lemniscal system of tetrapods. Gustation in teleosts reaches the diencephalon and telencephalon via a medullary secondary gustatory nucleus, which is comparable to the parabrachial nuclear region of mammals. Finally, teleosts possess a direct spinal ascending somatosensory system similar to the mammalian anterolateral (protopathic) system in addition to indirect spinal ascending projections which are relayed at the obex level, comparable to the mammalian medial lemniscal (epicritic) system.

A notable difference between teleost and amniote ascending sensory circuitry is that the predominant diencephalic targets of teleostean ascending sensory projections are not in the dorsal thalamus, but in the preglomerular nuclei located in the lateral periphery of the posterior tuberculum (Wullimann, 1998; Northcutt, 2006). Specific sensory preglomerular nuclei exist for the auditory, the lateral line mechanosensory, the electrosensory, and the gustatory systems (Figure 5e). There is also a preglomerular nucleus relaying visual information from tectum to telencephalon, at least in some teleosts. Finally, somatosensory information is relayed in the preglomerular region (Finger, 2000). Furthermore, these preglomerular nuclei – and not the dorsal thalamic ones – provide the major diencephalic input to the pallial zones of the area dorsalis telencephali (Figure 5d, left side). Also, the preglomerular nuclei in teleosts clearly display a higher degree of cytoarchitectonic differentiation and interspecific variation compared to the dorsal thalamus. Thus, the functional similarities between the teleostean preglomerular region and the amniote dorsal thalamus are striking: both make up a large proportion of the diencephalon, are subdivided into many nuclei associated with specific sensory systems, and most of them have reciprocal connections with the pallium.

The teleostean telencephalon is divided into a subpallial ventral telencephalic and a pallial dorsal telencephalic area (shown, for the zebra fish, in Figure 5d, right side). Teleostean pallial masses are topologically different from the usual vertebrate location of medial, dorsal, and lateral pallium resulting from evagination of bilateral telencephalic hemispheres (illustrated, for sharks, in Figure 5b). In teleosts, pallial masses are everted (Nieuwenhuys and Meek, 1990) and a recently proposed theory of partial eversion attempts to explain this topology by a developmental mechanism (Wullimann and Mueller, 2004). For our purpose, it is important to note that the posterior zone of the dorsal telencephalic area (Dp) is the major recipient of secondary olfactory input and is considered as the homologue of the lateral pallium (or olfactory cortex). The pallial lateral zone has been described as a visual area, the lateral, central, and medial zones as lateral line mechanosensory, the lateral and medial zones as auditory, the medial and central zones as somatosensory, and the medial zone as gustatory recipient zones in various teleosts species (for review of original literature, see Wullimann, 1998 and Northcutt, 2006).

2.03.3.2.2 Chondrichthyans Historically, the smell-brain theory suggested that the telencephalon of fishes is largely dominated by secondary olfactory input. Thus, ascending sensory systems reaching the telencephalon were believed to exclusively characterize amniotes, or even mammals only. However, the situation summarized above suggests that there is a pattern of ascending sensory pathways to the telencephalon common to tetrapods and actinopterygians. Since sarcopterygians include tetrapods and are the sistergroup of actinopterygians (Figure 1a), it is pivotal to analyze the information on cartilaginous fishes as an outgroup to reveal the ancestral condition of ascending sensory pathways and centers in gnathostomes.

We will first consider the location of diencephalic sensory targets in cartilaginous fishes (for original literature, see Smeets *et al*., 1983; Wullimann, 1998; Hofmann, 1999). Visual information reaches the dorsal thalamus both directly from the retina and via the optic tectum. It is unclear which diencephalic region is involved in chondrichthyan audition, although autoradiographic deoxyglucose data suggest that it is the dorsal thalamus. Cartilaginous fishes also have a lateral lemniscal system. The ascending lateral line mechanosensory information reaches the dorsal thalamus, as well as the region lateral to the posterior tuberculum. Electroreception, on the other hand, does not reach the dorsal thalamus, but is represented in a ventral nucleus, lateral to the posterior tuberculum and in a hypothalamic nucleus, and both nuclei project to the telencephalon (Fiebig and Bleckmann, 1989). Further, directly ascending spinal somatosensory pathways exist up to the dorsal thalamus in chondrichthyans, though the presence of an indirect somatosensory system relayed at the obex level is unclear in these fishes. Ascending gustatory pathways have not been investigated in cartilaginous fishes.

These data in cartilaginous fishes suggest that a dual innervation of the diencephalon (dorsal thalamus/posterior tubercular region) by at least some ascending sensory systems is the ancestral pattern

for gnathostomes. Furthermore, it may be a gnathostome plesiomorphy that hair cell sensory organs in the labyrinth (audition, vestibular sense) are represented in the dorsal thalamus and the remaining hair cell sensory organs (mechanoreception, electroreception) are present in the posterior tubercular region. If so, the evolutionary loss of the latter sensory systems in amniotes may directly explain the dominance of the dorsal thalamus as the diencephalic sensory region in amniotes.

We turn now to the question where sensory systems are represented in the chondrichthyan telencephalon. Cartilaginous fish display evaginated telencephalic hemispheres with medial, dorsal, and lateral pallial divisions located dorsal to the subpallium (illustrated, in the spiny dogfish, by Northcutt, 1981; Figure 5b, left side). As noted above, many cartilaginous fish species have relatively large brains (Figures 1a and 4). In fact, galeomorph sharks and myliobatiforms (stingrays) among batoids show independent brain enlargement, while holocephalans, squatinomorphs, and squalomorph sharks, as well as most skates and rays other than stingrays, remain modest in brain size (Northcutt, 1978; cf. Figures 1a, 5b, and 5c). Apart from the cerebellum (compare *Squalus* and *Mustelus* in Figure 1a), particularly the telencephalon is also enlarged in these groups. Galeomorph sharks (e.g., *Mustelus canis*, the smooth dogfish; Figure 1a) display for example a conspicuous large central nucleus in the dorsal pallium (Figure 5c). Pioneer discoveries by Ebbesson and co-workers (summarized in Ebbesson, 1980) revealed that the telencephalon of the (galeomorph) nurse shark (*Gynglimostoma cirratum*) receives only very restricted secondary olfactory projections from the olfactory bulb to pallial (lateral pallium) and subpallial territories (Figure 5c) and, furthermore, that the nurse shark central pallial nucleus receives substantial contralateral and the lateral dorsal pallium receives ispilateral dorsal thalamic input (unspecified modality). Later, the central pallial nucleus of the nurse shark has been demonstrated to be recipient of a retino-thalamofugal and a retino-tecto-thalamofugal system (Luiten, 1981). Electrophysiological evidence also indicates that visual, somatosensory, and lateral line information is processed in the nurse shark central pallial nucleus (Bodznick, 1991). Furthermore, the medial pallium of the squalomorph spiny dogfish (*Squalus acanthias*) also receives dorsal thalamic as well as posterior tubercular inputs and it has been identified electrophysiologically as a multisensory region (vision, electrosense; Figure 5b right side, Smeets and Northcutt, 1987; Bodznick, 1991). These findings falsify the smell-brain theory because they show that the ancestral situation for gnathostome vertebrates is already characterized by ascending pathways of most, if not all, sensory systems reaching the telencephalon.

2.03.3.2.3 Agnathans Finally, turning to petromyzontids and myxinoids, we shall focus on the forebrain (original literature cited in Braun, 1996; Wicht, 1996). Both subpallial and pallial divisions may be recognized in the myxinoid telencephalon (*Eptatretus stouti*; Figures 1a and 5a). However, the myxinoid pallium apparently does not show three pallial divisions typical of gnathostomes (see the discussion above), but is rather homogeneously organized as a cortex, exhibiting five distinct neuronal layers throughout (Wicht and Northcutt, 1998). Olfactory bulb input covers most of the mediolateral extent of the hagfish pallium, but this input remains restricted to two pallial layers (P1, P5; Figure 5a). Secondary olfactory projections are also extensive – although to a lesser degree – in the lamprey pallium (Polenova and Vesselkin, 1993). However, all hagfish pallial layers receive additional dorsal thalamic input (Wicht and Northcutt, 1998). Furthermore, there are reciprocal connections both with the olfactory bulb and, importantly, with the dorsal thalamus. Also, the lamprey pallium receives dorsal thalamic input (Polenova and Vesselkin, 1993). An outgroup comparison of these findings may indicate that early vertebrates and craniates possessed a more olfactory dominated telencephalon or pallium than early gnathostome vertebrates. However, this is not to revive the smell-brain theory for two reasons. First, extant agnathans are very different from their ancestors (Carroll, 1988) and their life habits are seemingly very specialized for olfactory orientation, possibly representing later adaptive specializations. Second, and more importantly, the fact that diencephalic sensory input reaches the pallium in both lampreys and myxinoids demonstrates that, also in these highly olfactory guided animals, the telencephalon (and pallium) is not exclusively of olfactory nature as the smell-brain theory would predict.

2.03.3.3 Integrative and Motor Systems

Regarding the functional organization of two additional major integrative centers next to the telencephalon, namely optic tectum and cerebellum, surprising similarity is seen between gnathostome fishes and tetrapods. Also, both extant agnathan groups have an optic tectum that shares various inputs and outputs with those of gnathostomes. It

is beyond the scope of this contribution to review this information (for details on all groups, see Nieuwenhuys *et al*., 1998; for cartilaginous fishes, see Northcutt, 1978; Smeets *et al*., 1983; Hofmann, 1999; for teleosts, see Wullimann, 1998). The cytoarchitectonic and modular organization of the craniate optic tectum, its segregated multimodal input, and the topographical representation of this input and output to the reticular formation provide very likely an ancestral neuronal machinery apparently exquisitely designed for integrative orientation tasks, such as object identification and location, and coordinated motor control.

As noted above, a very rudimentary cerebellum may be identified in lampreys, but not in myxinoids. However, gnathostomes clearly have ancestrally a large cerebellum that exhibits the typical three-layered cortex with comparable cell types and internal circuits. Also the afferent and efferent connections of the chondrichtyan and actinopterygian cerebellum are similar to tetrapods, and this suggests that the cerebellum may have ancestral functions in motor learning and coordination in all gnathostomes (see also Evolution of the Cerebellum).

What remains to be discussed is how the fish brain manages to access the efferent structures, that is, the primary motor nuclei of brain and spinal cord, for displaying a particular behavior. Except for the long palliospinal and palliopontine tracts, which represent independently evolved derived characters of (some) mammals and birds, also the motor (spinal and cranial nerve motor nuclei; see the above discussion) and premotor systems of gnathostome fishes resemble those of tetrapods. As in mammals, descending spinal projections in chondrichthyans (Smeets *et al*., 1983; Cruce *et al*., 1999) and actinopterygians (reviewed in Wullimann, 1998) originate in all divisions of the reticular formation, in the caudal (inferior) raphe region (but not in the superior raphe), in vestibular and sensory trigeminal nuclei, and even in a nucleus ruber. Furthermore, the nucleus of the medial longitudinal fascicle is the locus of an ancestral craniate premotor system descending to medullary and spinal levels. Also, in both agnathan groups, all parts of the reticular formation, as well as vestibular and sensory trigeminal nuclei, give rise to descending spinal projections (Ronan, 1989). However, they both lack a nucleus ruber, likely related to the absence of extremities.

As noted above, both optic tectum and cerebellum act on various premotor centers, in particular onto the reticular formation. However, the forebrain control centers of fish spinal descending systems are less well understood compared to tetrapods. Even more than in the case of the ascending sensory systems, studies in cartilaginous fishes and agnathans are urgently needed in order to understand the ancestral gnathostome and craniate condition of multisynaptically descending (extrapyramidal) systems.

2.03.4 Neurochemical Organization

The adult functional fish brain anatomy of excitatory neurotransmitters, such as glutamate and aspartate, as well as of inhibitory neurotransmitters GABA and glycine, remains to be described in detail, although these are certainly involved in sensory, motor, and higher-order circuitry just discussed. Recent descriptions of GABA systems in lampreys or teleosts basically show a similar degree of conservation as seen in the sensorimotor networks they contribute to form. A more complete picture may be drawn here on modulatory neuroactive substances of the fish brain, such as dopamine, noradrenaline, serotonin, histamine, and acetylcholine, with a particular focus on the involvement of these transmitters in the ascending modulatory systems.

2.03.4.1 Dopaminergic, Noradrenergic, Serotoninergic, Histaminergic, and Cholinergic Systems

The neurons that synthesize monoamines and acetylcholine in the craniate brain include the neuromodulatory systems, which regulate all basic functions of the central nervous system (i.e., many aspects of motor programming and sensory processing; Nieuwenhuys, 1985). They are also the main substrate of more specific behavioral processes such as reward and motivation, awareness, aggression or escape, sleep, or thirst and hunger. Accordingly, modulatory systems send most of their projections anteriorly in a very divergent manner, likely accompanying the evolutionary invention of the telencephalon of craniates. They act in target cells through activation of membrane receptors, which belong to different classes (see Kapsimali *et al*., 2003), and mediate different types of responses in the neural networks they control.

2.03.4.1.1 Actinopterygians Neurons synthesizing catecholamines (mostly dopamine and noradrenaline in craniates) were primarily studied by immunohistochemistry of tyrosine hydroxylase, the rate-limiting enzyme of catecholamine synthesis, and sometimes by direct analysis of dopamine or noradrenaline distribution in various

actinopterygian brains (for reviews, see Kaslin and Panula, 2001; Rink and Wullimann, 2001). Here, we will focus on the model animal zebra fish (*Danio rerio*), as there is complementary immunohistochemical information on the serotoninergic, histaminergic, and cholinergic systems, as well as on critical ascending modulatory forebrain and spinal connections.

The zebra fish noradrenergic system (Kaslin and Panula, 2001; Figure 6a) includes medullary cells close to the viscerosensory column/area postrema (comparable to mammalian groups A1/A2; Smeets and Reiner, 1994) and a locus coeruleus (mammalian A6). The other noradrenergic neurons corresponding to the A3–A5 and A7 groups of mammals are not distinguishable separately in the teleost brain. Neurons of mammalian A1/2 exert local control on the respiratory pacemaker and related functions (e.g., swallowing, response to pH changes). These mixed dopaminergic/noradrenergic neurons are highly conserved in vertebrates and probably induce very similar cell responses in a broad range of species. The axons of the zebra fish locus coeruleus (Figure 7b) mainly project anteriorly, with a smaller contingent going to the hindbrain and spinal cord (Ma, 1997). Anterior projections reach virtually all midbrain and forebrain structures as they do in other gnathostomes. In mammals, the locus coeruleus is crucial for two basic components of behaviors, namely arousal (as opposed to sleep or resting states) and awareness, the latter being necessary for focusing on specific aspects of sensory perceptions. Three receptor classes mediate the effect of noradrenaline, α_1, α_2, and β, each of which comprising typically 3–4 subtypes, highlighting the large variety of cellular actions promoted by this neurotransmitter. No precise distribution of all receptors are available yet in teleosts, although α_{2A} and β_2 receptors seem to be more concentrated in anterior pallial areas and β_1/β_2 receptors in pretectal and cerebellar target areas (Zikopoulos and Dermon, 2005). The remaining tyrosine hydroxylase positive cells rostral to the locus coeruleus in the zebra fish brain are dopaminergic (Ma, 1997; Kaslin and Panula, 2001).

Some dopaminergic cell clusters in the zebra fish posterior tuberculum with a ventral telencephalic (likely striatal) projection were recently proposed to be homologous to the most anterior part (now interpreted as basal diencephalic instead of mesencephalic) of the amniote substantia nigra/ventral tegmental area (mammalian A9/A10; groups 1, 2, 4 of Rink and Wullimann, 2001; Figures 6a and 7a). Posterior tubercular zebra fish dopamine neurons project to telencephalic ventral (septum) and dorsal (basal ganglia) divisions, but some projections from the posterior tuberculum may also reach dorsal (pallial) areas, as is the case in mammals and other amniotes. The action of dopamine on these structures is mediated by classes of receptors (D1 and D2), comprising also 2–4 subtypes. In subpallial structures, two receptor subtypes are mainly found, the D_{1A} and D_2 subtypes, which are located on different populations of neurons (Kapsimali *et al.*, 2003). They likely mediate integration of sensorimotor cues in automatic programs of movements (dorsal striatum), as in other gnathostomes. In contrast, the D_{1B} receptors are clearly present in an area located at the Dm–Dl junction, which has been proposed to be homologous to the mammalian hippocampus (Kapsimali *et al.*, 2000; Salas *et al.*, 2003). In addition, some dopaminergic cells in the zebra fish posterior tubercular area project to the spinal cord (McLean and Fetcho, 2004) and, thus, may correspond to A11. Other diencephalic zebra fish dopamine cells include a ventral thalamic group corresponding to mammalian zona incerta (A13; group 0 of Rink and Wullimann, 2001; Figure 6a). Preoptic zebra fish dopamine cells may partially correspond to group A14, as there are strong preoptic projections to the ventral telencephalon (likely to septum; Rink and Wullimann, 2004). Other preoptic dopamine cells in teleosts project on the GnRH producing cells of the ventral hypothalamus, where they exert a highly variable, mostly inhibitory effect on gametogenesis and ovulation via D_2 receptors (Dufour *et al.*, 2005). A homologue of A15 as seen in some mammals additionally in the preoptic region is doubtful in the zebra fish. As all vertebrates, teleosts possess olfactory bulb (A16) and retinal (A17) dopamine cells, where dopamine acts mostly on D2-like receptors likely to increase discrimination for the two sensory pathways as in amniotes.

Clearly, teleostean posterior tubercular and hypothalamic dopamine populations are more numerous (groups 3, 5–7, 9–11; Figure 6a) than those of amniotes and they include three distinct cell types. Liquor-contacting cells (zebra fish groups 3, 5, 7, 10; Figures 6a and 7a) are absent in mammals (but present in all other vertebrates). Zebra fish large pear-shaped dopamine cells are long-distance projections neurons (see above and Figure 7a). Thus, only small round dopamine cells remain as possible candidates for an A12 (mammalian hypothalamic dopamine cells) homologue. Accordingly, numerous nuclei in the ventral and dorsal hypothalamic regions are targets of dopamine neurons. In some teleosts, the D_{1A} and D_{1B}

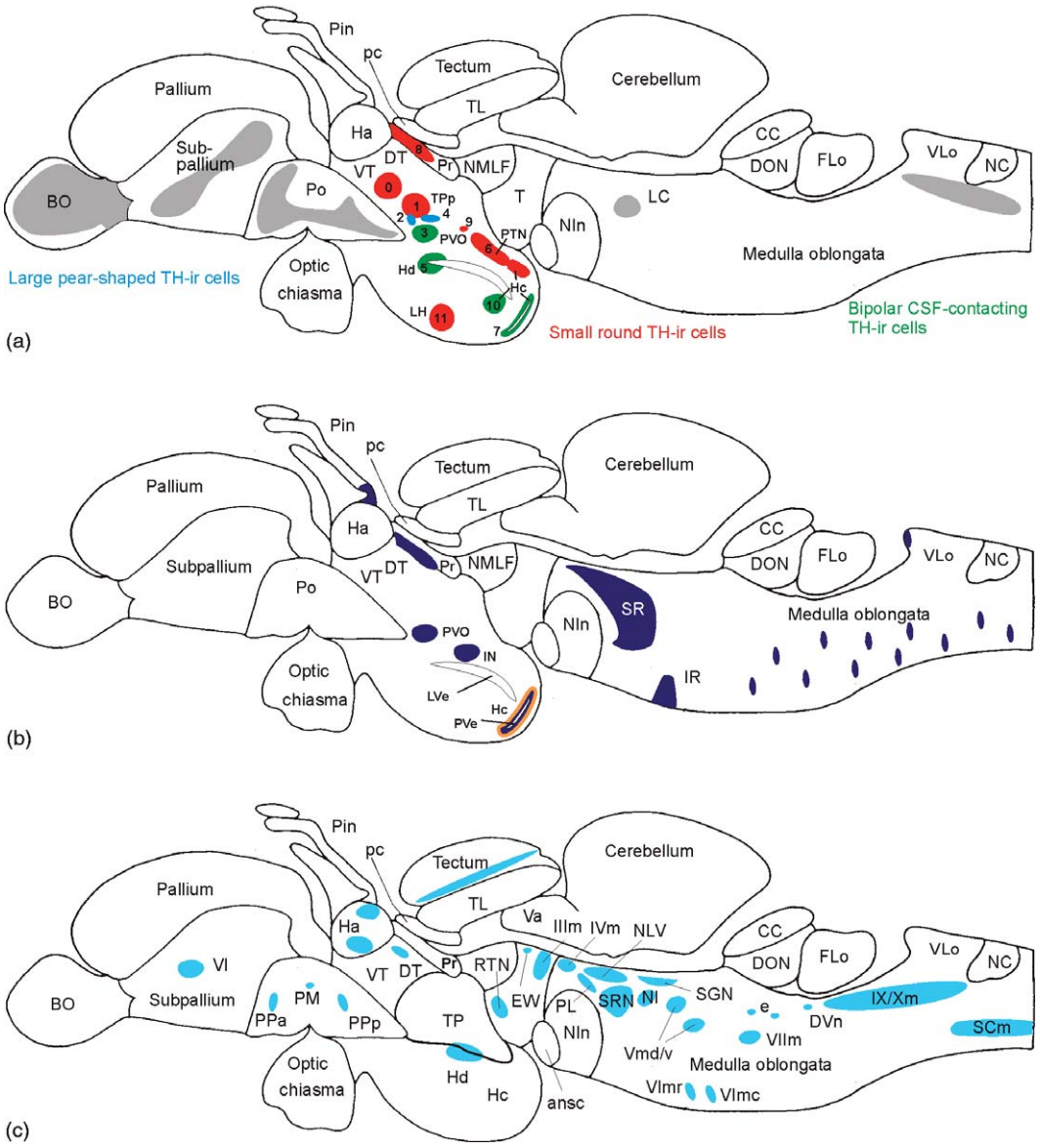


Figure 6 Adult neurochemical organization of the teleost brain (zebra fish). a, Dopaminergic and noradrenergic systems revealed by tyrosine hydroxylase distribution. Noradrenergic cells are only present in locus coeruleus and medulla oblongata. b, Serotoninergic and histaminergic (orange) systems. c, Cholinergic system revealed by choline acetyltransferase distribution. ansc, ansulate commissure; BO, olfactory bulb; CC, cerebellar crest; DON, descending octavolateralis nucleus; DT, dorsal thalamus; DVn, cholinergic neurons associated with DV; e, two medullary populations of efferent octavolateralis cells; EW, Edinger-Westphal nucleus; FLo, facial lobe; Ha, habenula; Hc and Hd, caudal, dorsal periventricular hypothalamic zones; IN, intermediate nucleus (of Rink and Wullimann, 2001); IR, inferior raphe; LC, locus coeruleus; LH, lateral hypothalamus; LVe, lateral recess ventricle; NC, commissural nucleus of Cajal; NI, nucleus isthmi; NIn, interpeduncular nucleus; NMLF, nucleus of medial longitudinal fascicle; NLV, nucleus lateralis valvulae; pc, posterior commissure; Pin, pineal organ; PL, perilemniscal nucleus; PM, magnocellular preoptic nucleus; Po, preoptic region; PPa, anterior part of parvocellular preoptic nucleus; PPp, posterior part of parvocellular preoptic nucleus; Pr, periventricular pretectum; PTN, posterior tubercular nucleus; PVe, posterior recess ventricle; PVO, paraventricular organ; RTN, rostral tegmental nucleus; SCm, spinal cord motoneurons; SGN, secondary gustatory nucleus; SR, superior raphe; SRN, superior reticular nucleus; T, tegmentum; TL, torus longitudinalis; TP, posterior tuberculum; TPp, periventricular nucleus of posterior tuberculum; Va, valvula cerebelli; Vl, lateral nucleus of area ventralis telencephali; VLo, vagal lobe; VT, ventral thalamus (prethalamus); IIIm, oculomotor nerve nucleus; IVm, trochlear nerve motor nucleus; Vmd/v, dorsal/ventral trigeminal nerve motor nucleus; Vlmr, rostral abducens nerve motor nucleus; Vlmc, caudal abducens nerve motor nucleus; VIIm, facial nerve motor nucleus; IX/Xm, glossopharyngeal/vagal nerve motor nucleus. a, Adapted from Ma, P. M. 1997. Catecholaminergic systems in the zebrafish. III: Organization and projection pattern of medullary dopaminergic and noradrenergic neurons. *J. Comp. Neurol.* 381, 411–427; Rink, E. and Wullimann, M. F. 2001. The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). *Brain Res.* 889, 316–330. b, Adapted from Kaslin, J. and Panula, P. 2001. Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (*Danio rerio*). *J. Comp. Neurol.* 440, 342–377. c, Adapted from Mueller, T., Vernier, P., and Wullimann, M. F. 2004. The adult central nervous cholinergic system of a neurogenetic model animal, the zebrafish *Danio rerio. Brain Res.* 1011, 156–169.

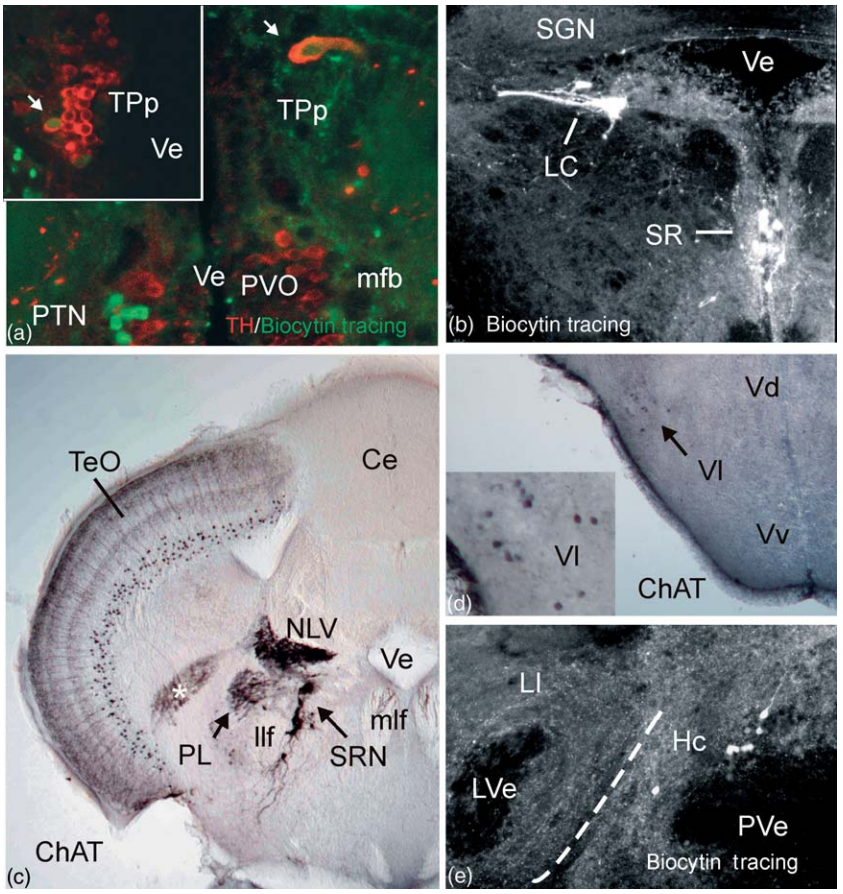


Figure 7 Ascending modulatory systems in the zebra fish brain shown in transverse sections. a, Photomontage shows tyrosine hydroxylase (TH)-containing (i.e., dopaminergic) small (left arrow) and large (right arrow) neurons in periventricular posterior tuberculum which were traced at the same time for projections to ventral telencephalon. b, Telencephalic projection neurons in noradrenergic locus coeruleus and serotoninergic superior raphe. c, Cholinergic neurons in superior reticular nucleus. d, Cholinergic neurons in lateral nucleus of ventral telencephalic area. e, Telencephalic projection neurons in caudal hypothalamus (likely histaminergic). These neurons, as well as neurons in periventricular posterior tuberculum, locus coeruleus, superior raphe, and superior reticular nucleus are all telencephalic projection nuclei (Rink and Wullimann, 2004), but double-label experiments for showing both neurochemical nature and projections of a given cell are only available for TH-containing neurons in the zebra fish. Ce, cerebellum; ChAT, choline acetyl transferase; Hc, caudal periventricular hypothalamus; LC, locus coeruleus; Ll, inferior lobe; llf, lateral longitudinal fascicle; LVe, lateral recess ventricle; mfb, medial forebrain bundle; mlf, medial longitudinal fascicle; NLV, nucleus lateralis valvulae; PL, perilemniscal nucleus; PTN, posterior tubercular nucleus; PVe, posterior recess ventricle; PVO, paraventricular organ; SGN, secondary gustatory nucleus; SR, superior raphe; SRN, superior reticular nucleus; TeO, optic tectum; TPp, periventricular posterior tuberculum; Vd, Vl, and Vv, dorsal, lateral, ventral nuclei of area ventralis telencephali (subpallium); Ve, ventricle. a, Adapted from Rink, E. and Wullimann, M. F. 2001. The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). *Brain Res.* 889, 316–330. c and d, After Mueller, T., Vernier, P., and Wullimann, M. F. 2004. The adult central nervous cholinergic system of a neurogenetic model animal, the zebrafish *Danio rerio*. *Brain Res.* 1011, 156–169.

receptor transcripts have been detected in preoptic nuclei and in the dorsal and ventral periventricular hypothalamic areas. In addition, the D_{1C} receptor, a dopamine receptor subtype which has been lost in mammals, is found in a few restricted areas of the dorsal hypothalamus, including the liquor-contacting cells. Pretectal dopamine cells (group 8; Figure 1a), which project to tectal layers where D_{1A} receptors are found, likely are ancestral for sarco- and actinopterygians, as they are absent in chondrichthyans and agnathans (and, again, absent in mammals). Telencephalic (subpallial) dopamine cells occur ancestrally in agnathans, chondrichthyans, and actinopterygians and are lost in tetrapods, while mammals appear to evolve convergently subpallial dopamine cells.

Turning now to serotoninergic zebra fish brain populations, there are also some striking correspondences to amniotes (Kaslin and Panula, 2001; Figure 6b). However, although the five classes of serotoninergic receptors, which have been isolated in mammals, also exist in teleosts, very few studies have addressed the relationship of serotonin projections and receptor localization. The large

serotoninergic population in the superior raphe, which has a telencephalic projection (Rink and Wullimann, 2004; Figure 7b), is almost certainly homologous to mammalian dorsal and central superior raphe nuclei (B6–8; Nieuwenhuys, 1985). The main target areas of these raphe neurons are probably hypothalamic nuclei where a high amount of receptor binding sites have been evidenced, as well as striatal and pallial areas. Serotonin cells in the zebra fish inferior raphe and in the more caudolaterally located reticular formation (both with spinal projections; Wullimann, 1998) may correspond to mammalian nucleus raphes magnus (B3) and nuclei raphes pallidus/obscurus (B1/2), respectively. There is a distinct population of serotonin cells in the posterior tuberculum, and two more in the hypothalamus of the zebra fish (Figure 1b). The situation in the amphibian posterior tuberculum and hypothalamus is similar (Dicke *et al*., 1997), and sauropsids – but not mammals – also have serotonin cells in the posterior tuberculum (Smeets and Steinbusch, 1988; Challet *et al*., 1996). The zebra fish hypothalamic intermediate nucleus exclusively exhibits serotonin cells, while the paraventricular organ as well as the caudal hypothalamus contain both dopamine and serotonin cells in the zebra fish (Figure 6b). Although absent in amniotes, serotonin cells seen in the teleost pretectum may be ancestral for vertebrates, as they occur in amphibians (Dicke *et al*., 1997), chondrichthyans, and lampreys (see the discussion below), but those in the pineal stalk may be unique to actinopterygians. As in tetrapods, histaminergic cell populations in the zebra fish brain are present exclusively in the most caudal hypothalamus (Kaslin and Panula, 2001; Figures 6b and 7e).

Also the cholinergic system of the zebra fish easily reveals great similarity to the amniote pattern (Figure 6c; for discussion see Mueller *et al*., 2004). First, all motor cranial nerve nuclei (as discussed above) expectedly are cholinergic. Second, there are cholinergic subpallial as well as brainstem neurons possibly corresponding to amniote cholinergic basal forebrain (Figure 7d) and ascending reticular (i.e., pedunculopontine-laterodorsal tegmental; Figure 7c) systems. Also, the zebra fish secondary gustatory nucleus is at least partially cholinergic and projects to the hypothalamus. Furthermore, a cholinergic isthmic nucleus (comparable to the mammalian parabigeminal nucleus) projects to the optic tectum.

2.03.4.1.2 Chondrichthyans The noradrenergic system of cartilaginous fishes also exhibits rhombencephalic groups including cells close to the viscerosensory column and a locus coeruleus (summarized by Smeets and Reiner, 1994). Chondrichthyan dopamine cells are present in olfactory bulb, subpallium, preoptic region, zona incerta, posterior tuberculum, and hypothalamus, where the situation is very comparable to actinopterygians (Smeets and Reiner, 1994). However, elasmobranchs (sharks and skates/rays) have large dopamine cell groups in the mesencephalic tegmentum resembling the amniote substantia nigra/ventral tegmental area. In contrast to all elasmobranchs investigated, *Hydrolagus collei* (a holocephalian, the sistergroup of elasmobranchs) lacks basal mesencephalic dopamine cells (Stuesse and Cruce, 1991); such cells are restriced to the directly adjacent posterior tuberculum (similar to actinopterygians) and this possibly represents the ancestral vertebrate condition (see discussion below). Unfortunately, there is practically no functional information on this system in cartilaginous fishes. Interestingly, chondrichthyans exhibit dopamine cells in the pallium and habenula, features they share (apparently convergently) only with mammals (pallial cells also with some reptiles).

Chondrichthyan serotoninergic cells are abundant in the extensive raphe region and reticular formation (Stuesse *et al*., 1990, 1991; Stuesse and Cruce, 1991). Also, similar to actinopterygians, posterior tuberculum and hypothalamus contain many serotonin cells. The pretectum contains serotonin in some, but not all, chondrichthyan species. There is no report on histamine in chondrichthyans.

The cholinergic system in chondrichthyans (summarized by Rodríguez-Moldes *et al*., 2002) shares many ancestral features with that in actinopterygians, that is, the motor nuclei, potential cholinergic basal forebrain (subpallial) cells, brainstem reticular ascending cholinergic system, an isthmic nucleus, and a possible secondary viscerosensory nucleus. Interestingly, there are pallial cholinergic cells in chondrichthyans, otherwise only seen in mammals.

2.03.4.1.3 Agnathans Noradrenergic cells in lampreys are definitely present in a brainstem group (corresponding to a locus coeruleus) that projects to the telencephalon (nucleus reticularis medius; Pombal *et al*., 1997). The dopamine system of lampreys includes retinal, olfactory bulb, preoptic, and possibly some subpallial cells (Pombal *et al*., 1997). The pattern in the posterior tuberculum and hypothalamus is similar to that in chondrichthyans and actinopterygians. In particular, a projection of dopaminergic posterior tubercular cells to the striatum has been shown, supporting the ancestral presence in vertebrates of a diencephalic homologue of the substantia nigra/ventral tegmental area, as

also seen in some chondrichthyans and all actinopterygians investigated.

In hagfishes, tyrosine hydroxylase containing cells are present in a brainstem group, likely representing a noradrenergic locus coeruleus, as well as in hypothalamic and posterior tubercular positions, presumably representing dopaminergic cells (Wicht and Northcutt, 1994).

The serotoninergic system in lampreys (Pierre *et al.*, 1992) and hagfishes (Kadota, 1991) includes neuronal groups apparently corresponding to raphe nuclei, posterior tubercular, and hypothalamic populations; lampreys also contain a pretectal one, as seen in other vertebrates.

Histaminergic neurons in lampreys have been reported in the hypothalamus and, unlike all other vertebrates, in the midbrain–hindbrain boundary region (Brodin *et al.*, 1990).

Regarding cholinergic systems in the lamprey brain, they also exhibit many ancestral characteristics, that is, motor nuclei of cranial nerves, nucleus isthmi, possibly a secondary viscerosensory population, and even a small cholinergic basal forebrain group (Pombal *et al.*, 2001).

2.03.5 Conclusions

Modern comparative research in developmental biology, functional neuroanatomy, and neurochemical central nervous system organization has fundamentally changed our view of vertebrate brain evolution. The metaphor of the vertebrate brain climbing slowly up the ladder of progress from fish to human has been replaced by the common theme of a largely conservative Bauplan of vertebrate brain organization, upon which uncounted variations are independently generated along various major phylogenetic lines.

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2.04 Evolution of the Amphibian Nervous System

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Glossary

basal optic neuropil (BON) Situated in the ventral tegmentum. It obtains afferents from all quadrants of the retina. Neurons of the BON are sensitive to horizontal and vertical direction of stimulus movement and, together with the thalamus and pretectum, constitute the circuitry for optokinetic responses.

basolateral amygdala It is disputed whether amphibians possess an amygdalar complex homologous to the mammalian basolateral amygdala of pallial origin.

bed nucleus of the stria terminalis (BNST) Part of the extended central amygdala.

central amygdala Occupies the caudal ventral telencephalon around the ventricle medial to the caudal pole of the striatopallidum in frogs. In salamanders, it is located more rostrally extending ventral to the striatopallidum. It is characterized by reciprocal connections with visceral-autonomic brain centers.

cerebellum Composed of the corpus cerebelli, the auricular lobes, and the cerebellar nucleus. A mossy fiber and a climbing fiber system are present. The cerebellar nucleus is considered homologous to the deep cerebellar nuclei of mammals. Like that of other vertebrates, the cerebellum is involved in sensorimotor integration and motor coordination.

dorsal column nucleus (DCN) Situated in the transition zone between the medulla oblongata and medulla spinalis. It receives somatotopically organized input from the skeletal system. The ascending tracts reach ipsi- and/or contralateral mesencephalic and diencephalic structures.

dorsal pallium Forms the dorsal part of the telencephalon and consists of a dorsomedial and a dorsolateral portion. Neurons of both portions display only intratelencephalic projections. It has associative-limbic functions.

dorsal striatopallidum Occupies the ventrolateral wall of the telencephalic hemisphere; its neurons resemble the medium-spiny neurons of the mammalian caudate-putamen. The rostral portion of this complex is now regarded dorsal striatum proper and the caudal portion dorsal pallidum.

hypothalamus Part of the diencephalon consisting of a preoptic and an infundibular region, which

have wide connections with nuclei of the limbic system and brainstem nuclei. It consists of the preoptic region, the partly cholinergic magnocellular preoptic nucleus, the suprachiasmatic nucleus, the posterior entopeduncular nucleus, the periventricular dorsal, ventral and lateral nucleus, the posterior tubercle, and the periventricular organ.

isthmic nucleus Situated in the caudal tegmentum and essential for object localization and selection. It is homologous to the parabigeminal nucleus of mammals. Retinotectal transmission is facilitated by a cholinergic isthmotectal projection, which is topographically organized and in register with the retinal map.

lateral line system Present in fully aquatic species and in species with biphasic lifestyle during larval stages, but absent in direct-developing or life-bearing taxa. It is involved in directional current detection and current-related postural adjustments.

lateral pallium Occupies the dorsolateral portion of the telencephalon. It is divided into a rostral-intermediate, precommissural, and a caudal postcommissural part. Neurons of the former portion project to the medial, dorsal, and ventral pallium and to the main olfactory bulb, while those of the latter portion send their dendrites and axons along the olfactohabenular tract to the dorsal and medial pallium and to the septum.

main olfactory amygdala Region in the ventrolateral part of the caudal pallium dorsolateral to the vomeronasal amygdala. It is connected to olfactory structures and to the hypothalamus.

medial pallium Occupies the dorsomedial portion of the telencephalon. The dorsal portion of the medial pallium is considered homologous to the mammalian Ammon's horn and the ventral portion to the subiculum; a dentate gyrus seems to be absent. It is believed to be involved in learning and memory formation.

motor nuclei Classically divided into viscero-, branchio-, and somatomotor nuclei. Motor pools display a somatotopic organization and form a medial and a lateral column in the spinal cord of most amphibian species.

nucleus accumbens/ventral striatopallidum Found in the rostral ventromedial telencephalon. It extends caudally to what is now considered the ventral pallidum.

nucleus of the diagonal band of broca Situated ventral to the medial septal nucleus and now believed to be part of the medial septal complex.

pallidum The caudal part of the dorsal striatum is now considered the dorsal pallidum. The ventral pallidum is situated in the ventromedial telencephalon. It is a shell-like caudal continuation of the nucleus accumbens/ventral striatum.

parabrachial nuclei The nucleus visceralis secundarius of amphibians is considered homologous to the parabrachial nuclei of amniotes.

pedomorphosis A form of heterochrony, in which traits that characterize larvae or juveniles of ancestral taxa are maintained in the adult stage of descendant taxa. It involves different degrees of retardation, reduction, or absence of traits in otherwise fully developed organisms.

posterior tubercle Situated in the caudal ventral diencephalon. It contains dopaminergic cells and is homologous to the mammalian substantia nigra pars compacta.

preoptic area/region See hypothalamus.

pretectum Transition zone between diencephalon and mesencephalon (also called synencephalon). Deep and laterally migrated neurons are distinguished with reciprocal connections with other visual centers. Neurons are directionally selective and involved in optokinetic nystagmus.

raphe nuclei Situated along the ventral midline of the entire brainstem. They have extensive ascending projections to all parts of the brain. The exact contribution of

the different raphe nuclei for targets in the forebrain is unknown.

reticular formation — Situated in the brainstem and composed of a median, medial, and lateral zone. These zones differ in the distribution of neurotransmitters. Numerous descending pathways converge onto the zones. Nuclei of the amphibian reticular formation are assumed to correspond to that of mammals.

rostral pallium — Occupies the rostral pole of the pallium and projects to all other pallial regions and, like the ventral pallium, to the dorsal edge of the striato-pallidum.

secondary simplification — Arises from pedomorphosis. A mosaic of fully adult, weakly expressed and missing traits appears at terminal ontogenetic stages. Accordingly, brains have fewer cells, a lower degree of morphological differentiation and reduced cellular migration, but retain the plesiomorphic organization found in other vertebrates.

septum — Located between medial pallium and nucleus accumbens. A medial complex including a ventrally situated nucleus of the diagonal band of Broca, a lateral, and a central complex are now distinguished.

solitary tract — Runs inside the dorsolateral medulla oblongata and receives general and gustatory viscerosensory fibers from the IXth and Xth cranial nerves. It is accompanied by the nucleus of the solitary tract.

striatopallial transition area (SPTA) — Located dorsal to the striato-pallidum. It is considered as part of the ventral pallium. Projects to the (lateral) vomeronasal amygdala and hypothalamus.

tectum mesencephali — Laminated structure forming the dorsal midbrain. It is the main center for visual perception and visuomotor functions. It possesses neuronal types with specific connections to the forebrain and/or brainstem. Here, like in amniotes, object recognition is based on population coding and occurs in a parallel-distributed fashion.

tegmentum mesencephali — Forms the ventral mesencephalon and consists of a dorsal and ventral part with a classical distinction of tegmental nuclei comparable to mammals.

thalamus — The dorsal thalamus contains an anterior, central, and posterior periventricular and an anterior and posterior lateral nucleus. Sensory afferents terminate in the ventral thalamus consisting of a periventricular nucleus and a number of migrated nuclei. In contrast to mammals, the dorsal thalamus does not process unimodal sensory (lemnothalamic) information. The anterior dorsal nucleus combines traits of the mammalian anterior, dorsomedial, midline, and intralaminar nuclei. The central dorsal nucleus of amphibians is regarded homologous to the nucleus rotundus of reptiles and birds.

torus semicircularis — Consists of a principal, laminar, and magnocellular nucleus. It is the major audiomotor interface and the center of convergence of ascending auditory, vestibular, somatosensory, and lateral line pathways as well as descending pathways from the forebrain.

ventral pallium — Situated between lateral pallium and striatopallidum and includes the SPTA. It projects to the accessory olfactory bulb, the vomeronasal amygdala and preoptic region, and hypothalamus.

vestibular nuclei — Situated in the medulla oblongata and divided into four nuclei, which receive projections from sensory epithelia of the canal ampullae, utriculus, sacculus, and lagena of the inner ear.

vomeronasal amygdala — Situated in the caudal ventrolateral telencephalon. It is continuous with the SPTA covering the area formerly called 'lateral amygdala' and is characterized by its massive input from the accessory olfactory bulb and its projections to the preoptic area and hypothalamus via the stria terminalis.

2.04.1 Introduction

In this article, we give an overview of the central nervous system (CNS) (see Basic Nervous System Types: One or Many?, Origin and Evolution of the First Nervous System), i.e., spinal cord and brain, of amphibians in a comparative and evolutionary context. A comprehensive description of sense organs and the CNS of amphibians is beyond the scope of this article, and we restrict a more detailed description to those parts of the CNS that are best studied and of greatest interest for a comparative and evolutionary approach, namely (1) the visual system including retina, optic tectum, pretectum, and thalamus; (2) thalamotelencephalic pathways; (3) telencephalic pallial regions; and (4) telencephalic limbic centers including the basal ganglia. For a more extended overview of the amphibian nervous system, the reader is referred to volume 2 of Nieuwenhuys *et al.* (1998). However, in our article, we include substantial data from more recent studies.

2.04.2 Phylogeny of Amphibians

Modern amphibians, the *Lissamphibia*, form the three orders Anura (anurans: frogs and toads; presently 29 families, about 5086 species), Urodela (urodeles: newts and salamanders; 10 families, about 545 species), and Gymnophiona (caecilians; six families, 170 species) (Frost, 1985; Duellman and Trueb, 1986; Amphibiaweb, 2006). Members of the order Anura are distributed worldwide; those of the order Caudata are found in the northern hemisphere of Eurasia as well as in North America, Central America, and the northern part of South America; and the order Gymnophiona is restricted to the tropics and subtropics of the Old and New World.

Most authors now assume that lissamphibians form a monophyletic group, but a minority assumes a polyphyletic origin for the three orders (cf. Pough *et al.*, 2001). It is assumed that the lungfishes (Dipnoi, six species) are the living sister group to tetrapods and the closest nontetrapod relatives of modern amphibians (Zardoya *et al.*, 1998; Tohyama *et al.*, 2000; Brinkmann *et al.*, 2004).

The earliest limbed vertebrates, the labyrinthodontids (stegocephalians), appeared in the Upper Devonian. Living amphibians have ancient roots and each may have originated in the Paleozoic (San Mauro *et al.*, 2005). Different authors consider them to be either a sister group or descendants of the temnospondyls (living about 340 Mya) (Ruta *et al.*, 2003) or the even more ancient microsaurs (Laurin, 1998a, 1998b). Most temnospondyls were clumsy-looking animals, up to several meters long with a thick, scaly skin, but some, the dissorophoids, were small and gracile and thought by some to be ancestors of the lissamphibia. Modern amphibians are mostly small to very small animals with thin, mostly smooth skin allowing cutaneous respiration: hence the name Lissamphibia (i.e., amphibians with smooth skin). They exhibit many additional traits that distinguish them from their paleozoic ancestors; this suggests that they have undergone substantial evolutionary transformation in the process of pedomorphosis, as will be discussed below.

The relationship between the three amphibian orders is still controversial, but most authors adhere to the hypothesis that salamanders and caecilians are more closely related to one another than to frogs (cf. Pough *et al.*, 2001). Despite their presumed monophyly, the three amphibian orders differ greatly in skeletal structure and way of life. While urodeles retained much of the bodily appearance of ancestral amphibians, anurans have a greatly reduced vertebral column and strongly developed hind limbs. Caecilians, finally, evolved a highly ossified skull and lost their limbs. The ancestors of amphibians most probably underwent metamorphosis including an aquatic larval stage, but many taxa from all three orders developed direct development (i.e., loss of a larval stage), and some of them developed viviparity (Duellman and Trueb, 1986).

2.04.3 Structure and Function of the Amphibian CNS

The CNS of amphibians consists of the spinal cord and the brain, which is divided into five parts, i.e., medulla oblongata, cerebellum, mesencephalon, diencephalon, and telencephalon, as indicated in Figure 1.

2.04.3.1 Spinal Cord

2.04.3.1.1 Gross morphology The amphibian spinal cord possesses cervical and lumbar enlargements characteristic of tetrapods. In transverse sections, the gray matter of the spinal cord typically has an H shape in frogs and a compact, oval appearance in salamanders (cf. Figure 2). The frog spinal cord is divided into a dorsal, lateral, central, ventromedial, and ventrolateral field; the spinal cord of salamanders consists of dorsal, intermediate, and ventral zones. In frogs, the dorsal horns are separated by dorsal funiculi; the substantia gelatinosa is difficult to delimit within the dorsal horn. In salamanders, the dorsal horn mainly consists of the

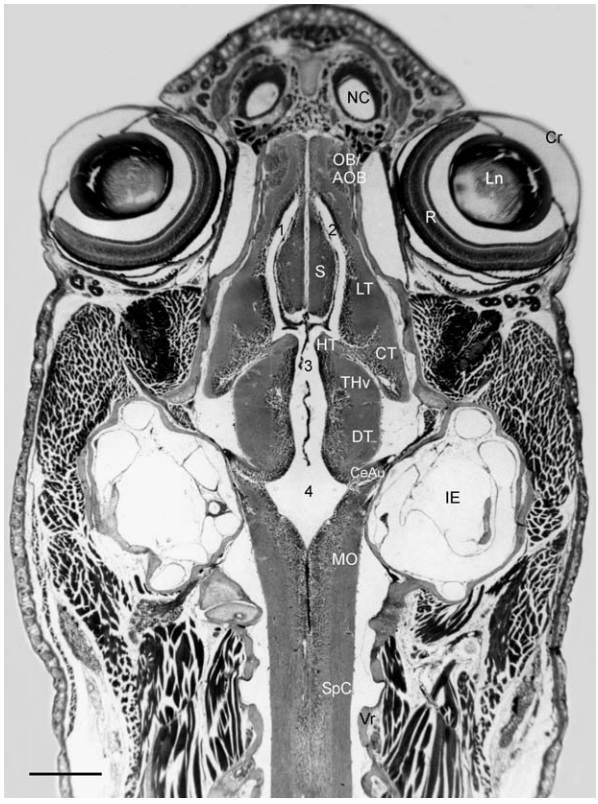

Figure 1 Horizontal section through the head of the salamander *Plethodon dunni* showing the gross anatomy of the brain and spinal cord, eye, nose, and inner ear. 1, 2, 3, 4, ventricles; NC, nasal cavity; OB, olfactory bulb; AOB, accessory olfactory bulb; Cr, cornea; Ln, lens; IE, inner ear; R, retina; S, septum; LT, lateral telencephalon; CT, caudal telencephalon; HT, habenular tract; THv, ventral thalamus; DT, dorsal tegmentum; CeAu, cerebellar auricle; MO, medulla oblongata; SpC, spinal cord; Vr, vertebra. Scale bar: 500 μm.

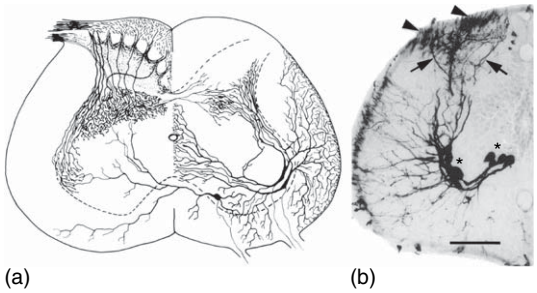

Figure 2 a, Schematic representation of a cross section through the lumbosacral region of the spinal cord of the frog. Motor neurons (right side) belong to the dorsolateral and ventromedial group of spinal motor neurons; their dendrites constitute spatially separate dendritic arrays. On the left side, the projection of dorsal root fibers is illustrated. A lateral bundle of fibers descends into the ventral horn, and establishes contact with motor neurons. The dotted area of the dorsal horn represents the substantia gelatinosa. b, Microphotograph of a transverse section through the spinal cord of *Plethodon jordani* at the level between the third and fourth spinal nerves. After HRP labeling of the superficial ramus of the brachial nerve, motor neurons (asterisks) and sensory fibers are stained. Some primary dendrites (arrows) extend to the dorsal or dorsolateral sensory fiber bundles (arrowheads). Scale bar: 100 μm. a, Reproduced from Frog Neurobiology, 1976, pp. 765–792, Organization of locomotion, Székely, G. and Czeh, G. With kind permission of Springer Science and Business Media. b, From Dicke, U. and Muhlenbrock-Lenter, S. 1998. Primary and secondary somatosensory projections in direct-developing plethodontid salamanders. *J. Morphol.* 238, 307–326.

substantia gelatinosa. The ventral zone comprises the motor neurons, which are arranged in motor columns along the rostrocaudal axis. Ependymal cells line the central canal, and radial glial cells send processes toward the pia. These types of cells are present throughout the CNS; inside the brain, they constitute the layer lining the ventricles.

2.04.3.1.2 Primary afferents Afferent fibers originate from end organs in the skin, joints, and muscles, from free nerve endings of the skeletal system and the inner organs. Dorsal root fibers entering the spinal cord bifurcate into descending and ascending fiber bundles; a lateral division of sensory fibers runs in a bundle comparable to the Lissauer tract of mammals. In the cervical spinal cord, afferent fibers cross the midline and terminate in the corresponding contralateral gray matter. In frogs and salamandrid salamanders, primary afferents ascend to the hindbrain and enter the cerebellum, whereas in plethodontid salamanders they reach the rostral medulla oblongata (Antal *et al.*, 1980; Muñoz *et al.*, 1997; Dicke and Muhlenbrock-Lenter, 1998). A somatotopic arrangement has been described for primary afferents that terminate in the dorsal column nucleus (DCN) situated in the rostral spinal cord (Muñoz *et al.*, 1994a, 1995, 1998). In frogs, primary afferents of the somatosensory system constitute a tract with thick myelinated fibers running in the medial dorsal horn, while thin myelinated fibers run ventrally and laterally to the entrance of the dorsal root. Cutaneous afferent fibers run within the dorsal tract and form a dorsal neuropil, while muscle afferent fibers form a ventral neuropil of the ventral tract, which is contacted by dendrites of motor neurons (Figure 2a). In plethodontid salamanders, both types of afferents form a dorsal and dorsolateral tract and corresponding neuropils, which are both contacted by motor neuron dendrites (Figure 2b).

A variety of neuropeptides involved in transmission of somatosensory and/or nociceptive stimuli (opioids, tachykinins, and FMRFamides) as well as serotonergic, histaminergic, and catecholaminergic fibers have been demonstrated in primary afferent fibers and/or in the dorsal spinal cord of amphibians (Lorez and Kemali, 1981; Danger *et al.*, 1985; Adli

et al., 1988; Salio *et al.*, 2001; Sanchez-Camacho *et al.*, 2001b; Partata *et al.*, 2002; Chartrel *et al.*, 2002; Guedes *et al.*, 2004) (see Somatosensory Specializations in the Nervous Systems of Manatees).

2.04.3.1.3 Autonomic neurons Sympathetic preganglionic somata are situated dorsal to the central canal and form a continuous column between the level of the third and the seventh/eighth spinal nerves; parasympathetic preganglionic neurons are situated in the most caudal part of the spinal cord.

2.04.3.1.4 Motor neurons In most amphibians, motor neurons are arranged in a medial and a lateral column (Matesz and Székely, 1978; Wake *et al.*, 1988; Kim and Hetherington, 1993) (Figures 2, 3d, and 3e). Among salamanders, interspecific differences exist; in bolitoglossines, for example, a clear distinction of the two motor columns is absent (Figures 3e and 3h, right). The medial motor column consists of pear-shaped cells and the lateral one of spindle-shaped cells. Primary dendrites of the latter motor neurons extend to the dorsal horn and overlap with primary afferent fibers and their neuropils. Within the motor columns, motor pools innervating different muscles considerably overlap in the rostrocaudal axis and in the transverse plane. Nevertheless, motor pools show a somatotopic organization in the sense that more caudally located motor neurons innervate more distally located limb muscles. Spinal circuits form building blocks for movement construction and have been described as isometric force fields. During limb behavior, motor elements are combined in chains and in combination contingent on the interaction of feedback and central motor programs (Giszter *et al.*, 1993; Kargo and Giszter, 2000).

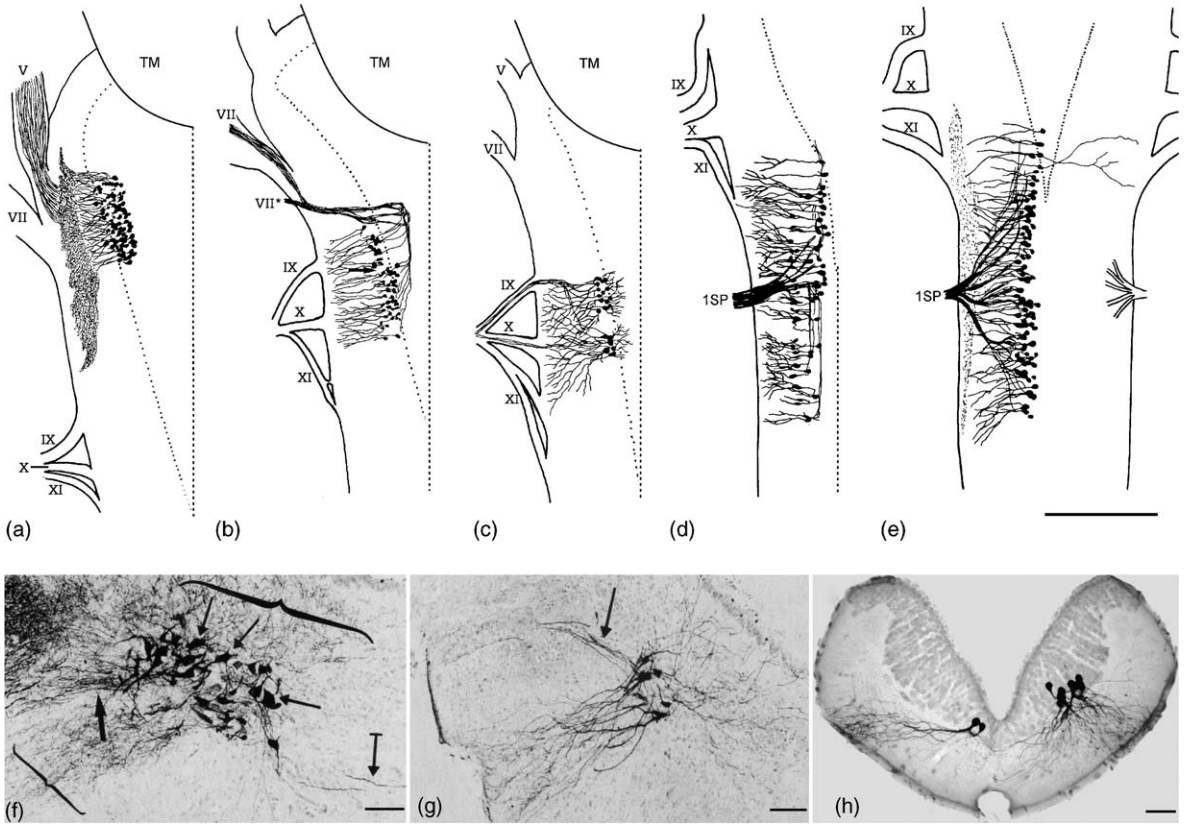

Figure 3 a–e, Camera lucida reconstruction of motor nuclei of salamanders after application of horseradish peroxidase (HRP) to the nerve stumps of the trigeminal (a) facial; (b) glossopharyngeal; (c) cranial nerves, the hypoglossal/first spinal; (d, e) in the species *Plethodon jordani* (a, c), *Salamandra salamandra* (b, d), and *Hydromantes italicus* (e). Note the presence of a medial and lateral motor column in (d) and the absence of a lateral motor column in (e). f–h, Microphotographs of transverse sections through motor nuclei. Motor neurons of the trigeminal (f) and facial (g) cranial nerve of *Rana esculenta*, and motor neurons of the Xth cranial nerve (h, left side) and the first spinal nerve (h, right side) of *Plethodon jordani*. Scale bar: 500 μm in a–e and 100 μm in f–h. V, VII, X, XI, XII, cranial nerves; VII*, lateral line nerve; TM, mesencephalic tectum; 1SP, first spinal nerve. a–e, From Roth, G., Rottluff, B., and Linke, R. 1988a. Miniaturization, genome size and the origin of functional constraints in the visual system of salamanders. *Naturwissenschaften* 75, 297–304. f and g, Reproduced from *Adv. Anat. Embryol.*, Vol. 128, 1993, pp. 1–92, The efferent system of cranial nerve nuclei: A comparative neuromorphological study, Székely, G. and Matesz, C. With kind permission of Springer Science and Business Media.

2.04.3.1.5 Secondary projections

2.04.3.1.5.(i) Descending projections from brain centers In all amphibian taxa, extensive descending pathways arise from the reticular formation, the octavolateral area, the locus coeruleus, the laterodorsal tegmental nucleus, the raphe nucleus, and sensory nuclei of the medulla oblongata. Descending projections from the cerebellum, mesencephalon (tectum, torus, and tegmentum), pretectum, posterior tubercle, ventral thalamus, hypothalamus, and the amygdaloid complex were likewise found (ten Donkelaar *et al.*, 1981; Luksch *et al.*, 1998; Dicke, 1999; Roth and Grunwald, 2000; Sanchez-Camacho *et al.*, 2001a, 2001b).

2.04.3.1.5.(ii) Ascending projections of the spinal cord Projections from the spinal cord ascend via the lateral and/or the ventral funiculus to the reticular formation, mesencephalon, and thalamus. Neurons of the DCN and the lateral cervical nucleus (LCN) form a contralaterally ascending ventral tract and an ipsilaterally ascending dorsal tract in frogs and in salamandrid salamanders. The contralateral tract reaches the level of the mesencephalon; the ipsilateral tract terminates at the level of the cerebellum in salamandrids and sparsely innervates mesencephalic and diencephalic structures in frogs. In plethodontid salamanders projections from the DCN and LCN ascend in three tracts, i.e., a contralateral ventral, a contralateral and an ipsilateral lateral one (spinal lemniscus), which ipsi- and contralaterally reach the cerebellum, tegmentum, torus, and tectum in the midbrain, posterior tubercle, pretectum, and ventral thalamus by a substantial number of fibers (Muñoz *et al.*, 1997; Dicke and Muhlenbrock-Lenter, 1998).

2.04.3.2 Medulla Oblongata and Cerebellum

2.04.3.2.1 Medulla oblongata

2.04.3.2.1.(i) The longitudinal zones In amphibians, as in other vertebrates, the medulla oblongata is an anatomically and functionally heterogeneous part of the brain. It contains primary and secondary relay stations for somato- and viscerosensory information as well as sensory input from the inner ear, and – when present – form the lateral line organs and ampullary organs (electroreception). Networks exist for the control of vital body functions such as respiration and blood circulation; the reticular formation is involved in the control of vigilance and attention. Finally, the medulla oblongata is the convergence zone of numerous descending pathways from all parts of the brain (for a synopsis of the sensory and motor cranial nerves and the reticular formation of vertebrates, see Butler and Hodos, 1996).

The division of the medulla oblongata of amphibians into four longitudinal zones is based mainly on the density and arrangement of cell masses and size and shape of somata, and this likewise holds for the division of the reticular formation into median, medial, and lateral zones. The existence of such longitudinal zones was demonstrated by characteristic differences in the distribution of neurotransmitters such as serotonin (raphe nuclei) or noradrenaline (locus coeruleus). Nuclei of the reticular formation were investigated by means of immunohistochemistry and tracer techniques in ranid frogs and assumed to be homologous to the classical distinction of reticular nuclei established in mammals (Marín *et al.*, 1996; Adli *et al.*, 1999; Stuesse *et al.*, 2001; Zhao and Debski, 2005). In salamanders, somata – except for giant cells such as Mauthner neurons – are more or less equal in size and rather evenly distributed throughout the cellular layer. However, tracer and immunohistochemical investigations of viscero- and somatomotor nuclei, sensory afferents, and transmitter-specific nuclei demonstrate that the longitudinal zones in the medulla oblongata of salamanders match those of other amphibian species (Dicke *et al.*, 1997; Landwehr and Dicke, 2005).

2.04.3.2.1.(ii) Motor nuclei The efferent system is situated in the basal plate of the medulla oblongata and consists of somatomotor and branchiomotor nuclei (Roth *et al.*, 1988b; Székely and Matesz, 1993) (Figures 3a–3c and 3f–3h). The hypoglossal nucleus and the motor nuclei innervating the external eye muscles (abducens nucleus, and the trochlear and oculomotor nuclei situated in the mesencephalon) constitute the somatomotor nuclei. Branchiomotor nuclei comprise the trigeminal and facial motor nuclei, the nucleus ambiguus (glossopharyngeal and vagal nucleus), and the accessory nucleus. The basic organization of the frog ambiguus nucleus is comparable to that of the rat, and differences in nuclear organization reflect differences in peripheral structures (Matesz and Székely, 1996). Motor neurons subserving different functions in tongue movements disclose characteristic morphological differences. Motor neurons innervating different groups of muscles involved in the movements of the tongue (protractor, retractor, and inner muscles) could be separated on the basis of the shape of dendritic arborization in the horizontal, frontal, and sagittal planes of the brainstem (Matesz *et al.*, 1999; Birinyi *et al.*, 2004).

2.04.3.2.1.(iii) Primary sensory afferents Cranial nerves V–XII terminate and/or originate in the rhombencephalon. The alar plate receives somatosensory fibers of the head, general visceral sensory and gustatory fibers, as well as afferents from the inner ear and the lateral line system (Fritzsch *et al.*, 1984; Kuruvilla *et al.*, 1985; Roth and Wake, 1985a; Fritzsch, 1989; Muñoz *et al.*, 1994b). The sensory fibers of the trigeminal nerve extend in the descending tract of the trigeminal nerve to the first and second spinal segment, where they cross to the contralateral side. Fibers and collaterals terminate continuously along the tract in an area containing small cells, i.e., the nucleus of the spinal trigeminal tract; gap junctional coupling was observed between fibers of the descending limb and their postsynaptic targets (Bacskai and Matesz, 2002). Primary afferents of the trigeminal nerve also terminate at the level of the obex in the principal sensory nucleus and in the mesencephalic nucleus of the trigeminal nerve. The latter nucleus is situated in the tectum mesencephali and is characterized by large, unipolar somata dispersed in the cellular layers. The solitary tract comprises mainly general and gustatory viscerosensory fibers of the IXth and Xth cranial nerves. This tract extends from the level of the trigeminal motor nucleus to the spinomedullary border and is accompanied by small, densely packed cells of the nucleus of the solitary tract. Afferents of the inner ear terminate at the level of the entrance of the VIIIth nerve in the dorsolateral nucleus, which is the first relay nucleus of the auditory system and homologous to the cochlear nucleus of mammals. Projections from sensory epithelia of the canal ampullae, utriculus, sacculus, and lagena of the inner ear reach the nucleus of the ventral octavus column, which in ranid frogs has been divided into four vestibular nuclei. GABA and glycine are the major inhibitory transmitters of neurons in the vestibular nuclear complex in frogs as well as in mammals (Reichenberger *et al.*, 1997). The lateral line system is present in fully aquatic species and in those with biphasic lifestyle during larval stages; it is lacking in direct-developing or life-bearing taxa. It is involved in both directional current detection and current-related postural adjustments in *Xenopus* (Simmons, 2004). Afferents enter via the VIIth and Xth cranial nerves (also via the Vth cranial nerve in urodeles) and bifurcate into descending and ascending branches within the medulla oblongata; collaterals terminate on a medially situated lateral line (octavolateral) column of cells that accompany the afferent tracts.

2.04.3.2.1.(iv) Afferents from other brain regions In frogs and salamanders, descending projections to the rostral medulla oblongata arise from at least 30 major cell groups situated in the telencephalon, diencephalon, synencephalon, mesencephalon, and cerebellum. The majority of afferent fibers originate from ipsilateral nuclei of these brain parts, and the white matter of the lateral and the medial medulla oblongata is reached by afferent fibers of different brain regions. Main afferents exclusively reaching the lateral white matter comprise fibers of the dorsal and ventral striatum and amygdalar nuclei of the telencephalon, the magnocellular nucleus of the preoptic area, the large neurons of the superficial pretectal nucleus, the mesencephalic nucleus of the trigeminal nerve, the red nucleus, and the cerebellar nucleus, while a substantial number of axons of the dorsal thalamus, lateral posteroventral thalamic nuclei, the dorsal hypothalamus, the nucleus of the longitudinal medial fascicle, and the superior and isthmic reticular nuclei exclusively descend to the medial white matter of the medulla oblongata. The lateral and the medial white matter receive extensive descending projections from the preoptic area, ventral thalamic and lateral posterodorsal thalamic nuclei, the deep pretectal nucleus, the tectum (see Section 2.04.3.4.9.(i)), the torus and tegmental nuclei, and the middle and lateral reticular nucleus. Fewer neurons of the ventral (ventral lateral) pallium, the central thalamic nucleus, the posterior tubercle, the nucleus Darkschewitsch and Edinger–Westphal likewise project to the white matter of the medulla oblongata (Naujoks-Manteuffel *et al.*, 1988; Dicke *et al.*, 1998).

2.04.3.2.1.(v) Ascending pathways of the medulla oblongata The raphe nuclei of the brainstem display extensive ascending projections to all brain parts, although the exact contribution of the different raphe nuclei, using combined immunohistochemistry and tracing, has mainly been studied in the mesencephalon and is lacking for targets in the forebrain. This also holds true for other reticular nuclei of the medulla oblongata (Dicke *et al.*, 1997; Stuesse *et al.*, 2001; Landwehr and Dicke, 2005; Zhao and Debski, 2005).

Second-order projections of the descending trigeminal nucleus reach the cerebellum, ventral mesencephalon, pretectum, and thalamus. The nucleus of the solitary tract projects to the nucleus visceralis secundarius (NVS) situated in the isthmic region and homologous to the parabrachial nucleus of mammals. The NVS/parabrachial nucleus in turn projects to the preoptic area, the amygdala, and ventral pallium (Moreno and González, 2004; Roth *et al.*, 2004). Second-order neurons of the auditory

pathway are situated in the contralateral dorsolateral nucleus, bilaterally in the nucleus of the superior olive and the torus semicircularis. Projections from the superior olive extend to the nucleus of the lateral lemniscus and to the torus, and some axons reach the posterior thalamus. The vestibular input is transmitted onto the vestibular nucleus complex with a remarkable specific convergence pattern, and a number of fundamental organization principles common to most vertebrates is found in the amphibian vestibular system (Straka and Dieringer, 2004). Ascending efferents from vestibular nuclei travel via the medial longitudinal fascicle to the cerebellum and to brainstem nuclei involved in oculomotor function. Projections from the lateral vestibular nucleus interconnect vestibular nuclei and reach tegmental nuclei, the nucleus of medial longitudinal fascicle and the anterior, central, and ventromedial thalamic nuclei (Matesz *et al.*, 2002). Neurons of the lateral line nucleus mainly project to the torus; in the aquatic toad *Xenopus*, the principal and magnocellular nuclei of the torus receive their major input from the lateral line nucleus (Edwards and Kelley, 2001).

2.04.3.2.2 Cerebellum Compared to that of most other vertebrates, the amphibian cerebellum is small, but exhibits the basic cerebellar circuitry typical of vertebrates. It is composed of the corpus cerebelli and the auricular lobes. The corpus cerebelli is the central part of the cerebellum and consists of a transverse plate, which contains a molecular and a granular layer; the Purkinje cells are aligned at the boundary between these two layers. The cerebellar nucleus, which is considered homologous to the deep cerebellar nuclei of mammals, is situated ventral to the corpus cerebelli. The granular layer contains the afferent fibers to and efferent fibers from the cerebellum. The mossy fiber-granule cell-parallel fiber system and the climbing fiber system are present and constitute excitatory input onto Purkinje cells. Somata of Purkinje cells and of stellate cells in the molecular layer are immunoreactive for GABA, and most of GABA-positive neurons in the granular layer appear to be Golgi cells. True basket cells are missing, stellate cells are fewer in number, and co-localization of GABA and glycine in Golgi neurons is encountered less frequently in frogs compared with mammals. In the bullfrog, Calbindin immunoreactivity (-ir) was observed in various populations of cells in the auricular lobe and interauricular granular band of the cerebellum, in the cerebellar peduncle, and in a bundle of interauricular commissural fibers. Cells in the granular layer of the ventral part (i.e., corpus cerebelli) of the cerebellar plate as well as fibers in the molecular layer of this region were not immunoreactive (Uray and Gona, 1999). The pattern of calbindin-ir in the auricular lobes and marginal part of the cerebellar plate differs distinctly in its origin, biochemistry, and connectivity from the corpus cerebelli.

The main input comes from the rhombencephalon and comprises fibers of the trigeminal and trochlear nerve, the vestibular nuclear complex, the glossopharyngeal-taste sensory system, the hypoglossal nerve (mediating sensory information of the tongue), the inferior olive, and primary and secondary afferents of the somatosensory system (Antal *et al.*, 1980; Amat *et al.*, 1984; Montgomery, 1988; Anderson and Nishikawa, 1997). The efferent cerebellar pathways extend to the lateral medulla oblongata and mainly reach the vestibular complex; they also descend to cervical and lumbar root fibers (Dicke *et al.*, 1998; Bacskai and Matesz, 2002). A small, distinct projection also reaches the ventral tegmentum at the level of the oculomotor nerve; reciprocal connections between the red nucleus and the cerebellum were described in frogs and salamanders (Montgomery, 1988; Naujoks-Manteuffel *et al.*, 1988; Larson-Prior and Cruce, 1992).

In *Rana pipiens*, a pathway from the hypoglossal motor nuclei to the cerebellar nucleus as well as an afferent projection from the peripheral hypoglossal nerve to the Purkinje cell layer of the cerebellar cortex was demonstrated by Anderson (2001). Anatomical convergence of these pathways in the medial reticular formation and a reciprocal connection between the trigeminal motor nuclei and the cerebellar nuclei as well as the medulla appear to be the anatomical basis for feeding reflex modulation. The neuronal circuitry for optokinetic responses includes both visual centers (thalamus, pretectum, BON) and the auricular lobe of the cerebellum (Fite *et al.*, 1992). In general, the cerebellum of amphibians, like that of other vertebrates, appears to be involved in sensorimotor integration and motor coordination.

2.04.3.3 Mesencephalon

2.04.3.3.1 Isthmic region and tegmentum The isthmic region (Figures 13c and 13d) is separated from the tegmentum by the sulcus isthmi, and the isthmic nucleus is situated ventral to the dorsocaudal end of the tegmentum, immediately rostral to the cerebellar corpus. The isthmic nucleus is a compact prominent nucleus; its dendrites extend laterally and form a conspicuous dendritic neuropil (see below for a more detailed description). The nucleus

visceralis secundarius, considered homologous to the parabrachial nucleus, is found dorsal to the isthmic nucleus, and neurons of the cholinergic laterodorsal tegmental nucleus are situated ventral to the nucleus isthmi (Marín *et al.*, 1997d). Neurons of the noradrenergic locus coeruleus are dispersed medially, ventrally, and/or caudally to the isthmic nucleus (Marín *et al.*, 1996). They have long processes directed ventrally or ventrolaterally and arborizing in the lateral reticular formation.

The tegmentum is divided into a dorsal and ventral tegmentum. The nucleus of the medial longitudinal fascicle is situated in the rostral dorsal tegmentum, while the dorsal tegmental nucleus is found throughout the rostrocaudal extent. The ventral tegmentum includes the oculomotor and trochlear motor nucleus and the accessory oculomotor nucleus Edinger–Westphal. The pedunculopontine tegmental nucleus is situated at the border of the dorsal and ventral tegmentum; its cholinergic part is present in frogs and plethodontid salamanders, but is absent in salamandrid salamanders (frogs and salamandrids: Marín *et al.*, 1997a; plethodontids: U. Dicke, unpublished data). The ventral tegmental nucleus is bordered by the ventrally located nucleus ruber, and the interpeduncular nucleus is situated in the median basal tegmentum.

The dorsal tegmental and the pedunculopontine tegmental nucleus have reciprocal connections with the tectum; neurons of the nucleus of the medial longitudinal fascicle and the nucleus ruber give rise to descending pathways to the medulla oblongata and rostral spinal cord (Naujoks-Manteuffel *et al.*, 1988; Dicke *et al.*, 1998). The interpeduncular nucleus receives olfactory input via the fasciculus retroflexus that descends from the habenula. Ascending and descending pathways of the brain run in the fiber layer of the dorsal and/or ventral tegmentum, and neurons of tegmental nuclei with their laterally directed dendrites are likely to receive input from a variety of brain regions. In general, the tegmental relay stations are poorly studied even though the tegmentum most likely constitutes a complex anatomical zone of interfaces, where the sensory, motor, and limbic systems of the brain meet.

2.04.3.3.2 Torus semicircularis The torus semicircularis is situated below the tectum. In frogs, it consists of three major auditory nuclei, the principal, laminar, and magnocellular nucleus (Potter, 1965). The laminar nucleus forms nearly a hemisphere and occupies the entire dorsal and rostral surface of the torus bordering the tectal ventricle (Figure 4). The somata of this nucleus are arranged in laminae (hence the name of the nucleus). The principal nucleus is situated caudally and ventrally of the laminar nucleus, which caudoventrally includes the magnocellular nucleus. In *Salamandra salamandra*, the subtectal dorsal tegmentum is divided into a dorsally located torus semicircularis and a ventrally situated dorsal tegmental nucleus. The torus of this species processes auditory and vibratory signals, and the hearing capabilities are comparable to those of anurans with extra-tympanic sound transmission (Manteuffel and Naujoks-Manteuffel, 1990).

The torus semicircularis is the center of convergence of ascending auditory (terminating primarily in the principal nucleus), vestibular (entire torus), somatosensory (lateral laminar nucleus), lateral line (if present, lateral principal nucleus) pathways and descending pathways from the forebrain, i.e., the central, ventromedial, and posterior thalamic nuclei, the anterior entopeduncular nucleus and suprachiasmatic nucleus terminating predominantly in the laminar and principal nucleus (Wilczynski, 1981; Feng and Lin, 1991; Matesz and Kulik, 1996; Edwards and Kelley, 2001). Weak descending afferents originate in the lateral septum and in the caudal striatopallidum (Marín *et al.*, 1997a; Endepols *et al.*, 2005).

Efferents from all toral nuclei run to the tectum, tegmentum, and isthmic nucleus; the principal nucleus projects to the posterior and central dorsal thalamic nuclei and the laminar and magnocellular nucleus to the telencephalon, predominantly to the striatopallidum (primarily the nucleus laminaris). All toral nuclei have descending projections to auditory brainstem nuclei (Neary, 1988; Feng and Lin, 1991; Luksch and Walkowiak, 1998; Endepols and Walkowiak, 2001).

The torus contains numerous neuromodulatory substances such as dopamine, bombesin, noradrenaline, enkephalin, substance P, somatostatin, neuropeptide Y, as well as steroid hormones (Endepols *et al.*, 2000). It contains neurons that either exhibit weak or no tonotopy and either simple or complex tuning curves (Walkowiak, 1980; Feng *et al.*, 1990). Whereas the majority of neurons can be driven by relatively simple auditory stimuli, some of them respond preferentially to complex sounds. In brief, the data underline the essential role of the torus semicircularis as the major audiomotor interface.

2.04.3.4 Neuroanatomy of the Retino-Tecto-Pretectal System

2.04.3.4.1 Retina The retina of amphibians, like that of other vertebrates, exhibits a typical five-layered

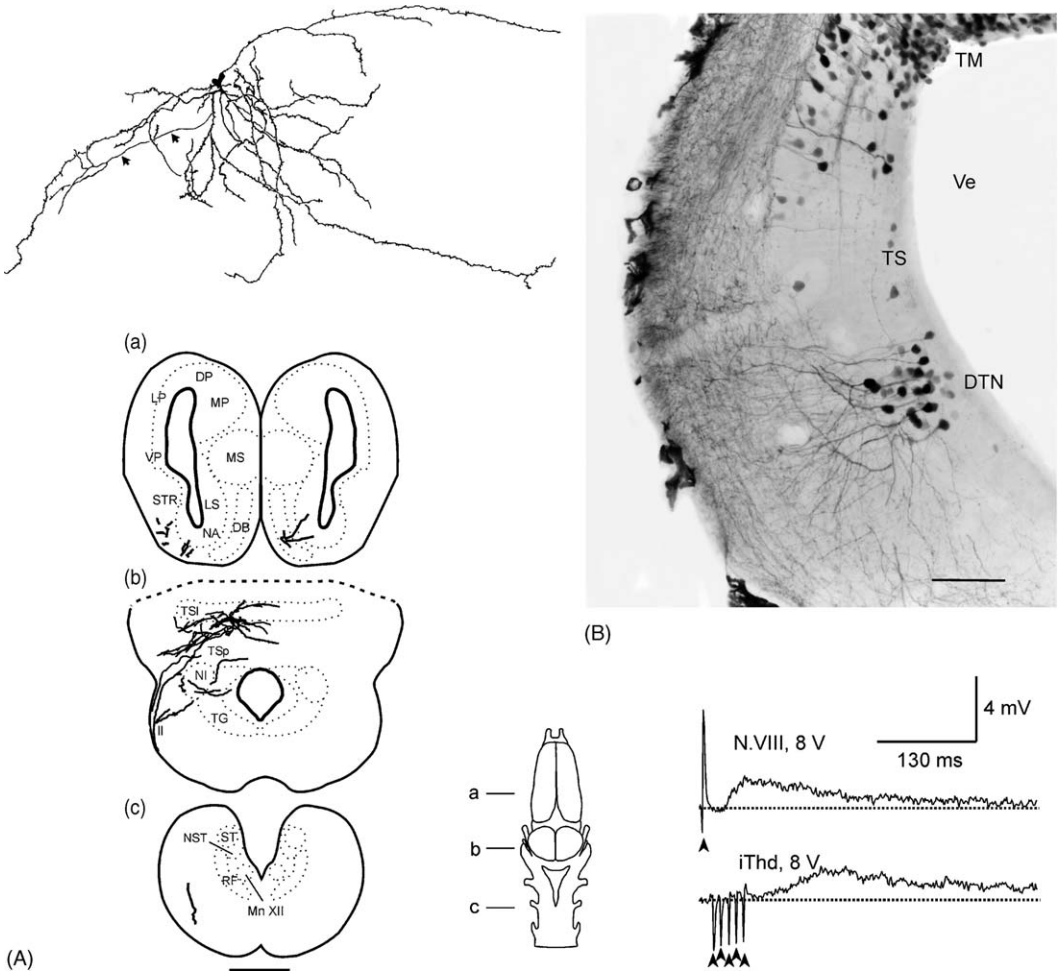

Figure 4 A, Reconstruction of two neurons in the torus of *Discoglossus pictus* recorded and subsequently labeled with neurobiotin. The reconstruction of the dendritic tree is shown at the top left; arrows point to the proximal part of an axon. Scheme of transverse sections (a–c): a, telencephalon showing the termination sites of two axons in its ipsi- and contralateral ventral part; b, location of the two neurons in the caudal laminar nucleus of the torus; the dorsal part of the tectum is cut off (broken outline); c, one axon descends laterally in the fiber layer down to the level of the obex. Responses at single stimulation of the contralateral auditory nerve (VIII) and repetitive stimulation of the ipsilateral dorsal thalamus (iThd) are given at the bottom right. Scale bar: 500 μm. B, Microphotograph of dorsal tegmental neurons of *Plethodon teyahalee* retrogradely labeled after tracer application to the tectum and forming a band extending rostrocaudally throughout the tegmentum. DP, dorsal pallium; LP, lateral pallium; VP, ventral pallium; STR, striatum; NA, nucleus accumbens; DB, diagonal band; LS, lateral septum; MS, medial septum; MP, medial pallium; TSl, laminar nucleus of the torus semicircularis; TSp, principal nucleus of the torus semicircularis; NI, isthmic nucleus; TG, tegmentum; II, XII, cranial nerves; NST, nucleus of the solitary tract; RF, reticular formation; Mn, hypoglossal motor nucleus; TM, mesencephalic tectum; Ve, ventricle; TS, torus semicircularis; DTN, dorsal tegmental nucleus. Scale bar: 100 μm. A, Reproduced from *J. Comp. Physiol. A*, Vol. 186, 2001, pp. 1119–1133, Integration of ascending and descending inputs in the auditory midbrain of a nurons, Endepols, H. and Walkowiak, W. With kind permission of Springer Science and Business Media.

structure. The outer and the inner nuclear layer and the layer of retinal ganglion cells (RGCs) are separated by the outer plexiform layer and the much thicker inner plexiform layer. The two plexiform layers are the main site of synaptic contacts between the five major types of retinal cells (photoreceptors, amacrine cells, bipolar cells, horizontal cells, and ganglion cells). The outer nuclear layer contains the inner segments of the photoreceptors and their nuclei. In most frog and salamander species, the rod nuclei in the outer nuclear layer are aligned at the distal side, and the cone nuclei are more proximal (Gordon and Hood, 1976). This is the contrary of the situation in most other vertebrates, in which the rod nuclei are vitread to the cone somata. Amacrine cells are concentrated at the vitread side of the inner nuclear layer, bipolar cells in the middle, horizontal cells at the sclerad side, and a few displaced RGCs at the vitread side. In most amphibians, the layer of RGCs contains more than one row of cells, and

axons of the RGCs bundle and constitute the optic nerve.

Amphibians have no specialized intraretinal structure like the fovea of primates or birds. However, in the frogs *Hyla raniceps* (Bousfield and Pessoa, 1980), *Heleioporus eyrei* (Dunlop and Beazley, 1981), and *Bufo marinus* (Nguyen and Straznicky, 1989), a streak of high cell density exists in the RGC layer along the nasotemporal meridian of the retina. The same is found in the inner nuclear layer (Zhu *et al.*, 1990) and the outer nuclear layer (Zhang and Straznicky, 1991). The increase in cell density is comparable to that of the visual streak in the reptilian retina (Wong, 1989; Wilhelm and Straznicky, 1992). In salamanders, differences in intraretinal cell density have not been found so far.

In plethodontid salamanders (Linke and Roth, 1989), four types of RGCs have been identified, while in frogs the number of RGCs varies among three major types (with 12 subtypes based on morphology of dendritic trees) in *Xenopus laevis* (Straznicky and Straznicky, 1988) and seven types in *R. pipiens* (Frank and Hollyfield, 1987).

The optic nerve contains myelinated and unmyelinated fibers. The highest number of optic nerve axons (and thus of RGCs) are found in anurans. In *Rana pipiens*, 470 000 fibers were counted, and the lowest number presently known among anurans was found in *X. laevis* with 68 000–80 000 fibers (Maturana, 1959; Dunlop and Beazley, 1984). On average, salamanders have 5–10 times fewer optic fibers. They range from 26 000 in *Batrachoseps attenuatus* (Linke and Roth, 1990) to 75 000 in the salamandrid *Notophthalmus viridescens* (Ball and Dickson, 1983). The percentage of myelination in adult amphibians is low compared to that of mammals; in *X. laevis*, the percentage of myelination is 11% (Dunlop and Beazley, 1984), whereas the lowest percentage is found in the plethodontid salamander, *B. attenuatus*, with less than 1% myelinated fibers (Linke and Roth, 1990).

2.04.3.4.2 Visual afferents to the brain The majority of fibers of the optic nerve cross in the optic chiasm and reach targets in the opposite diencephalon and mesencephalon (frogs: Lázár and Székely, 1969; Fite and Scalia, 1976; Montgomery and Fite, 1989; salamanders: Fritzsch, 1980; Rettig and Roth, 1986). Ipsilaterally projecting RGCs in *R. pipiens* include not more than 2.3% of the overall population in the ganglion cell layer and exist in the monocular as well as binocular parts of the retina (Singman and Scalia, 1990, 1991). Within the optic chiasm, a sequence of positional transformations occurs that result in the formation of multiple optic pathways (Montgomery *et al.*, 1998).

Staining of retinal afferents in frogs and salamanders reveals four thalamic neuropils: the neuropil of Bellonci (NB), the corpus geniculatum thalamicum (CGT) (Figures 5 and 6), the preoptic area, and the posterior thalamic neuropil. The latter neuropil is divided into a laterally situated pretectal neuropil and a medially situated uncinate field; in frogs the presence of such a division was reported in a study on *Rana* (Fite and Scalia, 1976). In the mesencephalon, the superficial and part of the deeper fiber layers of the tectum receive extensive visual afferents (Figure 7). A smaller number of RGCs projects to the basal optic neuropil (BON) situated in the tegmentum rostral to the root of the third cranial nerve. In salamanders, the neuropil of Bellonci can be clearly divided into a medial part and a lateral part (Figure 6) (Fritzsch, 1980; Rettig and Roth, 1986; Wiggers, 1999), and plethodontid salamanders have a substantial number of ipsilaterally projecting RGCs compared to other urodeles and anurans (cf. Figure 7a).

2.04.3.4.3 Organization of retinal projections In amphibians, the projection of RGCs is topographically organized onto diencephalic and mesencephalic targets. In the diencephalon, the topography of projections appears to differ in frogs and salamanders (Rettig and Roth, 1986; Montgomery and Fite, 1989; Montgomery *et al.*, 1998).

In frogs, contralateral projections of the retina distribute as follows: the anterior CGT, NB, and pretectal neuropils receive afferents from the ventral and nasal quadrants of the retina. Axons from the ventral quadrant terminate in the dorsal and those from the nasal quadrant terminate in the ventral portion of the thalamic targets. In the posterior CGT, NB, and pretectum, retinal axons of the temporal and dorsal quadrants terminate in the dorsal and ventral portion of the targets, respectively. The rostral and central tectum receives retinal afferents from the temporal quadrant, the medial tectum is reached by retinal axons of the ventral quadrant, and afferents from the dorsal and nasal quadrant of the retina project to the lateral and caudal tectum, respectively. The BON obtains afferents from the entire retina; the major retinal projection is contralateral, but a small, ipsilateral component was described in *R. pipiens* (Montgomery *et al.*, 1981). The basal optic root consists of a lateral and a medial fascicle. In *R. pipiens*, the lateral fascicle innervates the entire terminal field of the BON, while the medial fascicle innervates only the central and mediodorsal portions. The ventrolateral portion of the BON is innervated only by the lateral

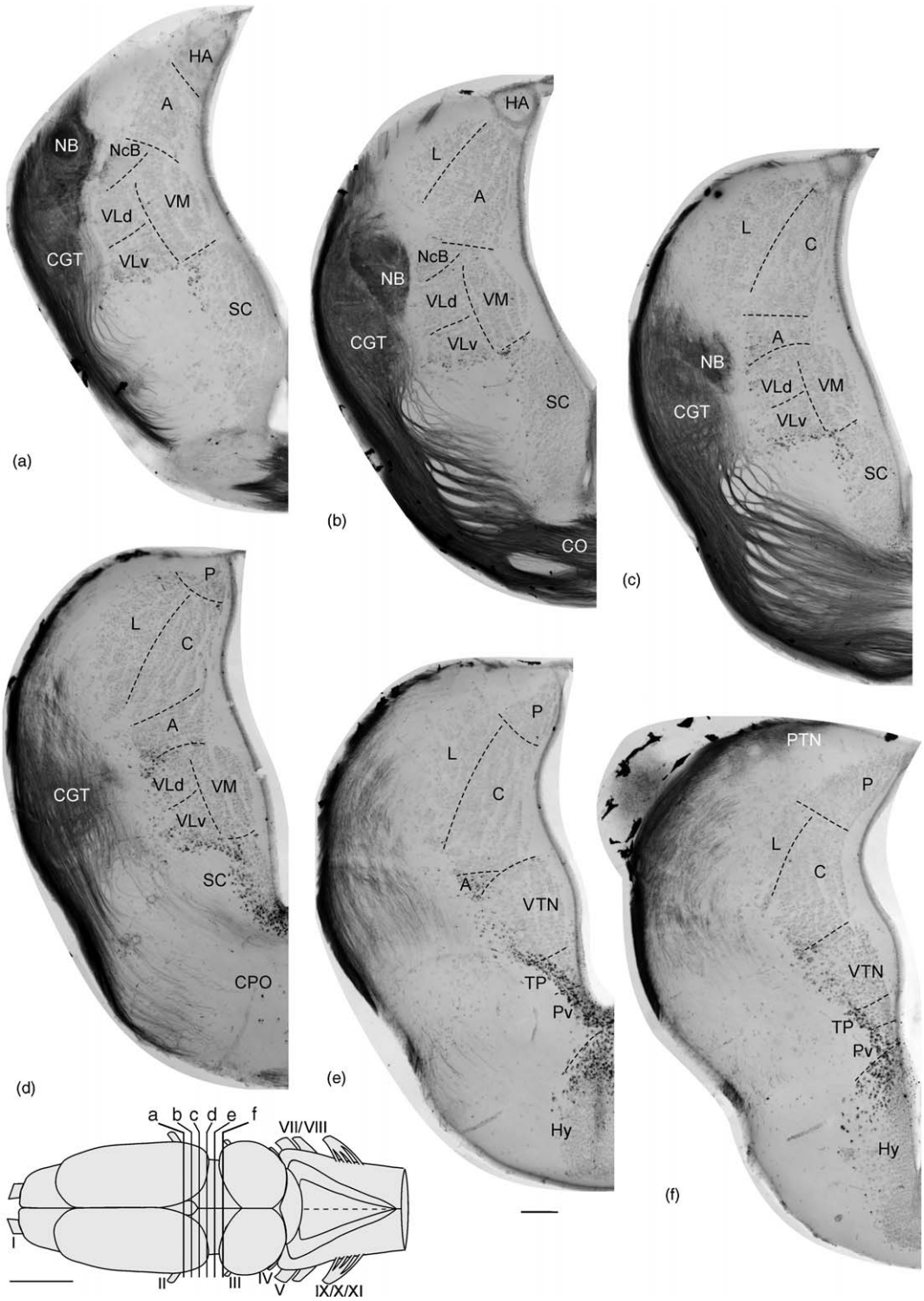

Figure 5 Microphotographs of transverse sections through the diencephalon of *Bombina orientalis* showing retinofugal neuropils (NB and CGT) revealed by anterograde tracing after application of biocytin to the optic nerve. Sites of sections are indicated in the inset. I, II, III, IV, V, VII, VIII, IX, X, XI, cranial nerves; CPO, postoptic commissure; HA, habenula; A, anterior dorsal thalamic nucleus; NB, neuropil of Bellonci; NcB, nucleus of Bellonci; VM, ventromedial thalamic nucleus; VLv, ventral portion of the ventrolateral nucleus; SC, suprachiasmatic nucleus; CGT, corpus geniculatum thalamicum; L, lateral dorsal thalamic nucleus; CO, optic chiasm; C, central dorsal thalamic nucleus; P, posterior dorsal thalamic nucleus; VTN, ventral thalamic nucleus; TP, posterior tubercle; Pv, paraventricular organ; Hy, hypothalamus; VLd, dorsal portion of the ventrolateral nucleus. Scale bar: 100 μm. From Roth, G., Grunwald, W., and Dicke, U. 2003. Morphology, axonal projection pattern and responses to optic nerve stimulation of thalamic neurons in the fire-bellied toad *Bombina orientalis*. *J. Comp. Neurol.* 461, 91–110.

fascicle and the medial region by both fascicles (Fite *et al.*, 1988).

In salamanders, contralateral projections of RGCs are arranged such that afferents from the nasal quadrant terminate in thalamic and pretectal neuropils, while those from the ventral quadrant reach the medial portion of the CGT, lateral NB, and pretectal neuropils. RGCs in the dorsal quadrant of the retina project to the lateral portion of the CGT, lateral NB, and pretectum; in the latter, the entire uncinate field is reached. Afferents originating from RGCs of the temporal retina terminate in the caudal portions of the CGT and lateral NB, in the entire medial NB, the caudal pretectal neuropil and the entire uncinate field. The topography of retinal projections to the contralateral tectum is identical with that found in frogs. Also, the BON receives afferents from all quadrants of the retina. Ipsilateral projections of RGCs reach the rostral tectum; more extensive projections are found in bolitoglossines (Rettig and Roth, 1986). The RGCs project ipsilaterally to all targets in the thalamus and pretectum, but the topic arrangement has not been investigated with modern tracers, which label neuronal structures more intensely.

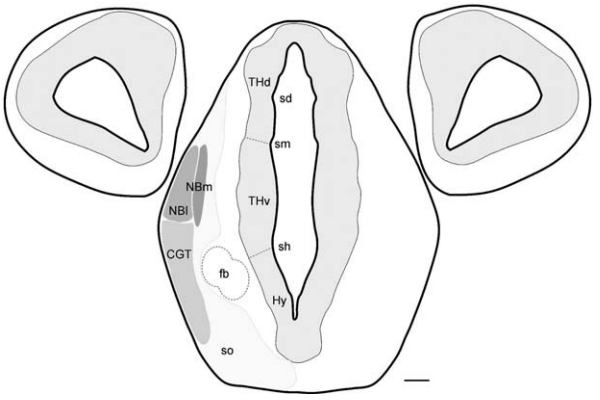

Figure 6 Drawing of a transverse section through the middiencephalon of *Plethodon jordani* showing the sites of retinofugal neuropils (NB pars medialis and pars lateralis and CGT). THd, dorsal thalamus; sd, dorsal thalamic sulcus; sm, medial thalamic sulcus; NBm, neuropil of Bellonci, medial part; NBl, neuropil of Bellonci, lateral part; CGT, corpus geniculatum thalamicum; fb, forebrain bundle; so, stratum opticum; Hy, hypothalamus; sh, hypothalamic sulcus; THv, ventral thalamus. Scale bar: 100 μm. Modified from Roth, G. and Grunwald, W. 2000. Morphology, axonal projection pattern and responses to optic nerve stimulation of thalamic neurons in the salamander *Plethodon jordani*. *J. Comp. Neurol.* 428, 543–557.

2.04.3.4.4 Projection specificity of retinal ganglion cells and morphology of terminal arbors In amphibians, the morphology of single terminal arbors was studied by means of intracellular labeling of RGCs and anterograde staining of the optic nerve (frogs: Stirling and Merrill, 1987; Hughes, 1990; salamanders: Wiggers, 1999). Axons of RGCs often have multiple terminal structures in the tectum and in the thalamic neuropils, while projections to the BON appear to originate from another type of RGC in plethodontid salamanders (Wiggers, 1999).

In frogs, terminal arbors of intracellularly HRP-labeled RGCs responding to the extinguishing of light were situated in the deep tectal fiber layers with a size of 400 and 200 μm in rostrocaudal and mediolateral axis, respectively. The axon gives off few branches to the pretectum before entering the tectum (Stirling and Merrill, 1987). In the Hughes (1990) study, HRP-labeled axons of RGCs were found in all superficial tectal fiber layers, but the

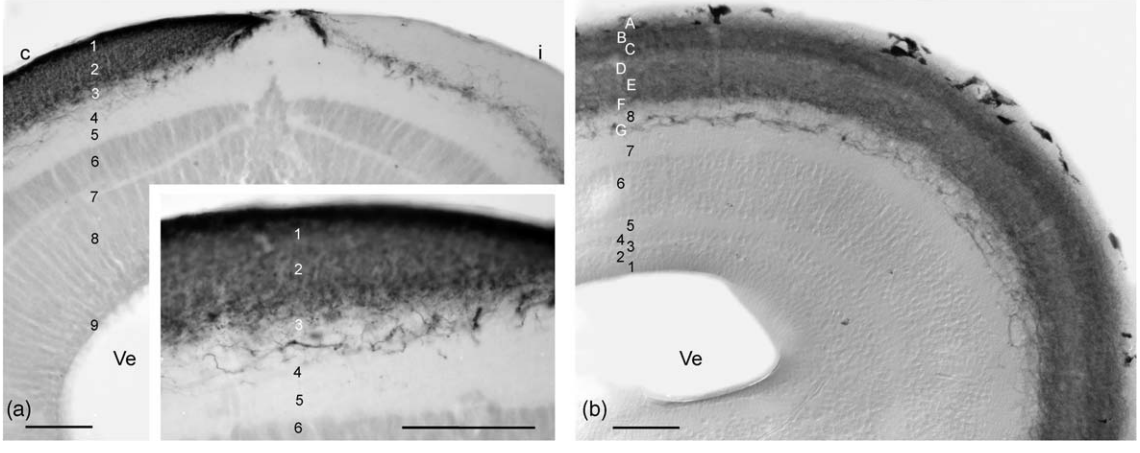

Figure 7 Microphotographs of transverse sections through the tectum of *Plethodon jordani* (a) and *Bombina orientalis* (b) showing retinal afferents after application of biocytin to the stump of the optic nerve. In (a), the contralateral afferents to the superficial tectal layers are shown on the left side and the ipsilateral afferents, mostly restricted to layer 3, on the right side. The inset shows the contralateral afferents at greater magnification. In (b), the contralateral afferents forming laminae A–G of layer 9 are shown. Ve, ventricle. Scale bars: 100 μm.

extent and morphology of arbors differed from layer to layer. Small and dense arbors with thin and beaded fibers were found in the superficial layer, whereas in the deep tectal fiber layer large arbors with sparse branching were labeled.

In a study on intracellularly biocytin-labeled RGCs of plethodontid salamanders by Wiggers (1999), the following types of projection pattern were frequently found:

1. RGCs with large terminal arbors and dense branching in thalamic neuropils (mainly in the medial NB) project to the deep tectal fiber layers with axons that are sparsely beaded and reveal no obvious terminal structures; pretectal neuropils are formed by sparse fields of axon collaterals.
2. RGCs with dense terminal arborization in the pretectal neuropils have additional sparse fields of collaterals in the thalamus, especially in the CGT and lateral NB; a projection to tectal fiber layers was not found.
3. RGCs with axons forming dense terminal arbors in the superficial fiber layer of the tectum reveal only few beads in the pretectal neuropils; neuropils in the thalamus were not found.
4. RGCs that have dense terminal fields in one of the two layers underneath the superficial tectal layer reveal additional sparse terminals in thalamic and pretectal neuropils. Axons of RGCs form terminals with moderate or sparse branching of collaterals in the CGT and in the lateral NB.

2.04.3.4.5 Cytoarchitecture of the tectum mesencephali In the tectum of frogs, nine layers are distinguished beginning from the ventricle (Potter, 1969) (cf. Figures 7b and 8a). Layer 1 contains ependymal glial cells with long processes extending toward the tectal surface, where they form the external limiting membrane. Cellular layers 2, 4, and 6 (stratum griseum periventriculare) together constitute the periventricular grey matter. These cellular layers are divided by deep fiber layers 3 and 5, consisting of unmyelinated afferent and efferent fibers and basal dendrites of the periventricular neurons. Fiber layer 7 (stratum album centrale) contains the bulk of efferent tectal fibers and a few scattered neurons. Layer 8 (stratum griseum centrale) consists of loosely arranged neurons embedded in a meshwork of dendrites of tectal neurons and afferent fibers. Layer 9 (stratum fibrosum et griseum superficiale + stratum opticum) contains relatively few neurons dispersed in the meshwork of retinal afferents and dendrites of tectal neurons. Layer 9 is further divided into seven laminae A–G. Lamina A (occurring only in the rostral tectum) and laminae B, D, and F plus lamina G in layer 8 contain myelinated and unmyelinated fibers (mostly retinal afferents), and C and E are cellular layers.

The tectum of salamanders, like that of caecilians and lepidosirenid lungfishes (Northcutt, 1977), shows an essentially two-layered structure consisting of a periventricular cellular layer and a superficial white matter consisting of dendrites of tectal neurons and tectal afferent and efferent fibers, in which only a few migrated neurons are dispersed. However, based on

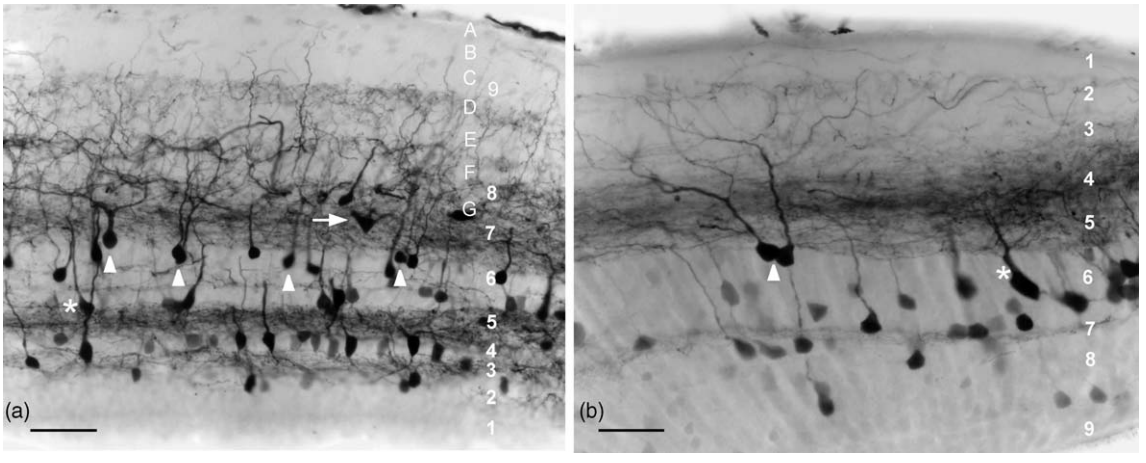

Figure 8 Microphotographs of tectal neurons type 2, 3, and 5 in *Discoglossus pictus* (a) and *Plethodon jordani* (b) labeled after injection of biocytin into the lateral medulla oblongata. The dendritic trees of neurons arborize in the middle layer of retinal afferents (arrowheads point to somata of the homologous type 3 of frogs and type 2 of salamanders) or in the deep, nonretinal afferent layers (asterisk indicates soma of type 5 in frogs and type 3 in salamanders). In (a), the arrow points to a spindle-shaped soma in layer 7; this type of neuron (type 2 of frogs) is only present in the frog tectum. Axons of the different types of neurons constitute the lateral uncrossed tectobulbospinal tract, and give rise to a nontopographic tectothalamic projection. From Dicke, U. and Roth, G. 1996. Similarities and differences in the cytoarchitecture of the tectum of frogs and salamanders. *Acta Biol. Hung.* 47, 41–59.

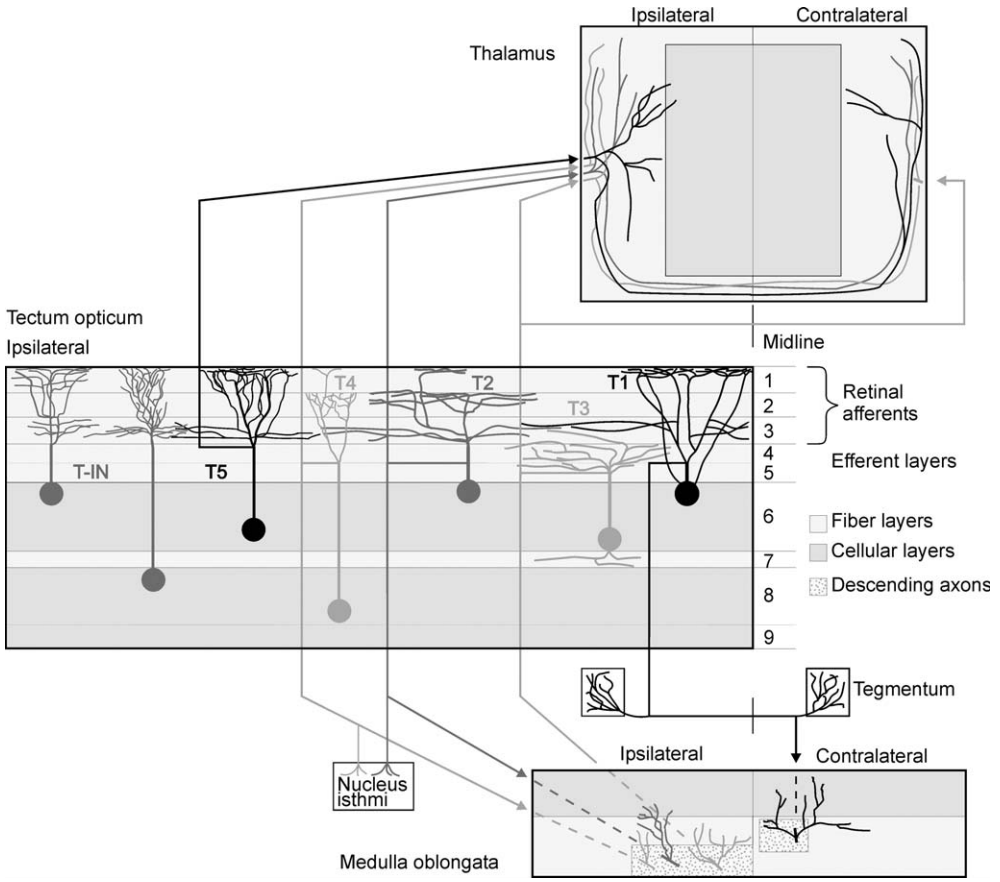

Figure 9 Schematic diagram of ipsilaterally and contralaterally ascending and descending tectofugal pathways constituted by different types (T1–T5) of neurons in salamanders. For further explanation see text. From Roth, G., Dicke, U., and Grunwald, W. 1999. Morphology, axonal projection pattern and response types of tectal neurons in plethodontid salamanders. II: Intracellular recording and labeling experiments. *J. Comp. Neurol.* 404, 489–504.

tracer experiments, the salamander tectum is divided from the surface to the ventricle into nine layers (Roth, 1987) (Figures 7a, 8b, and 9). Layers 1–3 contain retinal afferent fibers as well as afferents from other visual centers such as pretectum, thalamus, and isthmic nucleus; layers 4 and 5 contain efferent fibers and afferents from other senses, e.g. somatosensory, vestibular, and lateral line (if present). Layer 6 consists of the superficial cellular layer, while layer 7 (absent in miniaturized plethodontid salamanders) contains deep unmyelinated fibers. Layer 8 is the deep cellular layer, and layer 9 contains periventricular ependymal (glial) cells. In some salamanders, for example *Ambystoma mexicanum*, the periventricular grey matter regionally exhibits two to three sublayers.

On the basis of data from tracer studies on neuronal types (see below), tectal layers in salamanders and frogs can be homologized. Periventricular cellular and fiber layers 6–8 in salamanders are homologous to periventricular layers 2–6 in frogs, with cellular layer 6 being the most superficial of the periventricular layers in both groups. Dorsally, layer 6 is followed by the main efferent fiber layer(s), layers 4 and 5 in salamanders and layer 7 in frogs. Whereas in salamanders, only a few migrated neurons are found in the superficial part of the tectum (layers 1–3), in frogs such cells form a cellular band in layer 8 and are loosely arranged in layer 9. Retinal afferents terminate in layers 1–3 in salamanders and in laminae A–G of layers 8 and 9 in frogs.

2.04.3.4.6 Morphology and location of neuron types in the tectum A comparison of the morphology of dendritic trees of projection neurons and their targets in frogs and salamanders reveals that in both orders the same set of tectal projection neurons exists (frogs: Lázár *et al.*, 1983; Antal *et al.*, 1986; Dicke and Roth, 1996; salamanders: Roth *et al.*, 1990, 1999; Dicke and Roth, 1996; Dicke, 1999) (cf. Figures 8, 10, and 11). Types with descending axons are presented first and are ordered by their type of arborization from the superficial to the deeper fiber layers.

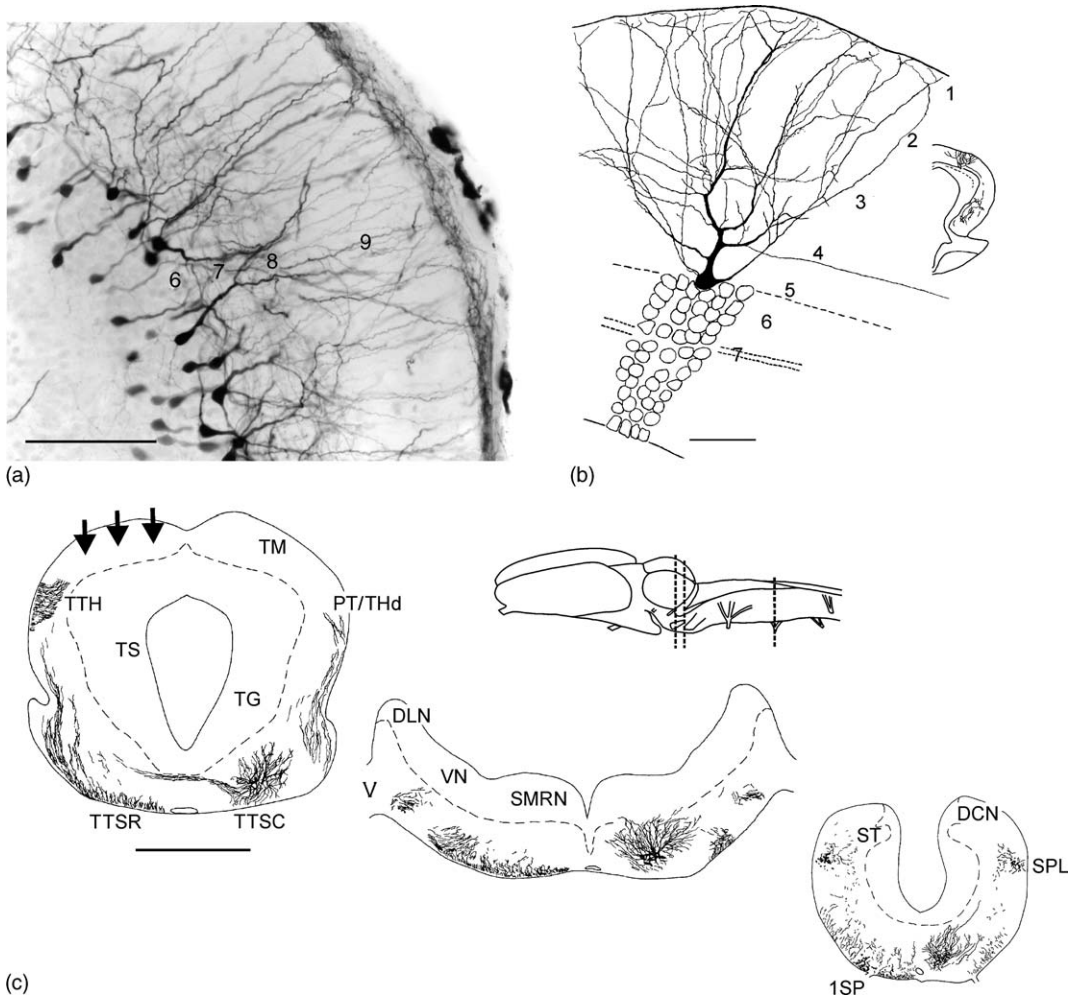

Figure 10 a, Microphotograph of the tectum of *Discoglossus* showing type-1 neurons labeled after application of biocytin into the medial medulla oblongata and projecting to the contralateral medulla oblongata. b, Camera lucida drawing of a type-1 neuron in *Plethodon jordani* labeled after application of biocytin into the medial medulla oblongata and projecting to the contralateral medulla oblongata. c, Drawing of descending tectobulbospinal tracts at levels indicated in the inset (tectum, level of Vth cranial nerve and 1st spinal nerve). Black arrows indicate the site of tracer application. 1, 2, 3, 4, 5, 6, 7, 8, 9, ventricles; TM, mesencephalic tectum; TTH, tecto-thalamic tract; TS, torus semicircularis; TTSR, uncrossed tecto-bulbo-spinal tract; TTSC, crossed tecto-bulbo-spinal tract; TG, tegmentum; PT/THd, descending tract of pretectum and thalamus; DLN, dorsolateral nucleus; VN, vestibular nucleus; SMRN, superior middle reticular nucleus; 1SP, first spinal nerve; SPL, spinal lemniscus; DCN, dorsal column nucleus. Scale bars: 100 μm in (a, b) and 500 μm in (c). Modified from Dicke and Roth (1996); Dicke (1999), Roth *et al.* (1999).

In frogs, type-1 neurons with large pear-shaped or pyramidal somata situated in layer 6 and occasionally in layers 7 and 8 have candelabrum-shaped dendritic trees that arborize predominantly in lamina A. They closely resemble type-1 neurons of salamanders with somata situated in the superficial cellular layer 6 or in efferent layers 4 and 5; their likewise candelabrum-shaped dendritic trees extend into layers 1–3. Axons descend contralaterally to the medulla, where they constitute the crossed tectobulbospinal tract (Figures 10a and 10b).

Type-2 and type-3 neurons of frogs have the same pattern of descending and ascending axonal projections, but the former type of cells have spindle-shaped somata situated in or immediately above the large efferent layer 7 (Figure 8a). Two to several thick dendrites originate directly from the soma and branch into thick secondary dendrites, which extend obliquely to the surface. Smaller dendrites either terminate in lamina F and less frequently in laminae B and D or terminate in lamina B. This type of tectal neuron, with ipsilaterally descending axons, was not found in salamanders.

Type-3 neurons (pear-shaped or pyramidal cells) in frogs and type-2 neurons in salamanders (Figures 8a and 8b) can be regarded as homologous, because their somata are situated in the superficial layer of the periventricular gray. Both types have

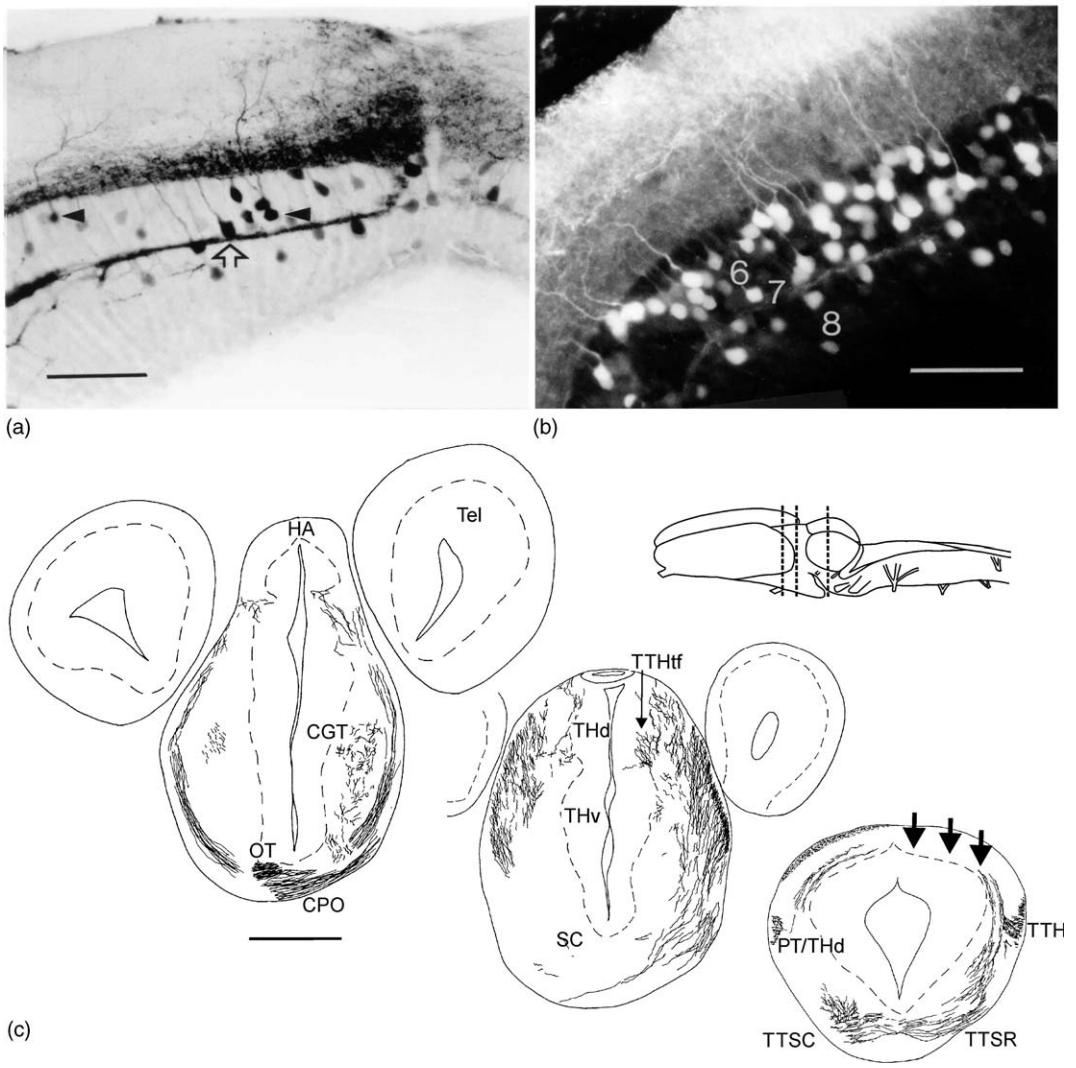

Figure 11 a, b, Microphotographs of transverse section showing tectal neurons labeled after tracer application to the postoptic commissure in *Plethodon jordani*. a, Type-3 tectal neuron (open arrow) and type-5 tectal neuron (black arrowhead) ipsilateral to the application site of biocytin. b, Type-5 neurons constituting the bulk of the ascending tectothalamic tract labeled ipsilateral to the application site of tetramethylrhodamine. c, Drawings of the ascending tectothalamic tract at levels indicated in inset. 6, 7, 8, ventricles; HA, habenula; CGT, corpus geniculatum thalamicum; OT, optic tract; CPO, postoptic commissure; Tel, telencephalon; TTHtf, terminal fields of tecto-thalamic tract; THd, dorsal thalamus; THv, ventral thalamus; SC, suprachiasmatic nucleus; PT/THd, descending tract of pretectum and thalamus; TTSC, crossed tecto-bulbo-spinal tract; TTSR, uncrossed tecto-bulbo-spinal tract; TTH, tecto-thalamic tract. Scale bars: 100 μm (a, b), and 500 μm (c). Modified from Dicke, U. 1999. Morphology, axonal projection pattern, and response types of tectal neurons in plethodontid salamanders. I: Tracer study of projection neurons and their pathways. *J. Comp. Neurol.* 404, 473–488.

wide to very wide dendritic trees, which arborize predominantly in the deeper retinorecipient laminae (lamina C or D in the frog, and layers 2 and 3 in the salamander tectum). In frogs, the somata are located in periventricular layers 2 and 4, a larger number in layer 6, and fewer in layers 7 and 8. In salamanders, somata are found in the upper part of layer 6. The axons of this type descend ipsilaterally, some of them forming contacts with the isthmic nucleus; in the medulla, axons run in a ventrolateral superficial position. They constitute the lateral part of the uncrossed tectobulbospinal tract. Axons ascend to the ipsi- and contralateral thalamus.

Type-5 neurons (pear-shaped cells) of frogs and type-3 neurons of salamanders can be considered homologous, because their somata are usually situated in the deeper part of the periventricular gray (Figures 8a and 8b). Their dendritic tree is flat and T-shaped and mostly confined to the efferent fiber layers (layer 7 in frogs, layers 4 and 5 in

salamanders). With their descending axons they either contribute to the lateral part of the uncrossed tectobulbospinal tract or constitute its medial part; they have ascending ipsilateral or bilateral projections to the pretectum and thalamus.

In frogs, the somata of the type-4 neuron (pear-shaped) are located in the deep cellular layer; their slender primary dendrite arborizes in lamina C. They resemble the rarely labeled type-4 neurons of salamanders with narrow dendritic trees arborizing predominantly in layer 2. Axons of this type descend ipsilaterally to the ventrolateral part of the rostral medulla oblongata; other axons or axon collaterals ascend to the ipsilateral thalamus.

In the frog tectum, cells with small pear-shaped somata situated in layer 8 and slender dendritic trees and arborizing either in lamina B or D were identified as rostrally projecting cells. They strongly resemble the slender type-5 neurons found in salamanders (Figures 11a and 11b). The somata of the latter are situated in layer 6 and less often in 8; the narrow dendritic trees arborize in fiber layer 1 or 2. Axons only ascend ipsi- and/or contralaterally to the pretectum and thalamus.

Interneurons are similar in the salamander and frog tectum. Their pear-shaped somata are situated mainly in the deeper part of the periventricular gray matter, and their dendritic trees are mostly very slender (Figure 9). The dendritic trees arborize in different retinorecipient laminae of the tectum. However, because of a much higher degree of cell migration in the anuran compared to the urodele tectum, in frogs many interneurons, i.e., small pear-shaped cells, occupy a superficial position. During ontogeny of the frog tectum, these neurons (like other tectal cells) originate in the periventricular germinal zone and then migrate toward the surface. In salamanders, this late ontogenetic migration process is either strongly reduced or completely abolished, with the consequence that cells remain within the periventricular cellular layer 6 (Schmidt and Roth, 1993).

2.04.3.4.7 The number of tectal neurons The salamander tectum comprises on average 100 000 cells; frogs possess 2–17 times more tectal cells (Roth *et al.*, 1995). The bulk of them is formed by interneurons; projection neurons make up roughly 5% independent of the absolute number of neurons (Dicke, 1999).

2.04.3.4.8 Nonretinal afferents The tectum mesencephali of amphibians, like that of other vertebrates, is a center for multisensory integration. It receives afferents from the thalamus and pretectum, which are also targets of primary visual afferents. In frogs, thalamic neurons projecting to the tectum were described recently in *Bombina* (Roth *et al.*, 2003). They are located in the dorsal portion of the ventral nucleus, the NB, and the central and posterior dorsal nucleus. Most of these neurons are characterized by a dendritic tree oriented dorsolaterally, sometimes also ventrolaterally entering the NB or the CGT neuropils. Their axons terminate in layer 7 of the tectum, the layer of tectal efferents. They are either restricted to the medial portion or extend throughout that layer and form collaterals. In the salamander *Plethodon*, the situation corresponds with that found in *Bombina* (Roth and Grunwald, 2000). Two groups of cells send axons to the tectum: one group of cells has its somata in the posterior dorsal thalamus around the sulcus medialis (corresponding to the central dorsal and posterior dorsal nucleus of frogs) and possesses a wide and flat dendritic tree that arborizes mostly in the medial white matter; dorsalmost dendrites reach the lateral surface. The other group consists of cells with somata in the ventral thalamus (ventral nucleus and NB in frogs), which forms relatively narrow dendritic trees extending laterally or ventrolaterally toward the surface. Neurons of the latter group constitute two nuclei, one at the level and another one ventrally to the sulcus medialis (Dicke, unpublished data) (Figure 12). In the three groups, terminals in the tectum are confined mostly to deeper tectal fiber layers 3–5 within the medial, intermediate, or lateral zone of the tectum. Furthermore, neurons of the pretectum project to the tectum (Marín *et al.*, 1997b); in salamanders, axons run ipsilaterally in the deep fiber layers of the tectum (Luksch *et al.*, 1998). Finally, afferents originate from a small number of neurons in the contralateral tectum.

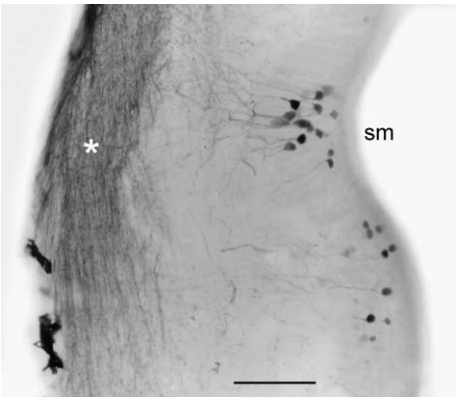

Figure 12 Microphotograph of ipsilaterally labeled ventral neurons in the mid thalamus of the salamander *Plethodon teyahalee* after application of biocytin to the tectum. Medial is to the right, dorsal is to the top. Asterisk indicates anterogradely labeled axons of tectal neurons as well as retinofugal axons of retinal ganglion cells that collateralize and run to the tectum and thalamus. Scale bar: 100 μm. sm, medial thalamic sulcus.

Neurons of the vomeronasal amygdala, dorsal tegmentum, isthmic nucleus, and medulla oblongata and spinalis project into tectal fiber layers; only few striatal neurons project to the deep fiber layers of tectum (Marín *et al.*, 1997c; Roth *et al.*, 2004). In *Rana*, there are mainly indirect connections of the striatum to the tectum constituted by pathways via the amygdala/entopecuncular nucleus, pretectum, and tegmentum (Marín *et al.*, 1997b; see also below).

A substantial tectal innervation arises in the dorsal tegmental nucleus, which projects ipsilaterally (entire nucleus) and contralaterally (only the posterior portion) to the tectum (Figure 4b); there is no information on the site of termination inside the tectum. In addition to retinal afferents from the retina, in amphibians as well as in other vertebrates, a major input to the superficial tectum originates in the isthmic nucleus (homologous to the parabigeminal nucleus in mammals). In frogs, neurons in the dorsal part of this nucleus project to the ipsilateral fiber layers 9 and 8 and those of the ventral part to the superficial contralateral fiber layer (Gruberg and Udin, 1978; Gruberg *et al.*, 1994). In salamanders, two subnuclei were likewise reported in a double-labeling study of Wiggers and Roth (1991), but intracellular labeling revealed that the majority of isthmic neurons project to both tectal hemispheres (Wiggers, 1998). However, an ipsilateral projection to several retinorecipient layers and a contralateral projection to only the superficial layer of retinal afferents is commonly found in all vertebrate tecta. The isthmotectal projection is topographically ordered and in register with the retinal map (Gruberg and Lettvin, 1980; Gruberg *et al.*, 1989; Wiggers 1998).

In amphibians as well as in all vertebrate species studied so far, neurons of the isthmic nucleus comprise acetylcholine as a major transmitter and are the principal source of cholinergic input to the tectum (Ricciuti and Gruberg, 1985; Wallace, *et al.*, 1990; Marín *et al.*, 1997a; Marín and González, 1999). In plethodontid salamanders and in the caecilian, *Dermophis mexicanus*, part of the cholinergic input to the tectum stems from tectal cells revealing ChAT-like immunoreactivity (-ir); their dendritic trees also reach superficial fiber layers (Wallstein and Dicke, 1998; Gonzalez *et al.*, 2002) (Figure 13). In frogs, the isthmic nucleus also contains GABA-ir somata, but the distribution varies among species. GABA-ir cells are evenly distributed in the isthmic nucleus of *R. pipiens* (Li and Fite, 1998), while in *Rana esculenta* the majority of them are found in the anterior one-third of the nucleus (0.5% of the total population), and a meshwork of GABA-immunostained fine-beaded axons fills the entire isthmic nucleus (Pollak *et al.*, 1999). Based on lesion experiments, it is assumed that the majority of GABA-positive fibers derives from local GABA-positive cells and the rest from tegmental GABAergic cells. In *Rana catesbeiana*, staining of GABA-ir cells is moderate to dense in the anterior and posterior part of the nucleus and absent in the central part, and the isthmic nucleus is sparsely GABA-immunoreactive in *X. laevis* (Hollis and Boyd, 2005).

Afferents from the medulla to the tectum include the auditory dorsal nucleus, the vestibular nucleus, the middle reticular nucleus and the raphe nuclei of the rostral medulla oblongata as well as nuclei of the medulla oblongata and spinal cord that mediate somatosensory information. Axons from sensory nuclei mainly innervate the contralateral tectum and run in the deep fiber layers containing efferent fibers of tectal neurons (layers 7 in frogs, and 4 and 5 in salamanders), whereas the majority of axons of the reticular nucleus ascend to the ipsilateral tectum and extend in all deep fiber layers of the tectum (3, 5, 7 in frogs; 3–5, 7 in salamanders). The distribution of transmitters in the nuclei with ascending projection was recently studied in the salamander, *Plethodon* (Landwehr and Dicke, 2005). Projection neurons of the dorsal and vestibular nucleus are glutamate-ir, and in the latter nucleus often reveal additional GABA- and/or glycine-ir. Projection neurons of the middle reticular nucleus reveal predominantly gly-ir, often co-localized with glu-ir; this nucleus appears to be homologous to the mammalian gigantocellular reticular nucleus (Figure 14b).

In salamanders, the serotonergic raphe nuclei strongly innervate the retinorecipient layers 2 and 3, and some axons of the raphe nuclei reach the efferent fiber layers 4 and 5 (Dicke *et al.*, 1997) (Figure 14a). In *Rana*, 5-HT is located in tectal layers 3, 5, 6, 7, and 9 (Liu and Debski, 1995), but the source of serotonergic innervation differs from that found in salamanders. The 5-HT-ir cells are labeled in cellular layers 2, 4, and 6 of the tectum and are assumed to contribute to the serotonergic innervation of the deeper fiber layers. The distribution of serotonergic neurons is most prominent in the lateral tectum and decreases significantly medially, but is largely constant in the rostrocaudal dimension (Debski *et al.*, 1995). The 5-HT-ir fibers in lamina A of layer 9 are mainly of retinal origin, while serotonergic fibers in the other laminae most likely originate from neurons in the midbrain tegmentum and the isthmic nucleus. The raphe nuclei of the reticular formation of the medulla project nontopographically to midtectal layers (Zhao and Debski, 2005). Neurons of the DCN and LCN situated in the caudal medulla oblongata and cervical spinal cord receive predominantly somatosensory

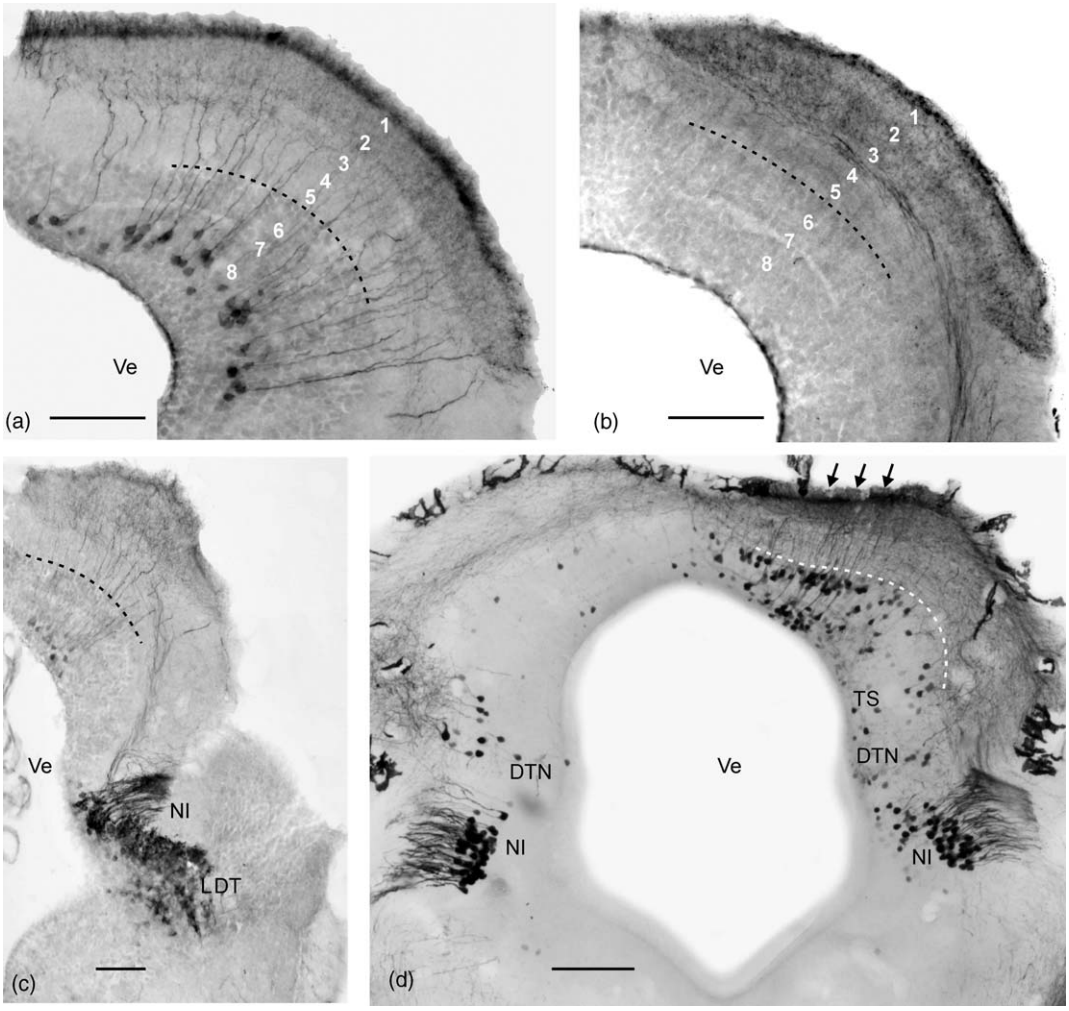

Figure 13 Microphotographs of ChAT-immunoreactive structures in the plethodontid salamander *Desmognathus ochrophaeus* ((a), (c)) and in the salamandrid *Salamandra salamandra* (b). In (d), retrograde labeling of bilateral tegmental and isthmic neurons after unilateral application of biocytin (black arrows) to the tectum is shown. Note that in *Desmognathus*, ChAT-ir tectal neurons are present that extend processes into the superficial layers, whereas in *Salamandra* only the isthmic neurons provide the tectal layers with cholinergic fibers. 1, 2, 3, 4, 5, 6, 7, 8, ventricles; Ve, ventricle; NI, isthmic nucleus; LDT, laterodorsal tegmental nucleus; DTN, dorsal tegmental nucleus; TS, torus semicircularis. Scale bars: 100 μm.

input. These neurons carry different sensory modalities and project to the tectum and the torus semicircularis. The pattern of ascending somatosensory projections differs between plethodontid salamanders on the one hand and *Pleurodeles waltl* and frogs on the other (Muñoz *et al.*, 1994a, 1994b, 1995, 1997; Dicke and Muhlenbrock-Lenter, 1998) (see Section 2.04.3.1.5.*(ii)* above). In the salamander, *Ambystoma tigrinum*, ipsi- and contralateral projections to the tectum have been reported (Gruberg and Solish, 1978; Gruberg and Harris, 1981).

In plethodontids, the tectum appears to be the main center for multimodal integration, while in frogs the torus is the main target of somatosensory afferents. The presence of a more elaborate secondary somatosensory system in terrestrial plethodontids compared to salamandrid salamanders with a larval stage as well as ranid and pipid frogs may be due either to phylogeny, differences in development (direct vs. biphasic), or functional adaptation (e.g., differences in quantity and distribution of transmitters in the tectum in the context of visuomotor and visual functions).

2.04.3.4.9 Tectal efferent pathways

2.04.3.4.9.(i) Descending pathways In order to elucidate descending tectal pathways, tracer studies have been carried out in frogs and salamanders using HRP or cobaltic lysine applied to the entire half of the medulla spinalis (ten Donkelaar *et al.*, 1981; Tóth *et al.*, 1985; Naujoks-Manteuffel and Manteuffel, 1988), and the existence of a crossed and uncrossed

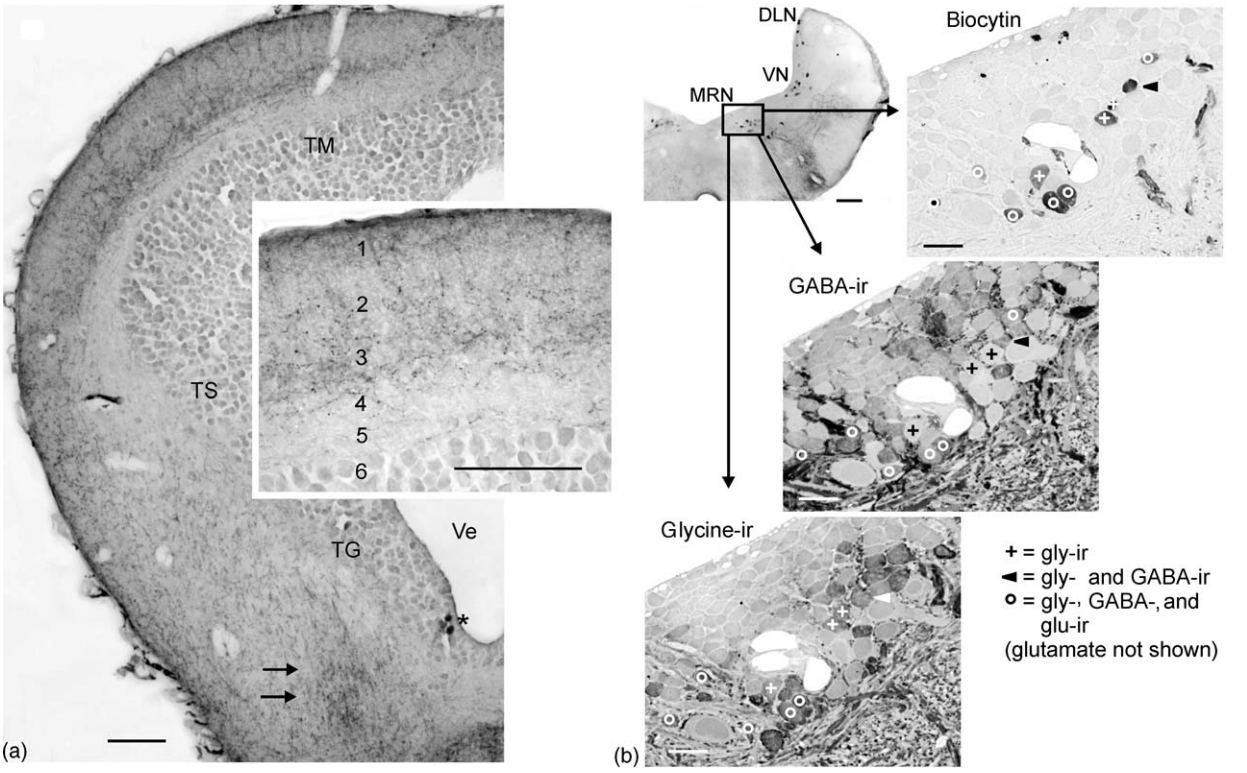

Figure 14 a, Microphotographs of transverse sections through the midbrain revealing 5-HT-immunoreactivity in the salamander *Plethodon jordani.* Arrows point to the ascending bundle and asterisk indicates labeled somata of the rostral raphe nucleus. Inset shows part of the tectum at higher magnification. b, Glycine-ir and GABA-ir in neurons of the middle reticular nucleus retrogradely labeled after biocytin application to the tectum. Consecutive semithin sections with cell bodies stained for the two transmitters and biocytin reveal that co-localization of transmitters occurs more frequently than previously assumed. TM, mesencephalic tectum; TS, torus semicircularis; TG, tegmentum; Ve, Ventricle; 1–6, ventricles; DLN, dorscelateral nucleus; VN, vestibular nucleus; MRN, middle reticular nucleus. Scale bar in (a) 100 μm and in (b) 50 μm for the section above left and 20 μm for semithin sections. a, Modified after Dicke, U., Wallstein, M., and Roth, G. 1997. 5-HT-like immunoreactivity in the brains of plethodontid and salamandrid salamanders (*Hydromantes italicus, Hydromantes genei, Plethodon jordani, Desmognathus ochrophaeus, Pleurodeles waltl*): An immunohistochemical and biocytin double-labelling study. *Cell Tissue Res.* 287, 513–523. b, Modified from Landwehr, S. and Dicke, U. 2005. The distribution of GABA, glycine and glutamate in neurons of the medulla oblongata and their projections to the midbrain tectum in plethodontid salamanders. *J. Comp. Neurol.* 490, 145–162.

tectal tract was consistently reported. Locally restricted application of biocytin to the ventromedial or ventrolateral medulla oblongata revealed that different populations of tectal neurons give rise to a crossed and one or two uncrossed descending tracts. In plethodontid salamanders, neurons in the ipsilateral tectum are labeled after ventromedial and ventrolateral tracer application, whereas in *Discoglossus* they are labeled only after ventrolateral tracer application (Dicke *et al.*, 1998; Dicke, 1999).

In plethodontid salamanders, the course of the descending axons and sites of collaterals to the medulla were investigated by biocytin application into the tectum as well as by intracellular biocytin injection into tectal neurons (Dicke and Roth, 1994; Dicke, 1999; Roth *et al.*, 1999) (Figures 10c and 9). The fibers of the crossed tectobulbospinal tract run to the ipsilateral ventral tegmentum and cross the midline in the rostral to caudal tegmentum. They further descend in the contralateral ventral tegmentum, where fibers form a neuropil extending inside the ventral white matter. In the medulla oblongata and spinalis, fibers run close to the midline within the ventral white matter below the surface. The tract thins out during its course to the spinal cord; axons terminate in the rostral medulla oblongata or reach at least the level of the third spinal nerve.

Axons of the uncrossed tract descend in the caudal tectum to the ventral tegmentum. During their course from the tegmentum to the ventral medulla, axons are distributed broadly in the medial white matter. More laterally, dense axon bundles are formed, which remain close to the lateral edge of the uncrossed tract inside the medulla; they originate from other types of neurons than the medially descending axons. In the medulla oblongata and spinalis, axons run in a superficial ventral position; in the transverse plane, they are distributed from the

midline to the ventrolateral part. The uncrossed tracts extend to rostral spinal cord levels, but only one-half to one-third of axons reach the level of the third spinal nerve.

During their course within the medulla oblongata and spinalis, axons of the descending tracts give rise to many fine collaterals that often carry boutons. Axons of single neurons give rise to a substantial amount of axon collaterals inside the medulla oblongata. The majority of collaterals extend inside the white matter. At the level of the IXth and Xth cranial nerves, prominent axon collaterals of the crossed tract extend dorsally and dorsolaterally into the gray matter; they often twist around each other. In the caudal medulla oblongata, some axon collaterals extend along the border of the gray matter to the dorsal side.

In salamanders as well as in frogs, the descending fibers of the crossed and uncrossed pathways show strong branching, but unlike in salamanders (and in other tetrapod vertebrates), in frogs only few contralaterally descending axons reach spinal levels. This may be a consequence of the condensation of the neck region and consequently of bulbar and cervical spinal motor nuclei that has occurred during the evolution of anurans.

2.04.3.4.9.(ii) Ascending pathways Systematic studies of the ascending axons of tectal neurons have been carried out in the frog *R. pipiens* and in the salamander *Plethodon jordani* (Montgomery and Fite, 1991; Dicke, 1999; Roth *et al.*, 1999). In *Rana*, the ascending projections from the dorsal mesencephalon to the thalamus and pretectum were investigated by means of HRP tracing. Axons of small pear-shaped neurons (type-5 in salamanders) in the superficial portion of tectal layer 8 exit the tectum through layer 9, travel in the superficial portion of the dorsal and ventral tectothalamic tracts, and innervate the nucleus lentiformis mesencephali (the large-celled part of the pretectal nucleus), the posterior lateral dorsal nucleus, and the CGT. Axons of small pear-shaped neurons in the lateral and caudal tectum ascend via the ventral tectothalamic tract, while those of neurons in the rostral and medium tectum run in the dorsal tectothalamic tract. Axons from pear-shaped neurons (type-5 of frogs homologous to type-3 in salamanders) in layer 6 and pyramidal neurons (type-3 of frogs homologous to type-2 in salamanders) in layer 8 leave the tectum through layer 7, travel in the dorsal or ventral tectothalamic tracts and are located medial to the axons of the pear-shaped neurons of superficial tectal layer 8. The majority of type-3 neurons project to the posterior lateral ventral nucleus and the anterior lateral nucleus, but terminals do not display a tectotopic organization. Another major projection to the thalamus originates from the pretectal gray and innervates the pretectal nucleus lentiformis mesencephali, the posterior lateral dorsal nucleus, the anterior lateral nucleus, dorsal and ventral divisions of the ventral lateral thalamus, and the nucleus of Bellonci. Other axons from the pretectal gray terminate in the contralateral medial portions of the posterior lateral dorsal thalamus, the ventral lateral thalamus, and the anterior lateral nucleus.

In *P. jordani*, ascending tectal projections were studied by tracer application and intracellular injection of biocytin into neurons (Figures 9 and 11c). Tracer application to the tectal hemisphere likewise labels retinal afferent fibers, and double labeling of the optic nerve and the tectum using different tracers reveals that the retinofugal axon bundles and ascending axons of tectal neurons take partially overlapping courses. The ascending axons of tectal neurons leave the tectum through fiber layers 3–5 and ascend superficially at the lateral edge of the tectum. During their course, axons give rise to many collaterals extending in the ipsilateral dorsal white matter. In the ipsilateral pretectum, two dense neuropils, a dorsolateral and a lateral one, are formed. In the ipsilateral thalamus, axons run laterally in the fiber layer, and collaterals are distributed in the entire white matter of the dorsal and ventral thalamus. They form a distinct neuropil at the border between the gray and white matter in the dorsal thalamus, where the neuropil and NB are situated. Many axons cross in the postoptic commissure to the contralateral thalamus, where they branch inside the ventral and dorsal white matter and again form a neuropil in the dorsal thalamus. Axon collaterals are labeled in the ipsi- and contralateral preoptic area, and only few extend to the ventral fiber layer of the ipsi- and contralateral caudal telencephalon.

In summary, the ascending pathways of amphibians are constituted by small-field neurons with only ascending projections and by two types of wide-field neurons with ascending and descending projections. The former neurons constitute the majority of ascending tectal projection neurons: they are regularly distributed throughout the tectum and give rise to a retinotopic tectal projection to the thalamus in the frog *Rana* and most likely in the salamander *Plethodon*. In contrast, the latter two types of neurons either are very low in number or are unevenly distributed in the tectum. They appear to give rise to a nonretinotopic tectal projection to the thalamus. Accordingly, in amphibians the ascending pathways may be divided into two functional systems, a retinotopically and a nonretinotopically organized system, which is comparable to the

situation found in reptiles and birds (see Evolution of the Nervous System in Reptiles).

In the tecta of reptiles and birds, efferent cells constitute ipsilaterally and contralaterally descending tracts as well as ipsi- and contralaterally ascending tracts (Reiner and Karten, 1982; Sereno, 1985; Sereno and Ulinski, 1985; Dacey and Ulinski, 1986a, 1986b; ten Donkelaar, 1990; Reiner, 1994). The morphology of neurons of origin of these tracts and their axonal projection patterns reveal similarities with the situation found in amphibians. One ascending pathway arises from neurons that possess wide dendritic fields in the retinorecipient layer; this pathway terminates in a nonretinotopic manner in the nucleus rotundus, the possible homologue to the pulvinar of mammals. The other pathway arises from radial neurons with long dendrites ascending to the stratum griseum superficiale (SGF); it ascends to the dorsal and ventral lateral geniculate nucleus (LGN) of the thalamus. In amphibians, we likewise find wide-field as well as small-field tectal neurons that give rise to pathways ascending to the thalamus. Axons of small-field neurons form neuropils in the dorsal thalamus, whereas wide-field neurons predominantly project to the ventral thalamus. In the thalamus, in turn, a large group of neurons project to the striatum, and a small group to the medial and dorsal pallium. Thus, a tectothalamopallial pathway exists in salamanders, but its thalamopallial part is formed by relatively few neurons. This will be discussed in greater detail below.

2.04.3.4.10 The distribution of transmitters and neuropeptides in the retinotectal system

2.04.3.4.10.(i) Retinal ganglion cells In amphibians, a large number of RGCs use glutamate as transmitter. Polysynaptic responses of tectal cells in *Rana* were found to be mediated by NMDA and non-NMDA receptors, and examination of monosynaptic currents revealed that retinotectal synapses express functional NMDA receptors that were voltage dependent and not responsible for the bulk of normal excitatory transmission (Hickmott and Constantine-Paton, 1993). GABA is likewise used as transmitter in retinotectal transmission; the number of GABAergic ganglion cells synapsing on tectal neurons differs across species. In *B. marinus*, roughly 3% of retrogradely labeled RGCs contained GABA; 88% of retinal axon terminals in the tectum revealed glutamate-ir, 6% GABA-ir, and 6% were negative for both GABA and glutamate (Gabriel *et al.*, 1992; Gabriel and Straznicky, 1995), while in *R. pipiens*, 15% of back-filled RGCs contained GABA (Li and Fite, 1998). Over 50% of cells in the retinal ganglion cell layer of *R. esculenta* contained calretinin, and many optic fibers were also labeled; a co-localization of calretinin and GABA was rarely observed in RGCs (Gabriel *et al.*, 1998). In *Ambystoma*, small populations of retrogradely labeled RGCs contained substance P (2%) or GABA (less than 1%); substance P and GABA were not co-localized in RGCs (Watt *et al.*, 1994). In the salamandrid salamanders *P. waltl* and *Triturus alpestris*, axons in the retinal radiation in the diencephalon are mainly GABA-negative (Naujoks-Manteuffel *et al.*, 1994).

2.04.3.4.10.(ii) Tectum mesencephali In *R. catesbeiana* and *X. laevis*, GABA-ir cell bodies are distributed throughout all tectal cellular layers, and form dense GABAergic populations in layers 2, 4, and 6, that in layer 6 probably has the densest population in the entire brain. In *Xenopus*, the laminar organization was less distinct and fewer labeled cells were present compared to *Rana* (Hollis and Boyd, 2005). In the tectum of *R. pipiens*, the synthesizing enzyme GAD is distributed with punctate structures in several laminae of the optic tectum, and the highest concentrations are found in layers 9 and 8 (Tyler *et al.*, 1995). GABA immunochemistry in this ranid species reveals that perikarya and fibers are labeled in superficial layers 8 and 9, but densely packed immunoreactive perikarya occur in deep tectal layers 2, 4, and 6 (Li and Fite, 1998). In the tectum of *R. esculenta*, nearly one-third of the total population of cells appears to be GABA-immunoreactive (Antal, 1991); the stained population consists of neurons with small perikarya, and the proportion is highest in layer 9 (61%), and lower in layers 7 (21%) and 6 (27%).

A substantial proportion of retinal axons terminate on GABA-containing tectal neurons. In *B. marinus*, 57% of retinal axons synapsed on GABA-ir and 5% on glutamate-ir tectal elements, while the remaining axons synapsed on dendrites revealing neither GABA- nor glutamate-ir (Gabriel and Straznicky, 1995). In *Xenopus*, an ultrastructural analysis of tectal layers 8 and 9 using labeling of retinotectal axon and GABA immunohistochemistry revealed that GABA-ir neurons participate in serial synaptic arrangements, in which retinotectal axons are the first element (Rybicka and Udin, 1994). Furthermore, retinotectal and isthmotectal axons do not synapse close to each other on the same dendrites. Surprisingly, axons of isthmotectal neurons relaying ipsilateral eye input to tectal cells mainly synapse onto GABA-ir interneurons (Rybicka and Udin, 2005).

In *R. esculenta*, a substantial portion of axons of RGCs that terminate in laminae B, C, and F of tectal layer 9 contain the calcium-binding protein calretinin. Also, approximately 10% of the tectal cells

were found to be immunoreactive for calretinin. Tectal neuron populations in layers 4, 6, 8, and 9 were labeled, and a few calretinin-positive cells were detected also in layer 2. Cells in layers 4, 6, and 8 belonged to projection neurons. Co-existence of GABA and calretinin was characteristic of cells in upper tectal layers, but was absent in neurons of deep layers of the tectum (Gabriel *et al.*, 1998). Several Met-enkephalin immunoreactive perikarya were found in tectal layer 6 of *R. esculenta*, and a third of these neurons showed GABA-ir in addition. Also, GABA and neuropeptide Y (NPY) were co-localized in half of NPY-immunopositive cells in layer 6, while only a few cells were double-stained in layers 9 and 4 (Kozicz and Lázár, 2001).

In salamanders, GABA-ir neurons are scattered in all cellular layers of the tectum in *Triturus cristatus* and *P. waltl*, and a rich GABergic innervation characterizes tectal fiber layers (Franzoni and Morino, 1989; Naujoks-Manteuffel *et al.*, 1994). In *P. jordani* and *Hydromantes italicus*, GABA-ir somata were found in one-third of tectal neurons, with the majority located in the deep cellular layer 8 and to a lesser degree in cellular layer 6. They were either immunoreactive for GABA only, for GABA and glutamate, or for GABA and glycine. About 80% of tectal somata revealed glutamate-ir, including those with GABA-ir in addition (one-fourth), and were situated in both cellular layers (Wallstein and Dicke, 1996). On the basis of immunochemical detection of co-localization of transmitters in tectal neurons with descending projections, the majority of the latter neurons appears to contain glutamate and a substantial part of them GABA in addition (S. Landwehr, unpublished data). It is unclear, however, to which degree glutamate appears as precursor of GABA.

In addition to the cholinergic and serotonergic innervation of the tectum already described above, catecholamines were investigated in the tectum of *Rana perezi* and *P. waltl* (Sanchez-Camacho *et al.*, 2002). Dopaminergic fibers were found primarily in deeper tectal layers of *Rana* (5–8) and in all tectal layers of *Pleurodeles*, whereas noradrenergic fibers predominated in superficial layers (7–9 in *Rana*; 1–5 in *Pleurodeles*); catecholaminergic somata were not found in the tectum. The catecholaminergic fiber input originates mainly from the pretectal area (*Pleurodeles*) and juxtacommissural nucleus (*Rana*), a smaller component from the suprachiasmatic nucleus, and in *Rana* also from the posterior tubercle as well as from the noradrenergic locus coeruleus. In plethodontid salamanders, the dopaminergic fiber input to the midbrain tectum appears to be less pronounced, since only few ir-fibers were found in the deep fiber layers 4 and 5 (Wallstein and Dicke, 1997). Furthermore, histochemistry of NADPH-diaphorase revealed labeled fibers and cell bodies in the tectum of *R. perezi* (Muñoz *et al.*, 1996).

The lamination of tectal cells and fibers is paralleled by another lamination established by peptides (cf. review of Lázár, 2001). A variety of peptides (23 of 28 peptides including those already mentioned above) is present in the frog tectum, and a substantial number of peptides was also localized in the lateral geniculate complex and pretectal area (13 and 15 of 28 peptides, respectively). Immunoreactivity of some peptides overlap tectal laminae or layers, while others partly overlap. Again other peptides share one lamina, but may be sharply separated within the lamina.

2.04.3.4.10.(iii) Pretectum and BON The BON contains a small number of lightly labeled GABA-ir perikarya, mostly located in its dorsal half (Li and Fite, 1998). GABAergic afferents extend from the retina to the contralateral tectum, from the BON to the ipsilateral pretectal nucleus lentiformis mesencephali, and a second-order pathway from the isthmic nucleus bilaterally to the optic tectum (Li and Fite, 2001). In *S. salamandra*, the pretectal and the accessory optic system display many GABAergic neurons (Naujoks-Manteuffel *et al.*, 1994). Labeled fibers and cell groups stained for the enzyme NADPH-diaphorase were observed in the pretectal area. NADPH-diaphorase and catecholamines were co-distributed in these areas; however, restricted co-localization in the same neurons was found (Muñoz *et al.*, 1996).

2.04.3.5 Neurophysiology of the Retino-Tecto-Pretectal System

2.04.3.5.1 Response properties of RGCs Based on recordings from the optic nerve and in the superficial layers of the tectum, five classes of RGCs have been identified in ranid frogs and three classes in toads and in salamanders (frogs: Maturana *et al.*, 1960; Grüsser and Grüsser-Cornehls, 1970, 1976; Ewert and Hock, 1972; Grüsser-Cornehls and Langeveld, 1985; salamanders: Cronly-Dillon and Galand, 1966; Norton *et al.*, 1970; Grüsser-Cornehls and Himstedt, 1973; 1976). The classes differ in the size of the excitatory and inhibitory receptive field (RF), the response to the on- and/or offset of illumination, the response to stationary or moving stimuli of different velocity, size, shape, or contrast, and the response to stimulation with light of different wavelengths. In brief, three universal classes of RGCs exist, which appear to constitute (1) a shape/color pathway, (2) a motion pathway, and (3) an ambient illumination pathway

(for an extended summary on the properties of RGCs, see Roth *et al.*, 1998).

Neurons of the first class of RGCs respond either to moving or nonmoving objects and require relatively high visual contrast. They can be classified as small-field edge-detector cells. They exhibit low conduction velocity due to unmyelinated fibers. Their axons terminate in the uppermost layer of the optic tectum. Class-1 and class-2 cells of frogs, R2 cells of toads, and layer-1 cells of salamanders belong to this class. They may include several subclasses differing in color sensitivity, responses to light ON or OFF, and erasability or nonerasability of stimulation by stationary edges. These cells are most probably involved in the detection of small, high-contrast objects such as prey. They are comparable to X-cells in cats and to P-cells in primates (Spillmann and Werner, 1990).

The second class of RGCs comprises neurons that respond to small changes in contrast and small dislocations of edges. They do not respond to nonmoving objects and are considered medium-field motion-detector or ON–OFF cells. They exhibit high-conduction velocity due to myelination of fibers. Axons run to the thalamus, pretectum, and tectum in parallel; in the tectum, they terminate in the intermediate layer of retinal afferents. They are represented by class-3 RGC in frogs, R3 cells in toads, and layer-2 cells in salamanders. The RGCs of this class are predominantly involved in motion and movement pattern detection. They correspond to Y-cells in cats and M-cells in primates.

RGCs of the third class respond well to large objects and to changes in illumination in larger parts of the visual field, but do not respond well to small- or medium-sized objects. They are classified as large-field dimming-detector or OFF cells. They show high-conduction velocity and have thick myelinated fibers, which project in parallel to the thalamus, pretectum, and tectum. Inside the tectum, they terminate in the deepest layer of retinal afferents. Class-4 and R4 cells of frogs and toads, respectively, and layer-3 cells of salamanders form this class. They may be involved in predator detection, optomotor behavior, or respond to changes in overall illumination.

2.04.3.5.2 Tectum

2.04.3.5.2.(i) Topic organization Retinal afferents from each retina form a contralateral and an ipsilateral two-dimensional representation or map in the tectum. In the plethodontid salamander *Hydromantes*, these maps were identified on the basis of data from single-cell recording and neuroanatomy (Wiggers and Roth, 1991; Wiggers *et al.*, 1995). Each visual hemifield is projected completely onto the contralateral tectal hemisphere. The ipsilateral retinotopic projection covers roughly the rostral two-thirds of the tectal surface. This representation is rotated 180° compared to that of the contralateral retinotopic projection such that the contralateral and ipsilateral retinotectal projection run in opposite directions. The ipsilateral tectal representation from one eye and the contralateral representation from the other eye are in register, whereas the left and right contralateral and the left and right ipsilateral representations are arranged opposite to each other. An object that moves from lateral to frontal in the visual hemifield shifts from rostral to caudal in the ipsilateral tectum, i.e., in a direction opposite to the contralateral projection from the same eye. When objects move along the *z*-axis, i.e., straight toward or away from the animal, the two contralateral tectal representations move in the same direction and opposite to the two ipsilateral representations. This topographic arrangement apparently is the basis for a precise localization of objects and is also important for depth perception based on retinal disparity (Wiggers and Roth, 1994; Eurich *et al.*, 1995; Wiggers *et al.*, 1995).

2.04.3.5.2.(ii) Neurons The visual field covers an area of 360°–400° in different frog species. Several classes of tectal neurons have been defined by the size and shape of their RFs as well as their location in the visual field, by their responses to stationary or moving objects of different velocity, size, shape, or contrast, and their responses to the direction of movement. The RFs of tectal neurons range from 1° to the size of the entire visual field, and seven different types of tectal neurons have been identified in various frog species (cf. Grüsser and Grüsser-Cornehls, 1976). Ewert and von Wietersheim (1974) and Roth and Jordan (1982) studied the response properties of tectal neurons in *Bufo bufo*, and Schürg-Pfeiffer and Ewert (1981) those of *Rana temporaria*. A detailed comparison of response properties of tectal neurons in frogs and salamanders is given in Roth (1987).

In salamanders, tectal neurons were classified in *S. salamandra* (Grüsser-Cornehls and Himstedt, 1973; Himstedt and Roth, 1980; Finkenstädt and Ewert, 1983a, 1983b; Himstedt *et al.*, 1987), in *H. italicus* (Roth, 1982), and in *Hydromantes* and *Bolitoglossa subpalmata* (Wiggers *et al.*, 1995). In plethodontid salamanders, the RFs of tectal neurons are not evenly distributed across the tectum, but concentrate in the frontal area of the visual field (Wiggers *et al.*, 1995). More than half of recorded neurons are situated in the rostral tectum, and their RFs are located in the binocular visual field. The size and shape of RFs does not differ between in the monocular contralateral visual field and in the

binocular field. In *H. italicus*, the size of the RFs ranges from 10° to 360°, with an average of about 40° (Wiggers *et al.*, 1995); in *Plethodon shermani* (formerly *P. jordani*) the RFs in the binocular visual field range from 6° to 200° with an average of 22° (Schuelert and Dicke, 2005).

A comparison between tectal response types in urodeles and anurans shows that similar types can be found in *Salamandra* and *Bufo*, on the one hand, and plethodontid salamanders and *Rana*, on the other. In the two former species, a worm-preference type was found, i.e., neurons responded best to the presentation of a horizontal rectangle moving in direction of its long axis inside the RF, with lower frequencies to the presentation of a square and with lowest frequencies to a rectangle moving perpendicular to its long axis. Such a preference for worm-like stimuli was absent in tectal neurons of *Rana* and plethodontid salamanders. This difference fits nicely with natural prey preferences of the species under consideration (cf. Roth, 1987).

In these studies on the response properties of tectal neurons, the relationship between neuronal responses elicited by a given object and the response of the behaving animal to such a stimulus remained unclear. In toads, a strong correspondence between the configural features of elongated visual objects and the response selectivity of one type of neuron was proposed (Ewert and von Wietersheim, 1974; Ewert *et al.*, 1978; Schürg-Pfeiffer and Ewert, 1981). Other studies demonstrated that a combination of stimulus features rather than a single feature determines the responses of tectal neurons (Himstedt and Roth, 1980; Roth, 1982; Roth and Jordan, 1982). From these latter studies it was concluded that visual features are encoded only ambiguously by the spike rate of a single neuron, and that object recognition is based rather on population coding (an der Heiden and Roth, 1987).

In a recent extracellular recording and labeling study of tectal neurons in *Plethodon* (Schuelert and Dicke, 2005), prey dummies differing in size, contrast, velocity, or movement pattern were presented either singly inside the excitatory RF or paired with one stimulus inside and another stimulus outside the excitatory RF (Figure 15). The authors found that

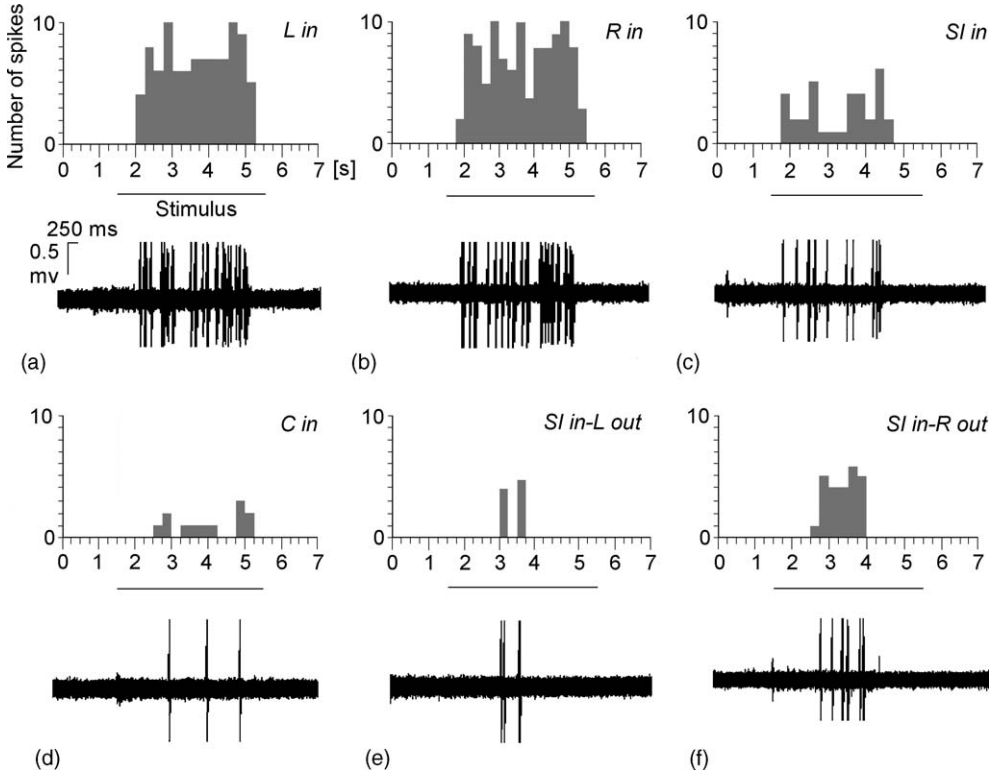

Figure 15 Responses of a tectal neuron in *Plethodon shermani* to the following stimuli: single presentation of a large-sized cricket (a; *L in*), a rectangle (b; *R in*), a still-image cricket (c; *SI in*) and a contrast-reduced cricket (d; *C in*) inside the excitatory receptive field (ERF). Although the number of spikes is comparable at single presentation of the L and the R, at paired presentation of the SI inside and the L (e; *SI in-L out*) outside the ERF, the spike number is much lower at presentation of *SI in-L out* compared to *SI in-R out*. Histograms of spike number at presentation of stimuli ($n=3$ for each stimulus type) are shown above the recording traces; the black line below the trace indicates the duration of stimulus presentation. From Schuelert, N. and Dicke, U. 2005. Dynamic response properties of visual neurons and context-dependent surround effects on receptive fields in the tectum of the salamander *Plethodon shermani*. *Neuroscience* 134, 617–632.

tectal neurons are not merely tuned to simple stimulus properties; rather, their responses are heavily influenced by the type of stimulus, i.e., the combination of single features, the familiarity of an object, and the context in which a visual stimulus occurs.

It became evident that in amphibians, visual object recognition involves much more complex spatial and temporal processing than previously assumed and concerns changes in spike number, temporal pattern, and dynamic changes in the size of the RF. Also, the response properties of tectal neurons indicate that these neurons integrate information across a much larger part of visual space than covered by the RF. For example, an inhibitory surround effect resulting in a decrease in the number of spikes and a reduction in RF size occurred at paired presentation, when a large-sized cricket or a rectangle was located outside the RF. However, this inhibition was significantly greater for the large-sized cricket stimulus than for the rectangle and indicates the biological relevance of the prey-like stimulus in object selection. This dynamic processing corresponds with the selection of stimuli in the visual orienting behavior of *Plethodon*, in which orientation toward a stimulus depended on the precise combination of different features (Schulert and Dicke, 2002). These findings suggest that prey recognition is guided by a number of visual features instead of a single feature and supports the idea of population coding as the basis for object recognition.

2.04.3.5.3 Nucleus isthmi Gruberg and co-workers found that unilateral lesions of the nucleus isthmi resulted in a scotoma to visually presented prey and threat stimuli in the contralateral monocular visual field (Gruberg *et al.*, 1991). A correlation exists between the size of the scotoma and the amount of nucleus isthmi ablated. Electrophysiological recording from positions within the area of the optic tectum including the scotoma reveal a roughly threefold increase in the size of the multi-unit RFs compared to mirror-image positions in the contralateral optic tectum. Across the entire extent of the isthmic nucleus, two superimposed maps exist, one representing the entire visual field of the contralateral eye, the other one representing the binocular visual field of the ipsilateral eye (Winkowski and Gruberg, 2002).

The role of the isthmic nucleus in enhancing intracellular calcium concentrations in retinotectal fibers was studied *in vitro*; results suggest that the input of the isthmic nucleus can facilitate retinotectal neurotransmission. This mechanism could be used to allow the frog to attend to a single prey stimulus in an environment of several prey stimuli (Dudkin and Gruberg, 2003). Recent recordings from the intermediate layer 7 of *Rana* revealed the existence of superimposed topographic maps of the monocular visual fields in the caudolateral tectum. The ipsilateral eye monocular visual field representation can be abolished by electrolytic ablation of the contralateral isthmic nucleus (Winkowski and Gruberg, 2005).

In the salamander *H. italicus*, the representation of the visual field and the properties of isthmic neurons were studied by Wiggers and Roth (1991). The RFs of isthmic neurons are centered in the frontal 100° field. The visual hemifield covered by neurons of each nucleus extends horizontally from 50° contralateral to 30° ipsilateral to the nucleus, and vertically from 36° below to 50° above the horizon. Thus, the projection fields of the isthmic nucleus overlap by 60°. About two-thirds of recorded neurons had their RFs within the upper part of the visual field, above eye level. The majority of neurons preferred stimulus sizes with edge lengths between 4.6° and 9.1°. The highest impulse rates, up to 25 impulses s^{-1}, were recorded at velocities between 20 and 36° s^{-1}. Half of isthmic neurons responded to stimuli from both eyes, whereas 35% responded only to contralateral stimulation. The remaining 14% responded weakly to ipsilateral stimulation and strongly to contralateral stimulation.

In summary, neurons of the isthmic nucleus reveal response properties that are similar to those found in tectal neurons; however, the representation of the visual space in the isthmic nucleus is not a simple copy of the tectal representation of visual space. The findings support the view of the isthmic nucleus as an essential structure involved in object localization and selection.

2.04.3.5.4 Pretectum In the pretectum of frogs and salamanders, a superficial nucleus (nucleus lentiformis in frogs) consisting of migrated, large-celled neurons and a deep subnucleus are found (Figure 16). Neurons in the pretectum in *R. pipiens* and *R. esculenta* are directionally selective and particularly sensitive to horizontal optokinetic patterns moving at velocities of 5–10° s^{-1} (Katte and Hoffmann, 1980). Neurons in the nucleus lentiformis mesencephali are involved in horizontal optokinetic nystagmus in *R. pipiens* (Fite *et al.*, 1989). At presentation of a large-field patterned stimulus at eight directions and three velocities of movement, all recorded units were spontaneously active and motion sensitive. Directional information appears to be encoded in the activity of a large population of motion-sensitive units, which includes both narrowly and broadly tuned individual response profiles. In contrast, neurons recorded in the nucleus/neuropil of the basal optic root respond more selectively to slowly

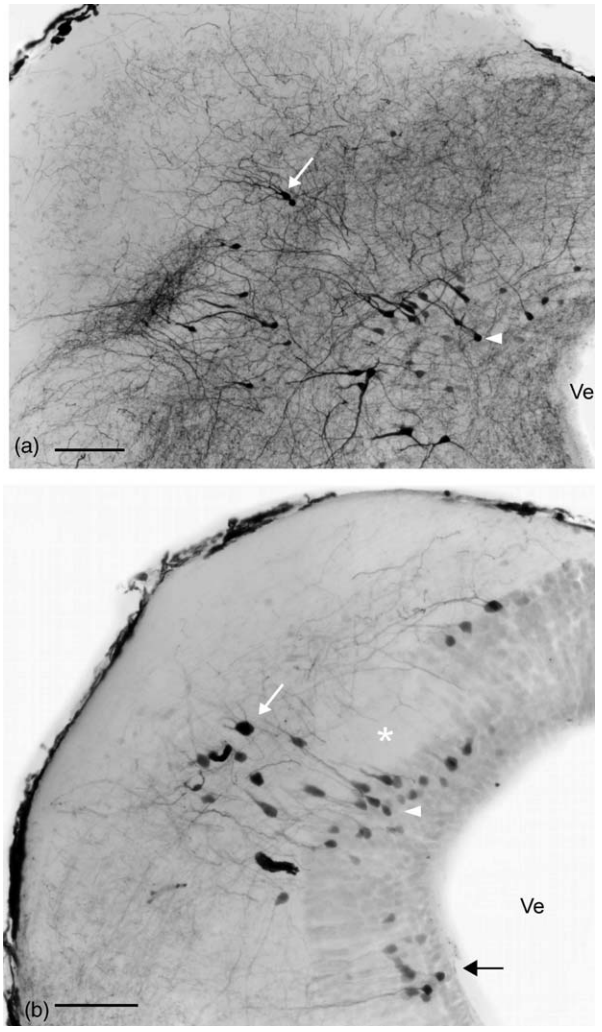

Figure 16 Microphotographs of transverse sections through the superficial and deep pretectal nucleus of *Discoglossus pictus* (a) and *Plethodon jordani* (b). Neurons were retrogradely labeled after application of biocytin to the ventral rostral medulla oblongata. Asterisk indicates the posterior commissure, white arrows and arrowheads point to neurons in the superficial and deep pretectal nuclei, respectively, and black arrow in B to ventral thalamic neurons. Ve, Ventricle. Scale bars: 100 μm. From Dicke, U., Roth, G., and Matsushima, T. 1998. Neural substrate for motor control of feeding in amphibians. *Acta Anat.* 163, 127–143.

moving vertical patterns, although horizontally sensitive neurons also have been reported (Katte and Hoffmann, 1980; Gruberg and Grasse, 1984).

Recordings of pretectal cells were also carried out in *S. salamandra* (Manteuffel, 1984a, 1984b, 1989; Sperl and Manteuffel, 1987). Two-thirds of recorded cells were sensitive to a temporonasal movement of stimuli; the majority preferred velocities between 1° and 5° s^{-1}. These neurons mostly possessed large RFs with a diameter of 82° and 34° in horizontal and vertical direction, respectively, and the RF center was situated always within the contralateral visual hemifield. Nearly half of pretectal cells were binocular and did not respond to ipsilateral stimulation. Cells in the BON of *Salamandra* and *T. cristatus* were also sensitive to temporonasal direction of stimulus movement, except one cell which was sensitive to the opposite direction (Manteuffel, 1982, 1984b). All neurons recorded from the BON or the nucleus of the oculomotor nerve were strictly monocular. Deeper and more caudal sites close to the BON cells that respond preferentially to vertical movement tend to be more numerous.

According to lesion experiments, the amphibian pretectum has proven to be essentially involved in control of gaze-stabilizing reflexes (Lázár, 1972; Montgomery *et al.*, 1982; Manteuffel *et al.*, 1983). In summary, the findings of recording and lesion experiments provide evidence that pretectal and accessory neurons are involved in oculomotor and optokinetic behaviors.

2.04.3.6 Diencephalon

The diencephalon of amphibians forms the walls surrounding the third ventricle and is, like that of all tetrapod vertebrates, traditionally divided into three parts, i.e., epithalamus, thalamus, and hypothalamus (Figures 17h–17j). The pretectum is considered to form either the posterior part of the thalamus or – together with the anterior portion of the tectum mesencephali – to represent a transition zone between diencephalon and mesencephalon surrounding the posterior commissure and called synencephalon (Kuhlenbeck, 1977).

2.04.3.6.1 Epithalamus and pineal complex The rostral part of the epithalamus contains the dorsal and ventral habenular nuclei and the habenular commissure. The ventral habenular nucleus is divided by the fasciculus retroflexus. Caudal to the habenular nuclei, the pineal gland or epiphysis, the posterior commissure, and below it the subcommissural organ (a gland) are found. The pineal gland is connected, via the pineal nerve, with the light-sensitive frontal organ located in the skin between the two eyes. Pineal gland and frontal organ project, among others, to the amygdala, pretectum, ventrolateral thalamic nucleus, and ventrolateral mesencephalic tegmentum.

2.04.3.6.2 Dorsal and ventral thalamus Dorsal and ventral thalamus are separated by the sulcus medialis, and the ventral thalamus is separated from the hypothalamus by the sulcus hypothalamicus (Figures 17h–17j, 5, and 6). In anurans, a number of thalamic nuclei can be distinguished morphologically. The dorsal thalamus is comprised of three

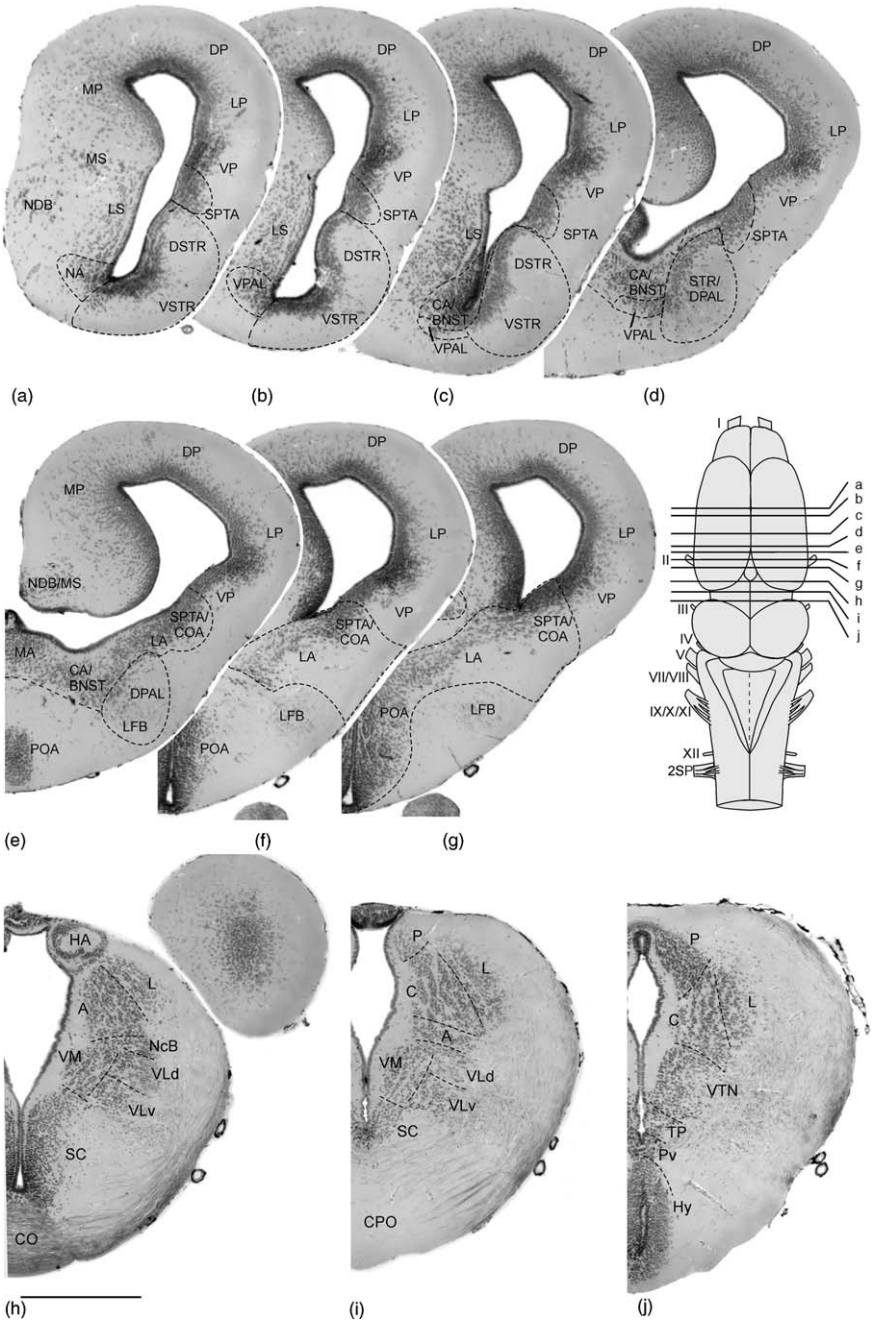

Figure 17 a–j, Transverse sections (Kluver–Barrera staining) through the telencephalon and diencephalon of *Bombina orientalis* at levels indicated in inset. Anterior mid-telencephalon (a), intermediate mid-telencephalon (b), posterior mid-telencephalon (c), anterior caudal telencephalon (d), mid-caudal telencephalon at the level of the foramen interventriculare (e), posterior caudal telencephalon at the level of the telencephalic commissures (f), posterior caudal telencephalon at the level of the magnocellular preoptic nucleus (g), anterior thalamus at the level of the optic chiasm and habenula (h), central thalamus at the level of the posterior commissure (i), and caudal thalamus at the level of the postoptic commissure (j). I, II, III, IV, V, VII, VIII, IX, X, XI, XII, cranial nerves; 2SP, second spinal nerve; MP, medial pallium; MS, medial septum; NDB, nucleus of the diagonal band of Broca; LS, lateral septum; NA, nucleus accumbens; VSTR, ventral striatum; DSTR, dorsal striatum; SPTA, striatopallial transition area; VP, ventral pallium; LP, lateral pallium; DP, dorsal pallium; CA, central amygdala; BNST, bed nucleus of the stria terminalis; VPAL, ventral pallidum; COA, cortical (olfactory) amygdala; LA, lateral (vomeronasal) amygdala; STR, striatum; DPAL, dorsal pallidum; LFB, lateral forebrain bundle; MA, medial amygdala; POA, anterior preoptic area; HA, habenula; VLd, dorsal portion of the ventrolateral nucleus; VLv, ventral portion of the ventrolateral nucleus; VM, ventromedial thalamic nucleus; SC, suprachiasmatic nucleus; L, lateral dorsal thalamic nucleus; A, anterior dorsal thalamic nucleus; CO, optic chiasm; CPO, postoptic commissure; C, central dorsal thalamic nucleus; VTN, ventral thalamic nucleus; TP, posterior tubercle; Pv, paraventricular organ; Hy, hypothalamus; P, posterior dorsal thalamic nucleus; NcB, nucleus of Bellonci. Scale bar: 500 μm. Modified from Roth *et al.* (2003, 2004).

periventricular nuclei, i.e., an anterior, a central, and a posterior (pretectal) nucleus, as well as of a migrated lateral nucleus with an anterior and posterior subdivision. The ventral thalamus consists of a periventricular ventromedial nucleus and a number of migrated nuclei, i.e., a ventrolateral nucleus subdivided into a dorsal and a ventral portion, a dorsally situated NB, and a superficial ventral nucleus (Neary and Northcutt, 1983; Roth *et al.*, 2003).

In salamanders, the dorsal and ventral thalamus consists – like the entire diencephalon – of a more or less compact cellular layer surrounding the third ventricle, with very few, if any, cells found in the white matter, and nuclei cannot be distinguished by morphological boundaries. However, on the basis of retrograde and anterograde tracing and intracellular labeling experiments, anterior, central, and posterior zones can be distinguished, with projection patterns that correspond to those found in anurans, but show great overlap. In the ventral thalamus, neurons with different projection patterns can be distinguished, which likewise correspond with the ventral thalamic divisions of anurans, but do not form distinct nuclei (Roth and Grunwald, 2000).

Sensory afferents to the thalamus include (1) primary visual afferents from the optic nerve and secondary visual afferents from the tectum, (2) secondary auditory afferents from the midbrain torus semicircularis, and (3) somatosensory and vestibular afferents from the spinal cord and the Vth and VIIth cranial nerves. As described above, the optic nerve/tract forms two neuropils in the thalamus, i.e., the NB and the CGT (Scalia *et al.*, 1968; Scalia and Gregory, 1970; Scalia, 1976; Levine, 1980; Roth *et al.*, 2003) (Figures 5 and 6). These two neuropils occupy most of the lateral white matter leaving a narrow zone between them and the gray matter of the thalamic nuclei. Here and in the entire lateral zone occupied by the retinofugal fibers, afferents from the optic tectum terminate (Dicke, 1999).

In the thalamus of *R. pipiens*, intensely labeled GABA-immunoreactive neurons and fibers were observed within the NB and CGT. In the pretectum, the posterior thalamic nucleus contained the most intensely labeled GABA-immunoreactive perikarya and fibers in the entire brain (Li and Fite, 1998). In *R. catesbeiana* and *X. laevis*, GABA-ir somata were distributed throughout the different areas of the thalamus. At mid-thalamic level, GABA-ir somata were arranged in columns that extended through the anterior, ventrolateral, and ventromedial thalamic nuclei (Hollis and Boyd, 2005). In the pretectum of *S. salamandra*, the transition zone of the dorsal to the ventral thalamus is almost completely devoid of GABA-ir cells (Naujoks-Manteuffel *et al.*, 1994), whereas the ventral thalamus contains a substantial number of GABAergic cells.

Numerous neurons stained for the enzyme NADPH-diaphorase were localized in the dorsal anterior, lateral anterior, central, and lateral posteroventral thalamic nuclei of *R. perezi*, and highly labeled terminal fields were found in the NB, the CGT, and the superficial ventral thalamic nucleus. The distribution of labeled cells did not correspond to any single known neurotransmitter or neuroactive molecule system. Abundant co-distribution of NADPH-diaphorase and catecholamines was found; double labeling techniques revealed restricted co-localization in the same neurons (Muñoz *et al.*, 1996).

Anterograde and retrograde tracing and intracellular labeling studies (Roth and Grunwald, 2000; Roth *et al.*, 2003; G. Roth *et al.*, unpublished data) reveal the projection pattern of dorsal thalamic neurons in anurans and urodeles (summary in Figure 18a–18c). These studies confirm earlier findings that the anterior nucleus or zone of the dorsal thalamus projects bilaterally or ipsilaterally via the medial forebrain bundle to the ventromedial, medial, dorsal, and dorsolateral part of the telencephalon (Kicliter, 1979; Neary, 1984) (Figures 19 and 20). Inside the anterior dorsal thalamic nucleus, the majority of neurons innervate the entire medial, dorsal and lateral pallium and most strongly the rostral portion of the medial and dorsal pallium. Many fibers form a dense terminal neuropil around the cellular prominence at the level of the sulcus rhinalis dividing the lateral and ventral pallium (Figure 15a, right). This thalamopallial tract as part of the medial forebrain bundle sends collaterals to the central and medial amygdala, nucleus accumbens, and septal nuclei (Figure 15a, right). A subpopulation of anterior dorsal thalamic neurons targets only the superficial neuropil in the ventral portion of the rostral medial pallium. These two pathways appear to supply sensory information to the pallium (with the exception of olfaction), because stimulation of the optic nerve (visual), spinal nerve (somatosensory), and torus semicircularis (auditory) elicits evoked potentials with shorter latency and higher amplitude in the rostral two-fifths of the medial pallium, from where responses flow into the caudal medial pallium and the dorsal and lateral pallium (F. Laberge, unpublished results).

An additional thalamotelencephalic pathway originating in the anterior dorsal thalamus and to a lesser extent from a region below runs, via the lateral forebrain bundle, to the caudal lateral pallium – a region that receives input from the main olfactory bulb (Moreno and Gonzalez, 2004).

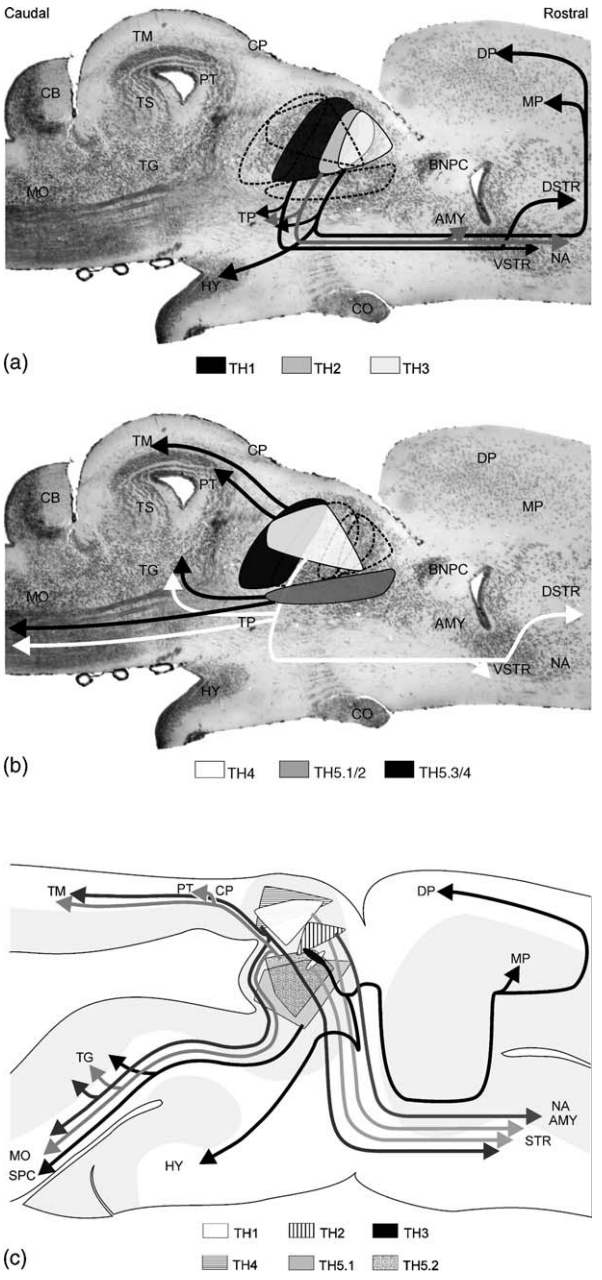

Figure 18 a, b, Diagrams of projection patterns of thalamic neurons combined with a lateral view of the caudal telencephalon, diencephalon, and mesencephalon in *Bombina orientalis*. c, Same in *Plethodon jordani*. CB, cerebellum; MO, medulla oblongata; TG, tegmentum; TM, mesencephalic tectum; TS, torus semicircularis; TP, posterior tubercle; CP, posterior commissure; DP, dorsal pallium; MP, medial pallium; BNPC, bed nucleus of the pallial commissure; DSTR, dorsal striatum; AMY, amygdala; NA, nucleus accumbens; VSTR, ventral striatum; PT, pretectum; CO, optic chiasm; Hy, hypothalamus; SPC, spinal cord. Modified from Roth and Grunwald (2000) and Roth *et al.* (2003).

The central dorsal nucleus/zone receives auditory input from the torus semicircularis (Hall and Feng, 1986; Mudry and Capranica, 1987; Feng *et al.*, 1990) and projects, via the lateral forebrain bundle, to the lateral amygdala and striatopallidum (Figure 21), where it contributes to the dense striatal neuropil (Figure 21B, right) (Wilczynski and Northcutt, 1983; Neary, 1988; Marín *et al.*, 1997a; Endepols *et al.*, 2004a). The ventromedial thalamic nucleus (probably homologous to the mammalian zona incerta), which receives auditory input from the torus semicircularis, likewise projects to the striatopallidum (frogs: Vesselkin *et al.*, 1980; Marín *et al.*, 1997a; Endepols *et al.*, 2004; salamanders: U. Dicke, unpublished data). The ventrolateral thalamic nucleus projects to the ventral pallium/SPTA. The lateral and posterior nuclei of the dorsal thalamus have no ascending but only descending projections to the pretectum, tectum, and tegmentum. (Roth and Grunwald, 2000; Roth *et al.*, 2003).

A long-standing question is whether the anterior and central nuclei/zones of the dorsal thalamus that project to the telencephalon receive direct or only indirect sensory afferents. Stimulation of the optic nerve in the salamander, *Plethodon*, and the frog, *Bombina*, elicited intracellular responses in the anterior dorsal thalamic nucleus, which showed either inhibition or excitation, but always at long latencies (about 35 ms on average). In contrast, short-latency, probably monosynaptic, responses could be recorded only from the ventral thalamus (Roth and Grunwald, 2000; Roth *et al.*, 2003) (Figure 22). Likewise, stimulation of the trigeminal and facial root (somatosensory and vestibular afferents) produced field potentials at long latencies in the anterior and central dorsal thalamus (Westhoff *et al.*, 2004), and the same holds for stimulation of the auditory pathway including the torus semicircularis (Mudry and Capranica, 1987; Hall and Feng, 1986; Feng *et al.*, 1990). Responses of neurons in the central dorsal thalamic nucleus at stimulation of auditory afferents show extreme habituation (Mudry and Capranica, 1987). This corroborates the view that the dorsal thalamus of frogs and salamanders does not receive substantial direct visual, auditory, somatosensory, and vestibular input. Rather, primary (only visual) or secondary (auditory, somatosensory) sensory afferents terminate in the ventral thalamus, which in turn projects to the dorsal thalamus (Dicke and Muhlenbrock-Lenter, 1998).

2.04.3.6.3 Response properties of thalamic neurons In anurans, thalamic and pretectal neurons were studied electrophysiologically in the toad, *Bufo americanus* and *B. bufo* (Ewert, 1971; Ewert and von Wietersheim, 1974). Ten classes of neurons were distinguished; eight of them responded to visual stimulation. One type of neuron responded best to large moving stimuli and had RF sizes between 30°

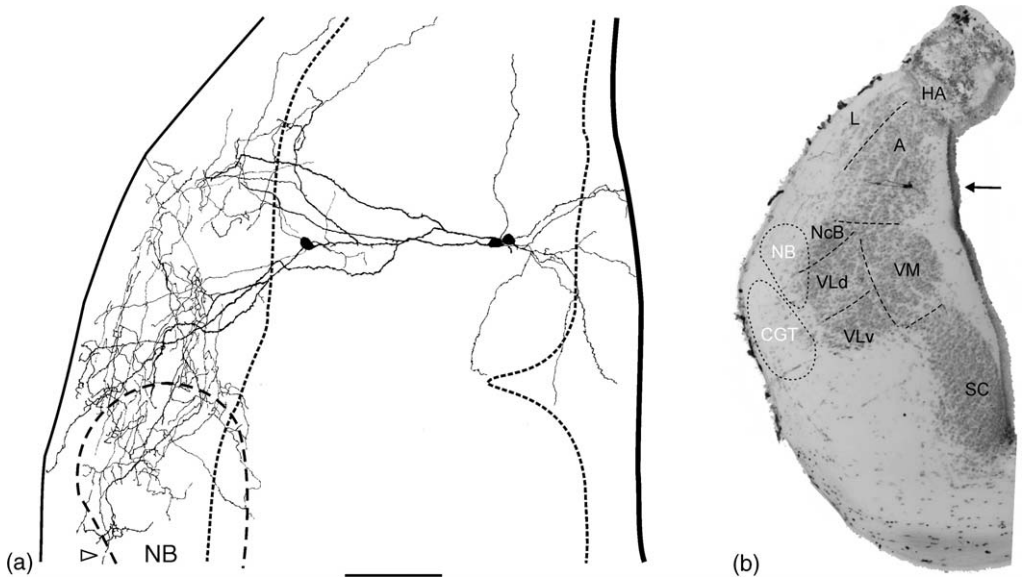

Figure 19 Camera lucida drawings of intracellularly labeled neurons in the dorsal thalamus of *Bombina orientalis*. a, Three neurons in the anterior dorsal thalamus, two of them in the periventricular anterior dorsal nucleus, with axons running to the medial and dorsal pallium, and one in the lateral anterior dorsal nucleus, without ascending projections. Only the dendrites of the latter enter the NB. The site of the neurons is indicated by a black arrow in (b). NB, neuropil of Bellonci; CGT, corpus geniculatum thalamicum; HA, habenula; A, anterior dorsal thalamic nucleus; L, lateral dorsal thalamic nucleus; NcB, nucleus of Bellonci; VLd, dorsal portion of the ventrolateral nucleus; VLv, ventral portion of the ventrolateral nucleus; VM, ventromedial thalamic nucleus; SC, suprachiasmatic nucleus. Scale bar: 100 μm. Modified from Roth, G., Grunwald, W., and Dicke, U. 2003. Morphology, axonal projection pattern and responses to optic nerve stimulation of thalamic neurons in the fire-bellied toad *Bombina orientalis*. *J. Comp. Neurol.* 461, 91–110.

and 46°. Another type responded selectively to motion; its RFs covered the contralateral or the entire visual field. Some of these neurons adapted quickly to stimuli, while others did not. Furthermore, one type of neuron responded best to stimuli approaching the toad along the *z*-axis, while another type represented luminance or darkness detectors.

Himstedt *et al.* (1987) studied the response characteristics of thalamic visual neurons in *S. salamandra* using a square, a horizontal, and a vertical rectangle as well as a random-dot pattern at different velocities. Neurons in the rostral thalamus, at the level of the NB and CGT, mostly had RF sizes between 36° and 50° and responded preferentially to the horizontal or vertical rectangle moved horizontally at low stimulus velocity. At intermediate velocity, most neurons responded best to the horizontal rectangle or square, and some responded best to the random-dot pattern. At high velocity, most neurons responded only to the horizontal rectangle or responded best to the square; one neuron responded best to the random-dot pattern. In the caudal dorsal thalamus, rostral to the pretectal neuropil and to the posterior commissure, RF sizes of neurons were comparable to those measured in the rostral thalamus. Likewise, in the majority of neurons the preferences of responses were similar to those of neurons in the rostral thalamus, but neurons did not respond to the random-dot pattern.

In summary, a topographically ordered representation of the visual space has not been reported in thalamic neurons. In general, the physiological properties of thalamic visual neurons differ from tectal neurons by their larger RFs and/or by profound adaptation to visual stimuli.

2.04.3.6.4 Hypothalamus The hypothalamus is divided into a preoptic and an infundibular region divided by the chiasmatic ridge (Neary and Northcutt, 1983). The preoptic region consists of the anterior preoptic area and the magnocellular preoptic nucleus. This nucleus is part of the cholinergic basal forebrain and also contains neurons belonging to the lateral, vomeronasal amygdala (see below). Therefore, it should be considered part of the telencephalon (secondary prosencephalon *sensu* Puelles, 1996). The suprachiasmatic nucleus is separated from the anterior preoptic area by a cell-free zone and is situated below the anterior thalamus and above the optic chiasm. Laterally, the posterior entopeduncular nucleus is found. The infundibular hypothalamus consists of a periventricular dorsal, ventral, and lateral nucleus composed of scattered neurons. Between the ventral hypothalamus and the ventromedial thalamic nucleus, the small posterior tuberculum and the equally small nucleus of the periventricular

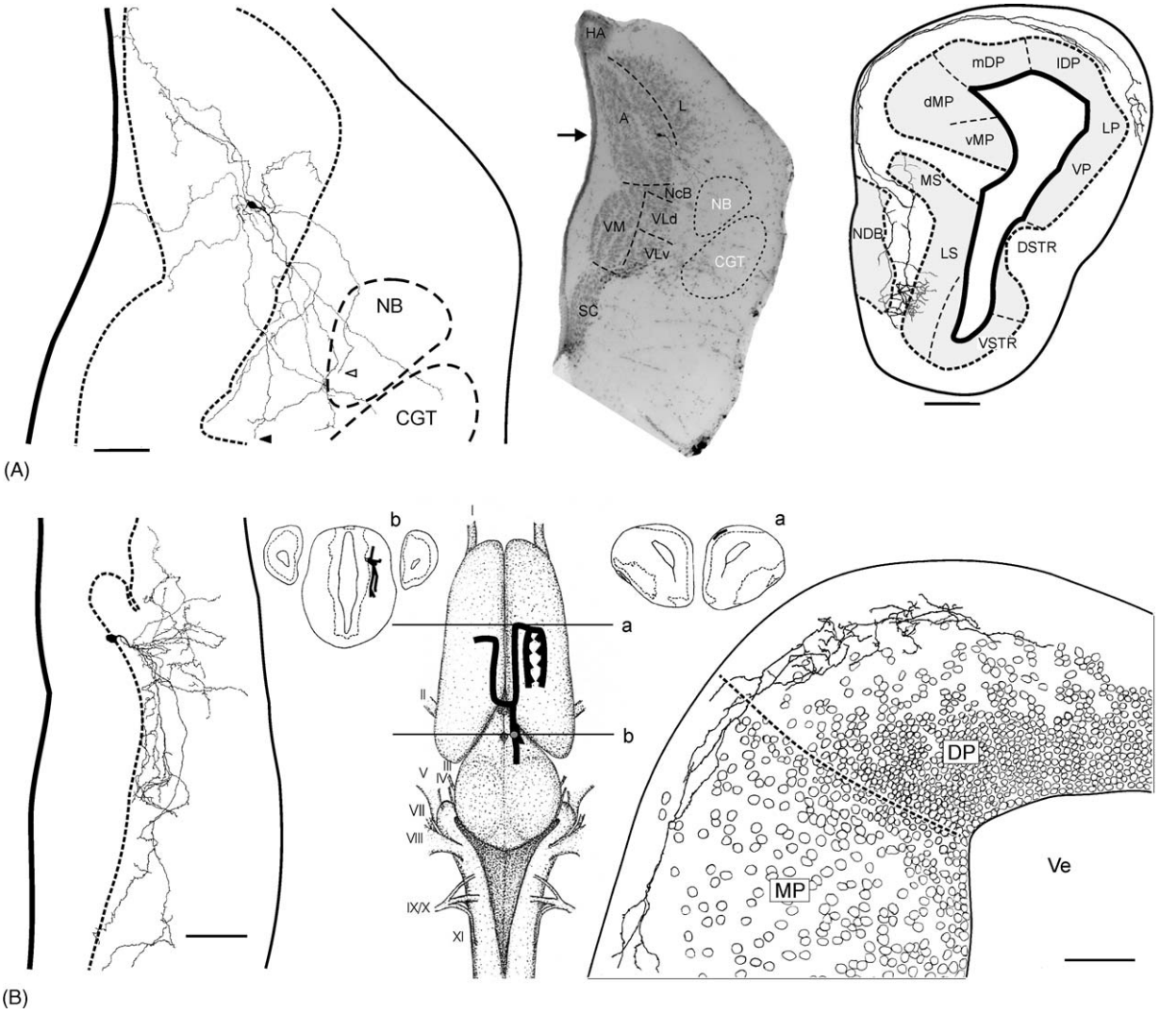

Figure 20 Camera lucida drawings of intracellularly labeled neurons in the anterior dorsal thalamus projecting to the medial and dorsal pallium of *Bombina orientalis* (A) and *Plethodon jordani* (B). In (A), middle, the site of the neuron is indicated by a black arrow; in (A), right, the course and terminal arborization of the axon in the septum, medial, dorsal, and lateral pallium is shown. In (B), middle, the site of the neuron (b) and the projection pattern of the neuron are indicated; in (B), right, the axon terminals in the medial and dorsal pallium at level (a) are shown. I–V, VII–XI, cranial nerves; NB, neuropil of Bellonci; CGT, corpus geniculatum thalamicum; A, anterior dorsal thalamic nucleus; HA, habenula; L, lateral dorsal thalamic nucleus; NcB, nucleus of Bellonci; VLd, dorsal portion of the ventrolateral nucleus; VLv, ventral portion of the ventrolateral nucleus; VM, ventromedial thalamic nucleus; SC, suprachiasmatic nucleus; NDB, nucleus of the diagonal band of Broca; LS, lateral septum; MS, medial septum; dMP, dorsal medial pallium; vMP, ventral medial pallium; mDP, medial dorsal pallium; lDP, lateral dorsal pallium; LP, lateral pallium; VP, ventral pallium; DSTR, dorsal striatum; VSTR, ventral striatum; Ve, ventricle; MP, medial pallium; DP, dorsal pallium. Scale bars in (A) left and (B) 100 μm, in (B) right 200 μm. Modified from Roth *et al.* (2003) and Roth and Grunwald (2000).

organ are found (Neary and Northcutt, 1983). The posterior tuberculum is considered to be homologous to the substantia nigra of mammals, because of the presence of dopaminergic neurons that show a distinct projection to the striatopallidal complex (Marín *et al.*, 1995).

The preoptic-hypothalamic region exhibits a pattern of wide connections, as revealed by biocytin tract tracing (Roth *et al.*, 2004) (Figure 23). The anterior preoptic area projects to the mediocentral amygdala, medial septum, nucleus of the diagonal band, suprachiasmatic nucleus, infundibular hypothalamus, ventral tegmentum, torus semicircularis, caudal tegmentum, and rostral medulla oblongata. Injection into the entire hypothalamus reveals projections to the periventricular zone of the central amygdala, ventral portion of the nucleus of the diagonal band,

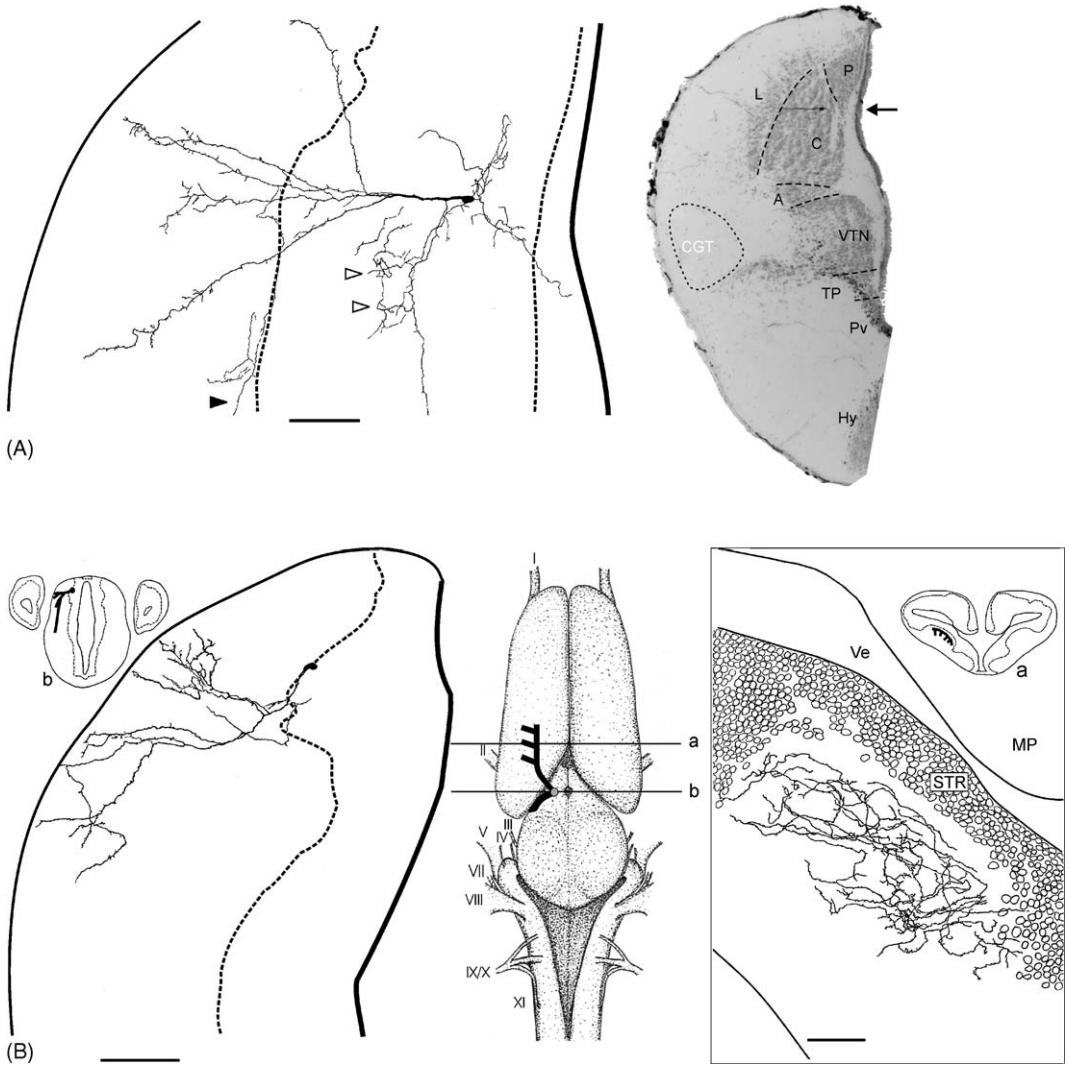

Figure 21 Camera lucida drawings of intracellularly labeled neurons in the central dorsal thalamus with ascending projections to the striatal neuropil in *Bombina orientalis* (A) and *Plethodon jordani* (B). Open arrowheads in (A) point to the ascending, black arrowhead to the descending axon. The site of the neuron is indicated by a black arrow in (A), right side. In (B), the site of the neuron is indicated by the left insert (b) and the projection pattern of the ascending axon in the middle with indications of the site of the striatal neuropil (a) and the neuron (a). Drawing in (B), right side, illustrates the terminal arborization of the axon inside the striatal neuropil at level a. I, II, III, IV, V, VII, VIII, IX, X, XI, cranial nerves; A, anterior dorsal thalamic nucleus; C, central dorsal thalamic nucleus; L, lateral dorsal thalamic nucleus; P, posterior dorsal thalamic nucleus; Pv, paraventricular organ; TP, posterior tubercle; CGT, corpus geniculatum thalamicum; Hy, hypothalamus; VTN, ventral thalamic nucleus; Ve, ventricle; STR, striatum; MP, medial pallium. Scale bars: 100 μm. Modified from Roth *et al.* (2003) and Roth and Grunwald (2000).

medial amygdala, the transition zone between medial pallium and dorsal septum, dorsal medial septum, nucleus accumbens, anterior preoptic area, and lateral (vomeronasal) amygdala; descending fibers run to the medulla oblongata and spinal cord. Injections of biocytin restricted to the dorsal hypothalamus reveals projections to the ventral pallidum, medial and central amygdala, ventral–lateral septum, caudal preoptic area, and suprachiatic nucleus.

Afferents to the anterior preoptic area originate in the nucleus of the solitary tract and the rostral medulla oblongata; the suprachiasmatic nucleus receives a sparse retinal input. The infundibular hypothalamus receives afferents from the ventral pallium, commissural medial pallium, medial and central amygdala, anterior preoptic area, suprachiasmatic nucleus, medial septum, nucleus of the diagonal band, lateral septum, lateral amygdala, magnocellular preoptic nucleus, dorsal and ventral thalamus, posterior tubercle, ventral tegmentum, torus semicircularis, locus coeruleus, and nucleus visceralis secundarius/parabrachial nucleus (Roth *et al.*, 2004).

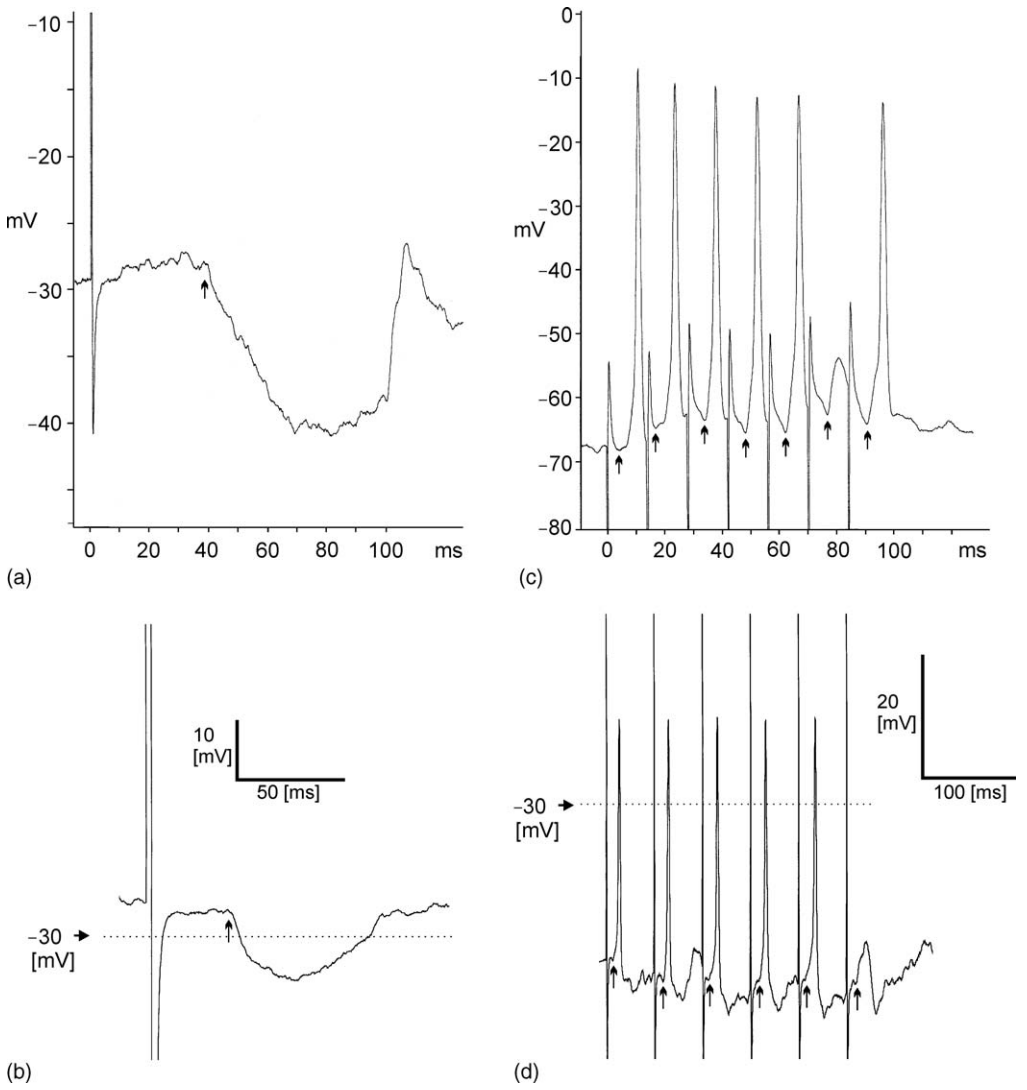

Figure 22 Responses of thalamic neurons to electrical stimulation of the optic nerve. Single sweeps are shown. Arrows indicate the onset of postsynaptic potentials. a, Inhibition in a dorsal thalamic neuron at long latency in *Bombina orientalis*. b, Same in *Plethodon jordani*. c, Short-latency response of a ventral thalamic neuron in *Bombina orientalis*. d, Same in *Plethodon jordani*. From Roth *et al.* (2003) and Roth and Grunwald (2000).

2.04.3.7 Telencephalon

The amphibian telencephalon, like that of all tetrapods, consists of pallial and subpallial regions (Figures 17a–17g). The pallium was traditionally divided into a medial, dorsal, and lateral pallium, but recent gene expression data led to a subdivision of the lateral pallium into a lateral and ventral pallium as different types of pallium (Puelles *et al.*, 2000; Puelles, 2001). Also, the rostral portion of the pallium appears to be a pallial region of its own. The subpallium includes a septal region below the medial pallium and a nucleus accumbens/ventral striatum situated in the rostral and central part of the ventromedial telencephalon, caudally tapering into a shell-like ventral pallidum situated below the septal region. The caudal ventral telencephalon of anurans is occupied medially and ventrally by the mediocentral amygdala and laterally by the lateral (vomeronasal) and cortical amygdala (Roth *et al.*, 2004). In salamanders, the mediocentral amygdala is situated more rostrally (Laberge and Roth, 2005). The striatopallidal complex is situated in the ventrolateral aspect of the telencephalon, bordered dorsally by the striatopallial transition area (SPTA).

2.04.3.7.1 Pallium

2.04.3.7.1.(i) Medial pallium The medial pallium of frogs and salamanders occupies the dorsomedial quadrant of the telencephalon and is characterized by extensive cell migration, which, however, does not

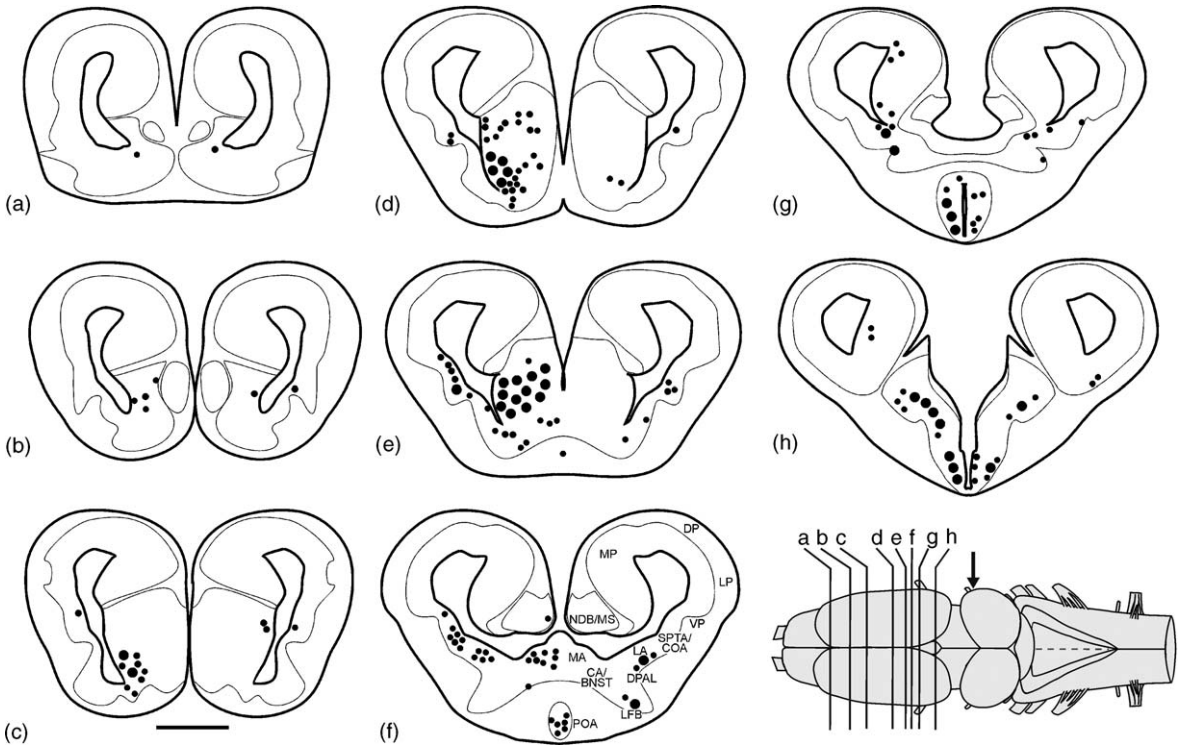

Figure 23 a–h, Schematic view of ipsilaterally labeled cell bodies after tracer application to the hypothalamus. Left, neurons retrogradely labeled after application of biocytin to the entire hypothalamus. Right, labeled neurons after application to the dorsal hypothalamus. Levels of sections and of tracer application (black arrow) are indicated in the inset. Large black dots represent 10 cell bodies, small black dots a single cell body. MP, medial pallium; LP, lateral pallium; VP, ventral pallium; DP, dorsal pallium; NDB, nucleus of the diagonal band of Broca; MS, medial septum; SPTA, striatopallial transition area; COA, cortical (olfactory) amygdala; LA, lateral (vomeronasal) amygdala; DPAL, dorsal pallidum; LFB, lateral forebrain bundle; BNST, bed nucleus of the stria terminalis; CA, central amygdala; MA, medial amygdala; POA, anterior preoptic area. Scale bar: 500 μm. Modified from Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35–61.

show any lamination. Ventrally, the medial pallium is confined by the zona limitans medialis – a cell-free zone separating the pallium from the septal region.

The dorsal border of the medial pallium of anurans is difficult to assess because of the gradual decline in cell migration toward the dorsal pallium. Consequently, some authors draw the border between medial and dorsal pallium more medially, i.e., at the level of the dorsomedial telencephalic sulcus (Hoffman, 1963; Northcutt, 1974; Scalia *et al.*, 1991) and others more laterally (Scalia, 1976; Northcutt and Kicliter, 1980; Neary, 1990; Northcutt and Ronan, 1992; Westhoff and Roth, 2002). After tracer application to the medial pallium, labeled neurons and fibers form a rather sharp border at the level of the dorsal sulcus (S. Mühlenbrock-Lenter, unpublished data). This would speak in favor of a more medial border between medial and dorsal pallium.

In the anuran medial pallium, authors distinguish either two subdivisions, i.e., a ventral small-celled part and a dorsal large-celled part (Röthig, 1912; Hoffman, 1963), or three subdivisions, i.e., a small-celled part, a large-celled part, and a transitional part (Neary, 1990). The medial pallium of salamanders has likewise been proposed to consist of two subdivisions, a ventral small-celled and a dorsal large-celled portion that might correspond to those observed in anurans (Herrick, 1933a; Northcutt and Kicliter, 1980; Neary, 1990).

Intratelencephalic afferents to the medial pallium originate in the dorsal and lateral pallium, mediocentral amygdala, nucleus accumbens, ventral pallidum and dorsal and central septal nuclei, the nucleus of the diagonal band, and the bed nucleus of the pallial commissure (Endepols *et al.*, 2005). Extra-telencephalic afferents come from the anterior dorsal thalamic nucleus, thalamic eminence, preoptic region, hypothalamus, periventricular organ, ventral tegmentum, locus coeruleus, raphe nucleus, and nucleus of the solitary tract (Roth *et al.*, 2004).

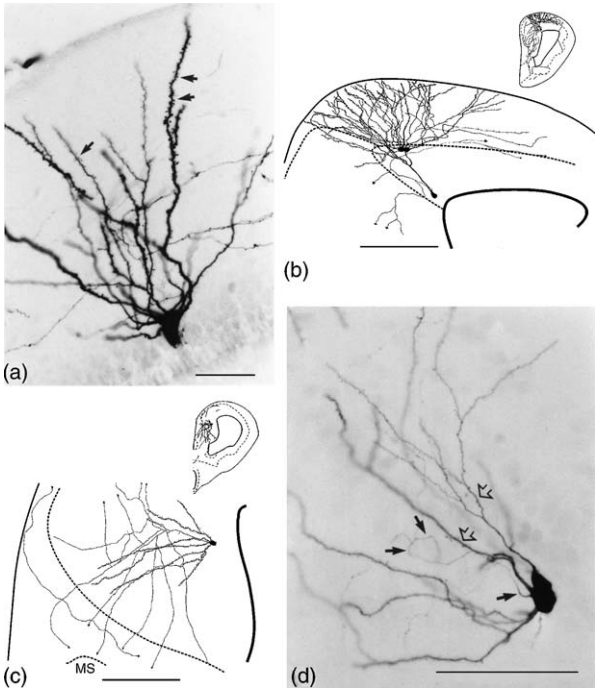

Figure 24 Microphotographs and reconstructions of intracellularly labeled neurons situated in the medial and dorsal pallium of *Discoglossus pictus* (a, c) and *Plethodon jordani* (b, d). a, Microphotograph of a cluster of two neurons situated in the dorsal pallium of *Discoglossus*; note that dendrites are heavily covered with spines (arrows). b, Camera lucida drawing of a cluster of neurons in the medial portion of the dorsal pallium (see inset) of *Plethodon*. c, Drawing of a type-1 neuron situated in the ventral half of the medial pallium of *Discoglossus* (see inset). d, Cluster of two neurons situated exactly at the border between medial and dorsal pallium, with one neuron in the medial and the other in the dorsal pallium. Dendrites of the dorsal pallial neuron are thinner than those of the medial pallial one (open arrows); filled arrows point to the primary axon of the medial pallial neuron. MS, medial septum. Scale bar: 50 μm in (a, d), 200 μm in (b, c). From Westhoff, G. and Roth, G. 2002. Morphology and projection pattern of medial and dorsal pallial neurons in the frog *Discoglossus pictus* and the salamander *Plethodon jordani*. *J. Comp. Neurol.* 445, 97–121.

Efferents of the medial pallium in the frogs *Discoglossus pictus* (Westhoff and Roth, 2002) and *Bombina orientalis* (G. Roth, unpublished data) have been studied by intracellular labeling (Figure 24). In *Discoglossus*, three types of medial pallial neurons were identified:

1. Neurons in the ventral medial pallium with bilateral projections to telencephalic areas including septum, amygdala, and striatum (weak), and diencephalic areas including the preoptic area, hypothalamus, anterior dorsal, and ventral thalamus (Figure 24c).
2. Neurons in the dorsal medial pallium with projections to the contralateral medial pallium and only ipsilateral projections to the dorsal and lateral pallium, septum, nucleus accumbens, amygdala, preoptic area, hypothalamus, anterior dorsal, and ventral thalamic nucleus.
3. Neurons at the border between medial and dorsal pallium with ipsilateral and contralateral projections to the medial and dorsal pallium, ipsilateral projections to the septum, and no extra-telencephalic projections.

In *Bombina*, of the neuron clusters labeled in the dorsal and intermediate medial pallium, all except one exhibited projections to the contralateral medial pallium and septum and ipsilateral projections to the dorsal pallium, septum, and nucleus accumbens. In addition, a substantial number of neurons projected to the dorsal portion of the ipsilateral lateral pallium. Two-thirds of them had extra-telencephalic to the suprachiasmatic nucleus, dorsal or ventral hypothalamus, and rostral tegmentum. Projections to the ventral pallium or to the dorsal portion of the striatopallidum originated only from neurons situated in rostral pallial regions. In contrast to the situation found in *Discoglossus*, neurons with and without extra-telencephalic projections showed no clear spatial separation. Of the 10 clusters labeled in the ventral-most part of the medial pallium, all projected to the dorsal septum and to the intermediate medial pallium, two to the dorsal medial and the medial part of the dorsal pallium, one to the nucleus accumbens, and four to the eminentia thalami. None projected to the contralateral side or to any extra-telencephalic target.

In the salamander, *Plethodon*, medial pallial neurons could be divided into a dorsal and a ventral group (Westhoff and Roth, 2002). Dorsal neurons project bilaterally to all telencephalic areas and to the preoptic area, ventral thalamus, and caudal hypothalamus. Ventral neurons project bilaterally to the medial pallium, medial septum, and nucleus accumbens, ipsilaterally to the dorsal pallium, and contralaterally to the anterior preoptic area and hypothalamus.

2.04.3.7.1.(ii) Dorsal pallium The delimitation of the amphibian dorsal pallium is likewise debated. While some authors denied the existence of a dorsal pallium in anurans (Kicliter and Ebbesson, 1976) or defined it as a narrow dorsal band between the medial and lateral pallium (Gaupp, 1899; Herrick, 1933b; Ariens Kappers *et al.*, 1936; Hoffman, 1963; Scalia *et al.*, 1991), other authors positioned the dorsal pallium (again as a relatively narrow band) more laterally, occupying the dorsal portion of the earlier lateral pallium (Northcutt, 1974; Northcutt and Kicliter, 1980; Northcutt and Ronan, 1992). Here, we adopt the above view that the border between medial and dorsal pallium is marked by the dorsomedial telencephalic sulcus and the border

between dorsal and lateral pallium by the rhinal sulcus.

The dorsal pallium consists of a periventricular cellular layer of densely packed somata and a number of migrated neurons which are substantial in number medially and decrease toward the lateral pallium. There is no sign of lamination.

Extra-telencephalic afferents to the dorsal pallium come from the anterior dorsal thalamic nucleus, hypothalamus, nucleus parabrachialis, and raphe nuclei (Roth *et al.*, 2003, 2004). Intra-telencephalic afferents originate mostly from the ipsi- and contralateral medial pallium and the ipsilateral lateral pallium, including the cellular prominence. Intracellular labeling experiments in the frogs, *Discoglossus* (Westhoff and Roth, 2002) and *Bombina* (G. Roth, unpublished data), reveal that dorsal pallial neurons in general lack extra-telencephalic and reveal only ipsilateral intra-telencephalic projections. They project ipsilaterally to the medial and lateral pallium and some of them also to the septal region, mostly to its dorsal part, as well as to the nucleus accumbens (Figure 3a).

In the salamander, *Plethodon* (Westhoff and Roth, 2002), neurons in the dorsal pallium are likewise defined by the absence of extra-telencephalic projections. Neurons in the medial part of the dorsal pallium project to the contralateral medial pallium and to the ipsilateral medial pallium, septum, nucleus accumbens, medial amygdala, and internal granular layer of the olfactory bulb (Figure 24b). Neurons in the lateral dorsal pallium have no contralateral projection; they project mainly to the ipsilateral medial pallium and about half of them to the ipsilateral septum. If we consider the medial portion of the dorsal pallium as representing the dorsal-most portion of the medial pallium, then the situation becomes similar in urodeles and anurans.

2.04.3.7.1.(iii) Lateral and ventral pallium
Traditionally, the lateral pallium has been divided into a dorsal and a lateral portion (cf. Kicliter and Ebbesson, 1976) divided by the rhinal sulcus and the lateral pallial cellular prominence. However, recent gene expression data demonstrate that these parts have to be considered different types of pallium called lateral and ventral pallium (cf. Puelles *et al.*, 2000; Puelles, 2001).

The lateral pallium receives essentially the same input as the dorsal pallium and some input from the ventral pallium as well. Intracellular labeling in *Bombina* (G. Roth, unpublished data) demonstrates that neurons in the precommissural lateral pallium are similar in morphology to those in the dorsal pallium and project to the dorsal and medial pallium and the dorsal septal region, often with extensive arborization, and a few axon collaterals extend to the ventral pallium. Neurons in the caudal, postcommissural portion of the lateral pallium likewise project to the dorsal and medial pallium, septum, and diagonal band of Broca. Neurons in the posterior part send axons rostrally at a subpial position inside the diagonal band of Broca, resembling the perforant path of mammals and terminating in the rostromedial pole of the telencephalon. In addition, neurons in the posterior part send dendrites and axons around the ventral caudal pole of the hemisphere accompanying the course of the lateral olfactory tract (olfactohabenular tract). This tract extends through the fiber layer of the lateral pallium close to the cellular prominence (and ventral to it); in the precommissural portion of the lateral pallium, it gives off only a few and in the postcommissural, caudal portion a substantial number of collaterals.

The ventral pallium is separated from the lateral pallium by the dorsolateral cellular prominence. The accessory (vomeronasal) olfactory tract runs through the periventricular cellular layer of the ventral pallium giving off numerous collaterals on its way to the vomeronasal amygdala. The ventral pallium terminates at the level of the pallial commissures where it merges with the vomeronasal amygdala. It receives intratelencephalic afferents from the dorsally adjacent lateral pallium, the caudal medial pallium, vomeronasal amygdala, nucleus accumbens, and striatopallidum. Extra-telencephalic afferents originate in the preoptic area, hypothalamus, anterior and central dorsal thalamus (weak), and the tegmental parabrachial nucleus (Moreno and González, 2004).

Intracellular labeling (Roth *et al.*, 2004, and unpublished data) reveals that neurons situated in the ventral pallium differ in morphology from those found in the lateral pallium in that they do not, or with only few dendrites, reach into the lateral pallium; rather, they extend their dendrites laterally or ventrolaterally (Figure 25). In most cases, ascending axons reach the accessory or main olfactory bulb; descending axons terminate in the neuropil of the vomeronasal and central amygdala, ventral pallidum, suprachiasmatic nucleus, and dorsal and ventral hypothalamus. Moreno and González (2004) consider the ventral portion of the ventral pallium (called SPTA by Marín *et al.*, 1998) a separate region, which they interpret as anterior amygdala (see below). However, there are no major differences in the morphology and projection pattern between the more dorsal portion of the ventral pallium and the SPTA (Roth *et al.*, 2004).

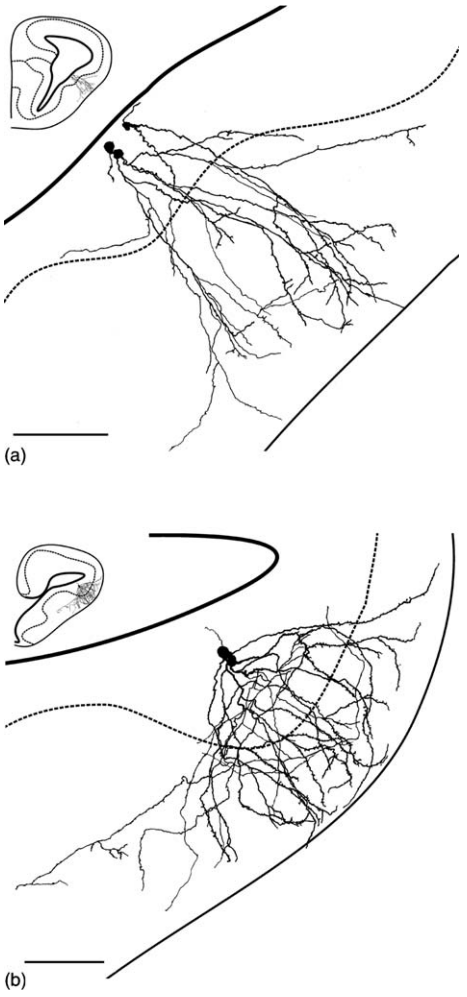

Figure 25 Reconstruction of an intracellularly labeled neuron situated in the ventral pallium/SPTA of *Bombina orientalis* (a) and *Plethodon shermani* (b). This type of neuron projects to the vomeronasal neuropil and the hypothalamus. Scale bar: 100 μm. From Roth *et al.* (2004) and Laberge and Roth (2005).

2.04.3.7.1.(iv) Rostral pallium Neurons situated in the rostral pole of the medial, dorsal, lateral, and ventral pallium receive the mass of dorsal thalamic afferents (see above) and differ in their projection pattern from neurons in more posterior regions in that all of them project to the main olfactory bulb and some of them to the SPTA and dorsal or dorsolateral edge of the dorsal striatal complex (G. Roth, unpublished data).

2.04.3.7.1.(v) Summary In summary, based on intracellular labeling, tract-tracing, and (immuno)-histochemical experiments, we can distinguish the following areas of the pallium of frogs and salamanders:

1. A medial pallium, which in many species is divided into a small-celled ventral and a large-celled dorsal portion. Neurons in this region, except those situated in the ventral part, generally have wide intra- and extra-telencephalic projections, the latter predominantly to the hypothalamus and ventral and dorsal thalamus. In frogs, ventral neurons, except those in the ventral-most part, exhibit mostly bilateral projections and dorsal neurons only ipsilateral extra-telencephalic projections. Most authors consider the dorsal portion of the medial pallium as homologous to the mammalian Ammon's horn and the ventral portion to the subiculum; a dentate gyrus seems to be missing.
2. A dorsal pallium separated from the medial pallium by the dorsal pallial sulcus. A dorsomedial portion (which could also be considered the dorsal portion of the medial pallium) contains neurons that have projections to the contralateral medial pallium via the anterior commissure and ipsilateral projections to the olfactory bulb to medial and ventral limbic centers and extra-telencephalic targets, mostly the preoptic region and hypothalamus; and a dorsolateral portion that contains neurons with projections confined to the ipsilateral dorsal septum, medial and lateral pallium, and are lacking extra-telencephalic projections.
3. A rostral-intermediate, or precommissural, lateral pallium, with neurons that project to the medial, dorsal, and ventral pallium and to the main olfactory bulb. The lateral olfactory tract runs through this region around the cellular prominence, but gives off only a few collaterals on its way to the caudal pallium.
4. A caudal, postcommissural, lateral pallium that receives massive input from the lateral olfactory tract and contains neurons that send their dendrites and axons along that tract (here the olfactohabenular tract) to the dorsal and medial pallium and to the entire septum.
5. A ventral pallium, including the SPTA containing neurons that project to the accessory olfactory bulb (AOB), the neuropil adjacent to the vomeronasal amygdala and preoptic region, striatopallidum, suprachiasmatic nucleus, and hypothalamus.
6. A rostral pallium occupying the rostral pole and projecting, unlike the medial, dorsal, and lateral pallium and like the ventral pallium, to the dorsal edge of the striatal complex.

The homologies and functions of the pallial regions mentioned are largely unclear. Most authors agree that the medial pallium is homologous to at least parts of the mammalian hippocampus,

i.e., Ammon's horn and subiculum, and that a dentate gyrus is lacking (for a more extended discussion see Westhoff and Roth, 2002). Extra- and intracellular recordings from neurons of the medial pallium in frogs including *B. orientalis* after stimulation of visual, somatosensory, and olfactory afferent pathways reveal only multimodal response properties (Supin and Guselnikov, 1965; Karamian *et al.*, 1966; F. Laberge and G. Roth, unpublished data). A few studies suggest that the medial pallium is involved in learning and memory formation (Finkenstädt and Ewert, 1988; Wenz and Himstedt, 1990; Papini *et al.*, 1995; Ewert *et al.*, 2001).

The function of the dorsal pallium is unclear. It receives essentially the same sensory and associative afferents as the medial pallium, but lacks extra-telencephalic and, with the exception of the dorsal septum, extra-pallial efferents. The response properties of neurons in the medial portion of the dorsal pallium are indistinguishable from those of the adjacent medial pallial neurons, which means that unimodal sensory areas are lacking. Thus, the dorsal pallium appears to have integrative-associative and limbic but no primary sensory functions.

The lateral pallium in the traditional sense (i.e., including the ventral pallium) is generally considered an olfactory pallium and homologous to the mammalian piriform cortex. However, although the zone around the cellular prominence dividing the lateral and ventral pallium receives collaterals from the lateral olfactory tract (originating in the main olfactory bulb), these collaterals are substantial only in the caudal, postcommissural portion. Thus, at least the caudal portion of the lateral pallium has to be considered olfactory pallium, while the function of the rostral and intermediate (precommissural) portion remains unclear.

The ventral pallium includes, together with the SPTA, the zone between the lateral pallium and the striatopallidal complex stretching from the olfactory bulb to the level of the telencephalic commissures, where it merges with the olfactory and lateral, vomeronasal amygdala. The ventral pallium is crossed by the accessory olfactory tract originating in the accessory olfactory or vomeronasal bulb; this tract gives off numerous collaterals on its way to the vomeronasal amygdala, where it forms a dense terminal neuropil. Thus, it is safe to consider the ventral pallium representing the vomeronasal pallium homologous to the mammalian posteromedial cortical amygdala (of pallial origin). Detailed functional studies are lacking.

2.04.3.8 Subpallium

2.04.3.8.1 Striatopallidal complex The dorsal striatum occupies the ventrolateral wall of the telencephalic hemisphere (cf. Figures 17a–17d). It is distinguishable from the ventral pallium/SPTA by neurons that extend their dendritic trees into the striatal neuropil (Roth *et al.*, 2003). The majority of the intracellularly labeled neurons are covered with spines and resemble the medium-spiny neurons of the mammalian caudate-putamen (cf., Heimer *et al.*, 1995) (Figure 26).

The dorsal striatopallidal complex receives afferents from a wide variety of brain regions including the medial pallium, ventral pallium-SPTA, vomeronasal amygdala, a number of mesencephalic and rhombencephalic nuclei, including the raphe nucleus, locus coeruleus, parabrachial nucleus, and the nucleus of the solitary tract, the hypothalamic-preoptic area, and the central dorsal and ventromedial thalamic nuclei (Marín *et al.*, 1997d). Efferents reach the medial and lateral amygdala, dorsal (sparse) and ventral thalamic nuclei, posterior tubercle, pretectum, tectum mesencephali, torus semicircularis, mesencephalic, and rhombencephalic reticular nuclei and the caudal brainstem (Marín *et al.*, 1997a). Endepols *et al.* (2004) as well as Roth *et al.* (2004) demonstrated that neurons with descending projections to the caudal tegmentum and rostral medulla oblongata are mostly found in the intermediate and caudal portion of the striatopallidal complex, and that more rostrally situated neurons project to the caudal portion of that complex (Figure 27). No projections to the medial or dorsal pallium are found.

The striatopallidum of amphibians and of tetrapod vertebrates in general (Reiner *et al.*, 1998) is characterized by the presence and co-localization of certain transmitters and neuropeptides such as glutamate, GABA, acetylcholine, dopamine, substance P, dynorphin, and enkephalin. GABAergic neurons are densely packed close to the ventricle, whereas cholinergic neurons typical of the amniote striatum are absent (Marín *et al.*, 1998; Mühlenbrock-Lenter *et al.*, 2005), but scattered ACh neurons are found in the dorsal pallidum (Marín *et al.*, 1998; Mühlenbrock-Lenter *et al.*, 2005). Thyroxin-hydroxylase-immunoreactive fibers indicating the presence of dopamine or noradrenaline are found in high concentrations in the cellular layer of the striatopallidum. Enkephalin-ir cell bodies are found in the rostral striatopallidum, i.e., the striatum proper. The striatal neuropil exhibits the highest density of Met+Leu-enkephalin-ir fibers, but many immunoreactive axons are found among cell bodies.

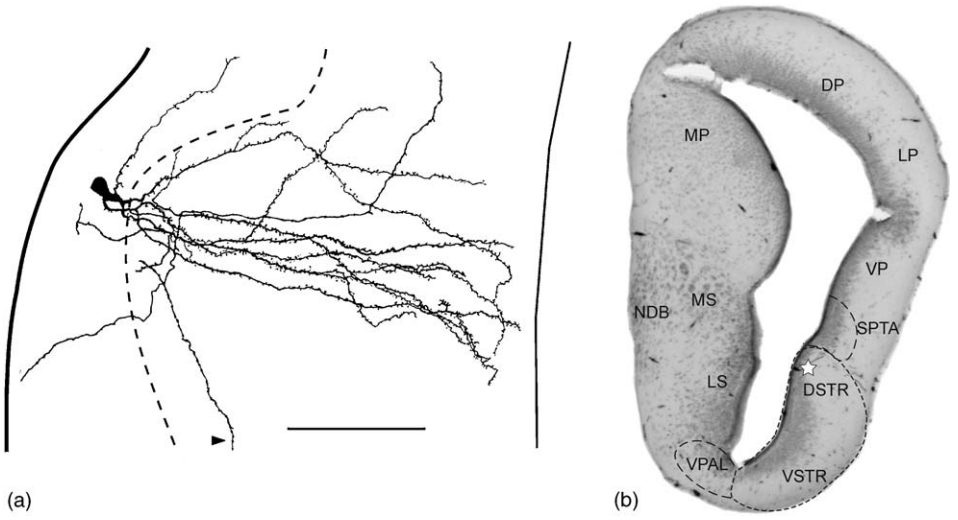

Figure 26 Reconstruction of an intracellularly labeled neuron situated in the striatopallidal complex of *Bombina orientalis*. Dendrites are heavily covered with spines. The site of the neuron is indicated by a white star in (b). This type of neuron has descending projection to the medulla oblongata. MP, medial pallium; MS, medial septum; NDB, nucleus of the diagonal band of Broca; LS, lateral septum; VPAL, ventral pallidum; VSTR, ventral striatum; DSTR, dorsal striatum; SPTA, striatopallial transition area; VP, ventral pallium; LP, lateral pallium; DP, dorsal pallium. Scale bar: 100 μm (a); 500 μm (b). From Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35–61.

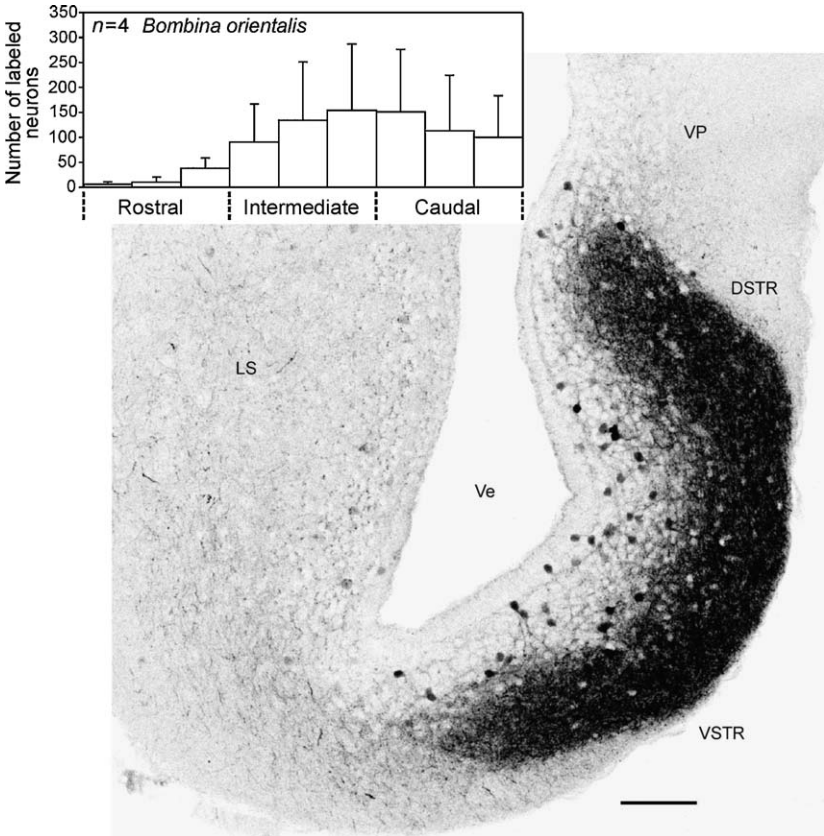

Figure 27 Inverted laser scanning image of labeled structures in the intermediate striatum of *Bombina orientalis* (transverse section) after tracer injection into the ipsilateral forebrain bundle. The inset demonstrates that the number of neurons with descending projections is low in the rostral and high in the intermediate and caudal portion of the striatopallidal complex; each column represents 150 μm. LS, lateral septum; Ve, Ventricle; VSTR, ventral striatum; DSTR, dorsal striatum; VP, ventral pallium. Scale bar: 100 μm. Modified from Endepols, H., Roden, K., and Walkowiak, W. 2004. Dorsal striatopallidal system in anurans. *J. Comp. Neurol.* 468, 299–310.

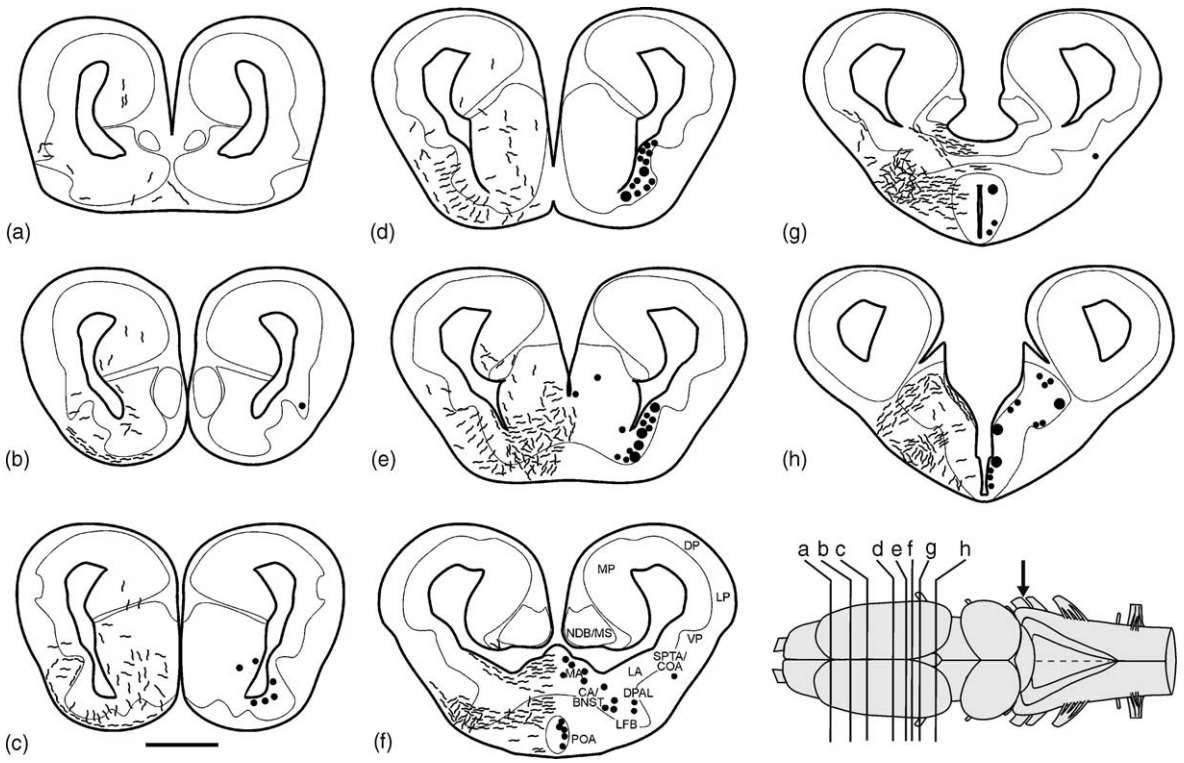

Figure 28 a–h, Schematic view of anterogradely labeled fibers (left hemisphere) and retrogradely labeled cell bodies (right hemisphere) in the telencephalon of *Bombina orientalis* after tracer application to the rostral medulla oblongata. Levels of sections and site of tracer application (black arrow) are indicated in the inset. Large black dots represent ten cell bodies, small black dots a single cell body. MP, medial pallium; LP, lateral pallium; VP, ventral pallium; DP, dorsal pallium; NDB, nucleus of the diagonal band of Broca; MS, medial septum; SPTA, striatopallial transition area; COA, cortical (olfactory) amygdala; LA, lateral (vomeronasal) amygdala; DPAL, dorsal pallidum; LFB, lateral forebrain bundle; BNST, bed nucleus of the stria terminalis; CA, central amygdala; MA, medial amygdala; POA, anterior preoptic area. Scale bar: 500 μm. Modified from Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35–61.

In contrast, Leu-enkephalin-ir fibers are sparse in the striatum proper. Its concentration increases along the rostrocaudal extent of the striatopallidum, with a maximum in the dorsal pallidum. Substance-P neurons are labeled in the rostral part of the striatal complex (Marín *et al.*, 1998; Endepols *et al.*, 2004; Mühlenbrock-Lenter *et al.*, 2005).

These immunohistochemical findings, together with the projection pattern of striatopallidal neurons mentioned, corroborates the view of Endepols *et al.* (2004) that the rostral portion of the dorsal striatopallidal complex can be regarded as dorsal striatum proper and the caudal portion as the dorsal pallidum, with a transition zone in between (Figure 28).In mammals, the striatum consists of functional compartments (striosomes and matrix) which differ in input and output as well as in immunohistological staining patterns (Graybiel and Ragsdale, 1978, 1983; Heimer *et al.*, 1995). Such compartments are absent in amphibians, but neurons and fibers of different immunoreactivity are arranged in layers (Mühlenbrock-Lenter *et al.*, 2005).

2.04.3.8.2 Ventral striatopallidal complex: nucleus accumbens/ventral striatum and ventral pallidum The ventral striatum *sensu* Northcutt and Kicliter (1980), i.e., the area ventrally adjacent to the dorsal striatopallidal complex (cf. Figures 17a–17d), most probably is not homologous to the ventral striatum of mammals. There is no difference in immunohistochemistry and the projection pattern between the dorsal and ventral parts of the dorsal striatopallidal complex (Mühlenbrock-Lenter *et al.*, 2005) and neurons in the two regions exhibit the same morphology and projection pattern (Roth *et al.*, 2004). Instead, the amphibian nucleus accumbens/ventral striatum is found in the medial rostral ventral telencephalon and extends caudally to what is now considered the ventral pallidum, which is confined to the superficial layer

surrounding the rostral central amygdala–BNST complex (BNST, bed nucleus of the stria terminalis) (Marín *et al.*, 1997a, 1997d, 1998; Roth *et al.*, 2004).

The nucleus accumbens receives afferents from the olfactory bulb, medial pallium, SPTA, medial amygdala, preoptic area and hypothalamus, dorsal and ventral thalamus, and posterior tubercle, i.e., from the same mesencephalic and rhombencephalic reticular nuclei that project to the striatopallidum, and from the anterior dorsal thalamic nucleus. Efferents run to the medial amygdala, preoptic area and hypothalamus, posterior tubercle, and to reticular brainstem nuclei (Marín *et al.*, 1997a, 1997d, 1998; Roth *et al.*, 2004).

In the ventral pallidum of anurans, as in other vertebrate species, a substantial number of numerous cholinergic neurons are found. Also, the nucleus accumbens/ventral striatum and the ventral pallidum are richly supplied by noradrenergic/dopaminergic fibers as well as by fibers containing substance P. In addition, somatostatin-ir fibers are found in the entire complex, and a strong enkephalinergic innervation exists in the rostral nucleus accumbens (Mühlenbrock-Lenter *et al.*, 2005). Nucleus accumbens/ventral striatum and ventral pallidum are closely interconnected (Marín *et al.*, 1997d; Roth *et al.*, 2004), but reveal only weak connections to the dorsal striatopallidum in the ventrolateral telencephalic wall – a situation similar to that found in mammals.

2.04.3.8.3 Septal region The amphibian septal region occupies the medial aspect of the telencephalon ventral to the medial pallium and dorsal to the nucleus accumbens/ventral striatum, ventral pallidum, and medial amygdala. Traditionally, the amphibian septal region is divided into a dorsally situated medial septum, a lateral septum bordering the ventricle and a nucleus of the diagonal band of Broca situated along the ventromedial surface of the telencephalic hemisphere (Ariens Kappers *et al.*, 1936; Kicliter and Ebbesson, 1976). Scalia (1976) distinguished a dorsal septum as a separate region. Additionally, the postolfactory eminence and the bed nucleus of the pallial commissure were thought to belong to the septum (Northcutt and Kicliter, 1980). More recent tracing studies distinguish (1) a medial complex consisting of a dorsally situated medial nucleus and a ventrally situated nucleus of the diagonal band of Broca, (2) a lateral complex consisting of a dorsolateral and a ventrolateral nucleus, and (3) a central complex consisting of a dorsal and a central nucleus. The postolfactory eminence, the bed nucleus of the pallial commissure, and the ventral portion of the septum are now excluded from the septal region (Endepols *et al.*, 2005; Roden *et al.*, 2005).

These two studies demonstrate that the central and medial septal nucleus receive direct input from the olfactory bulb, amygdala, and nucleus accumbens, whereas input from these regions to the lateral septal nucleus is less abundant or absent. The medial pallium projects to all septal nuclei, as does the anterior dorsal thalamic nucleus. The ventromedial thalamic nucleus/zona incerta of the ventral thalamus projects to the medial and lateral septal nucleus carrying visual, auditory, vestibular, and somatosensory information. The anterior preoptic, suprachiasmatic, and hypothalamic nuclei project to the central and lateral septal nucleus, and only the central septal nucleus receives input from the brainstem, particularly from the raphe nucleus (Roden *et al.*, 2005). All septal nuclei project to the medial pallium, the lateral and central nuclei, and to a lesser degree the medial nucleus projects to the olfactory nuclei, amygdala, nucleus accumbens, and hypothalamus, and the lateral septal nucleus also projects to sensory areas in the diencephalon and midbrain. Studies by Gonzalez and Lopez (2002) demonstrated that a cholinergic projection of the septum to the medial pallium is present in anurans, which the authors interpret as a forerunner of the mammalian cholinergic septohippocampal pathway. It appears that the amphibian septal region has essentially the same structural organization as the mammalian septum, but functional studies are lacking.

2.04.3.8.4 Amygdaloid complex The ventromedial, ventral, and ventrolateral part of the caudal telencephalon is occupied by the amygdaloid complex (cf. Figures 17c–17g, 22, and 27). Northcutt and Kicliter (1980), in their classical paper on the organization of the amphibian telencephalon, distinguished a medial amygdala caudal to the nucleus accumbens and ventral to the lateral septal nucleus, and a lateral amygdala starting rostrally as a lateral cellular prominence between lateral pallium and striatum, caudalward curving around the dorsal striatum in a C-shaped manner and eventually fusing with the anterior preoptic nucleus. The existence of a central amygdala, a BNST, and a dorsal and ventral pallidum was not discussed by the authors.

A new classification of the amygdaloid complex in anuran amphibians was presented recently by Marín and co-workers on the basis of histochemical and immunohistochemical data in the frog *Rana perezi* (Marín *et al.*, 1998). In their opinion, the lateral amygdala occupies the dorsal portion of the ventral pallium situated above an anterior amygdala that occupies the ventral portion of the ventral pallium, previously called SPTA (see above). More caudally, the ventral part of the ventral striatum *sensu*

Northcutt and Kicliter is considered by Marín *et al.* (1998) the dorsal and ventral pallidum and the lateral part of that complex the central amygdala. The medial amygdala now occupies part of the lateral amygdala *sensu* Northcutt and Kicliter, but rostralward curves around the striatum and joins the lateral and the anterior amygdala in the above sense. The medial amygdala *sensu* Northcutt and Kicliter, plus the ventral lateral septum, now becomes the BNST plus the ventral pallidum. Thus, compared to Northcutt and Kicliter, the entire amygdaloid complex is shifted laterally and dorsally in Marín *et al.* (1998).

In order to clarify this situation regarding the components of the amphibian amygdaloid complex and its possible homologies with that of mammals, it is useful to use a functional approach (Swanson and Petrovich, 1998) and look for four different functional parts:

1. A part receiving direct input from the main olfactory bulb (cortical amygdala of mammals).
2. A part receiving direct input from the accessory or vomeronasal olfactory bulb and projecting primarily to the hypothalamus (posteromedial cortical and medial amygdala of mammals).
3. A part with reciprocal connections with visceral-autonomic centers in the mesencephalic tegmentum, brainstem, and spinal cord (central amygdala-BNST of mammals).
4. A part with close connections to the pallium/cortex (basolateral amygdala of mammals).

The vomeronasal amygdala can be identified in the amphibian brain by its massive input from the AOB via the accessory olfactory tract that forms a distinct terminal neuropil in the caudolateral part of the telencephalon and by its projection to the preoptic area and hypothalamus via the ipsilateral stria terminalis (cf. Figure 23) and to the contralateral vomeronasal amygdala via the anterior commissure (commissural portion of the stria terminalis). Neurons in the telencephalon of both frogs and salamanders that exhibit these characteristics form a band of neurons continuous with the SPTA. This band covers the area called lateral amygdala by Northcutt and Kicliter (1980) and then stretches to the magnocellular nucleus of the periventricular preoptic area (Roth *et al.*, 2004; Laberge and Roth, 2005). Neurons in the vomeronasal amygdala extend most of their dendrites into the terminal neuropil of the accessory olfactory tract (Figure 29)

Based on its connections to olfactory structures and to the hypothalamus (Neary, 1990; Bruce and Neary, 1995; Roth *et al.*, 2004; Laberge and Roth, 2005), the region in the ventral part of the caudal pallium dorsolateral to the vomeronasal amygdala can be considered the main olfactory amygdala (Figure 30).

The extended central amygdala (i.e., central amygdala plus BNST) can be identified by reciprocal connections with visceral-autonomic brain centers, e.g., preoptic area, hypothalamus, posterior tubercle, periaqueductal gray, parabrachial nucleus, nucleus of the solitary tract, and DCN (Saper, 1995; Alheid *et al.*, 1995; Pitkänen, 2000). In *Bombina*, neurons fulfilling these criteria occupy the caudal ventral telencephalon around the ventricle medial to the caudal pole of the striatopallidum (Roth *et al.*, 2004) (Figure 23). In *Plethodon*, this visceral-autonomic amygdala is situated more rostrally, extending ventral to the striatopallidum (Laberge and Roth, 2005). Neurons in this zone distinctly differ in the morphology of their dendrites from those belonging to the striatopallidal complex (Roth *et al.*, 2004; Laberge and Roth, 2005). Many of them have a peculiar morphology in that one part of the dendritic tree is directed dorsally toward the ventricle and another one in the opposite, ventral direction (Figure 31).

It is disputed whether amphibians possess an amygdaloid complex homologous to the mammalian basolateral amygdala and it is assumed to be of pallial origin. As mentioned above, Moreno and González (2004, 2006), based on tracing experiments, recently proposed that the ventral pallium above the SPTA (which is believed by the authors to represent the anterior amygdala) is homologous to the mammalian basolateral amygdala.

2.04.4 Phylogenetic and Evolutionary Considerations

2.04.4.1 The Nervous System of Amphibians: Primitive or Simplified?

Traditionally, brains are viewed as having increased continuously in functional and morphological complexity during vertebrate evolution (Ariens-Kappers *et al.*, 1936; Romer, 1970; Kuhlenbeck, 1977; Bauchot, 1978; Ebbesson, 1980, 1984). This unilinear view of evolutionary progress has now been replaced by the concept that vertebrates have evolved independently in a radiative manner (cf. Northcutt, 1985; Nieuwenhuys *et al.*, 1998). Thus, it is no longer appropriate to speak of primitive and advanced organisms, arranged along a ladder of increasing complexity. One refers instead to primitive (plesiomorphic) and derived (apomorphic) traits of a taxon and to the shared derived states (synapomorphies) that can be used to infer patterns of genealogical relationship (Hennig, 1966). Nevertheless, it is still widely accepted that within vertebrate classes more recent taxa (teleosts vs.

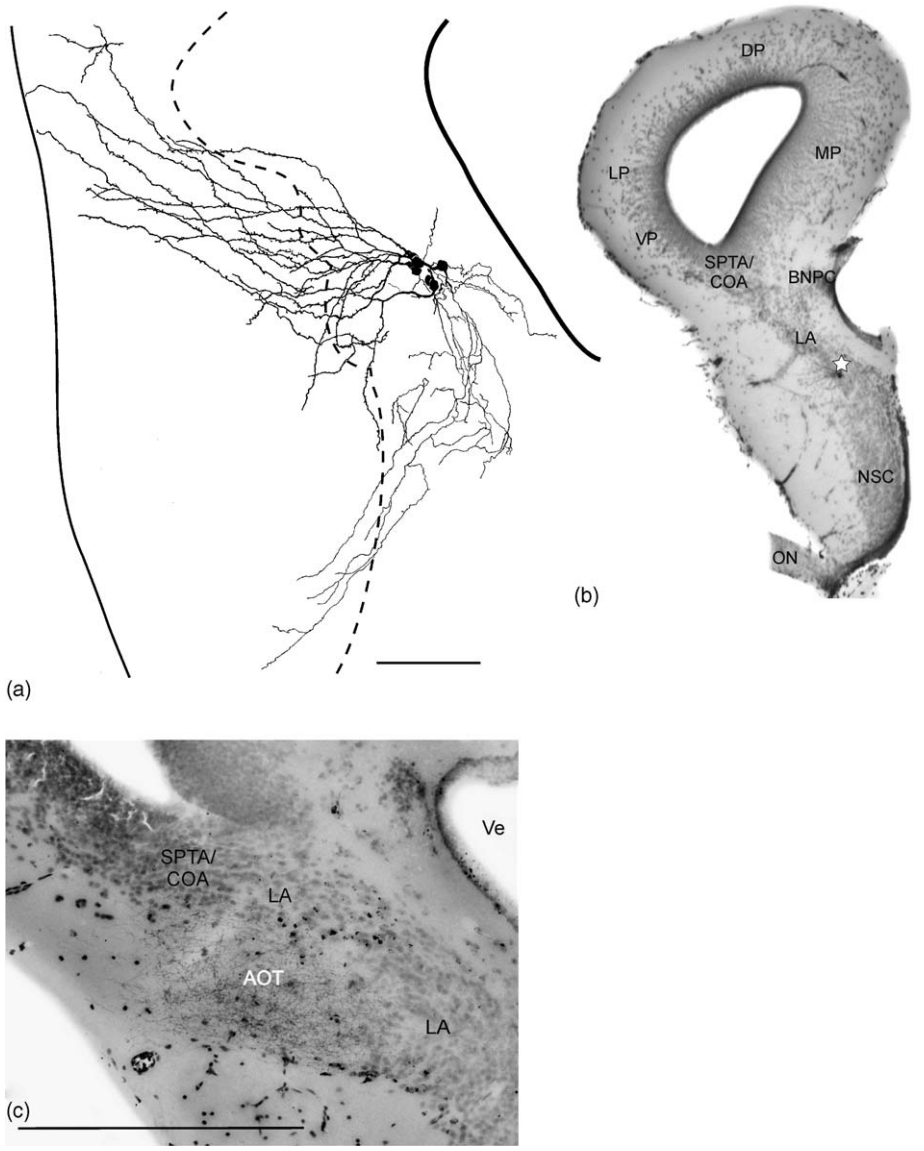

Figure 29 a, Camera lucida drawing of a cluster of intracellularly labeled neurons of the lateral, vomeronasal amygdala neuron of *Bombina* projecting to the rostral medulla. The majority of dendrites extend into the terminal neuropil of the accessory olfactory tract (cf. microphotograph in (c)), a minority into the preoptic region. In (b), the site of neurons is indicated by a white star. c, Microphotograph showing the terminal neuropil of the accessory olfactory tract (AOT) lateral to the vomeronasal amygdala. DP, dorsal pallium; LP, lateral pallium; MP, medial pallium; VP, ventral pallium; SPTA, striatopallial transition area; COA, cortical (olfactory) amygdala; BNPC, bed nucleus of the pallial commissure; LA, lateral (vomeronasal) amygdala; NSC, suprachiasmatic nucleus; Ve, ventricle; AOT, accessory olfactory tract. Scale bars: 500 μm. Modified after Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35–61.

chondrosteans, mammals and birds vs. reptiles and amphibians) possess relatively more complex brains.

In this context, the evolutionary status of the brains of amphibians has always created difficulties. The brains of frogs, and especially of caecilians (Kuhlenbeck, 1922) and salamanders (Herrick, 1948; Leghissa, 1962), appear to be simpler than those of chondrichthyans and osteichthyans, and even of cyclostomes in some respects. Despite an awareness that amphibians are tetrapods and thus not phylogenetically basal, their brains were viewed by leading comparative neuroanatomists as exemplifying the ancestral state of the vertebrate brain (Leghissa, 1962). However, Herrick (1948) suspected that the seemingly simple brains and sense organs of amphibians as well as many of their bodily characteristics had derived from a more complex ancestral state. This includes the reduction or loss

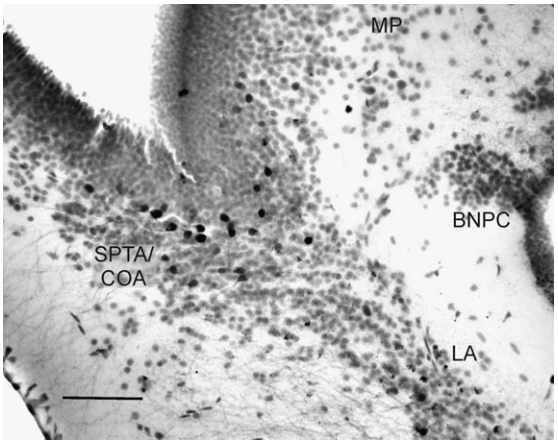

Figure 30 Microphotograph of a transverse section through the caudal telencephalon of *Bombina orientalis* showing retrogradely labeled neurons of the cortical (olfactory) amygdala after tracer application to the hypothalamus. For the level of section see Figure 28g. MP, medial pallium; BNPC, bed nucleus of the pallial commissure; SPTA, striatopallial transition area; COA, cortical (olfactory) amygdala; LA, lateral (vomeronasal) amygdala. Scale bar: 100 μm. Modified from Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35–61.

of ossification, limbs, a free-living larval stage, reduction of the inner ear, the electroreceptive, lateral line, and auditory system in a number of frogs, many salamanders, and caecilians. At the same time, among frogs, salamanders, and caecilians, derived traits can be found.

Today, it is widely accepted that lissamphibians have undergone secondary simplification and that secondary simplification arises from 'pedomorphosis', a form of heterochronic evolution in which traits that characterize larvae or juveniles of ancestral taxa are maintained in the adult stage of descendant taxa (cf. Gould, 1977). Pedomorphosis commonly involves different degrees of retardation, reduction, or absence of traits in otherwise fully developed organisms, as compared with phylogenetic outgroups. Thus, a mosaic of fully adult traits, weakly expressed traits, and missing characters appears in terminal ontogenetic stages. Accordingly, amphibian brains are expected to have fewer cells, a lower degree of morphological differentiation of cells, and reduced migration, but retain the plesiomorphic structural, functional, and developmental organization found among other vertebrates. However, this process has affected the three amphibian orders differently: anurans appear to be least and salamanders most pedomorphic, while caecilians exhibit an intermediate

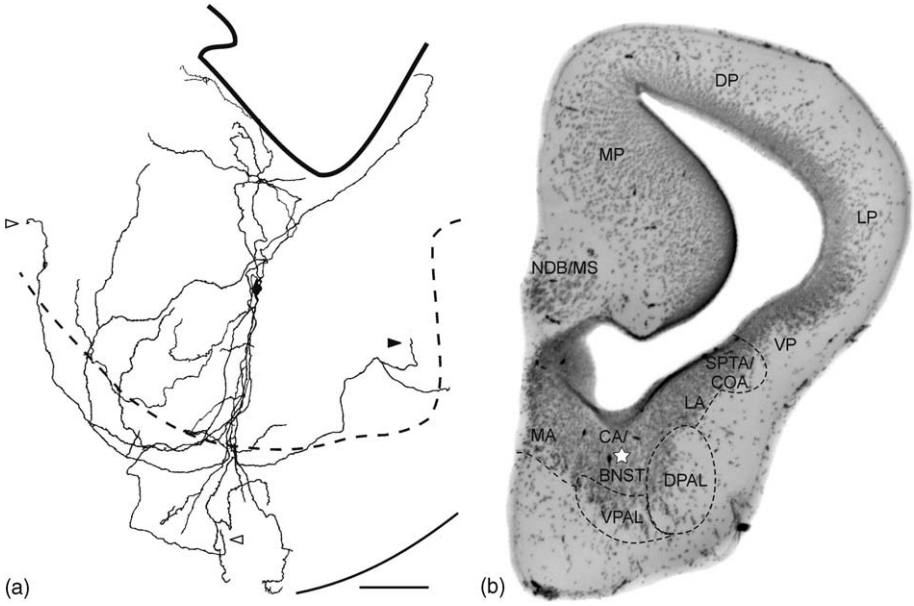

Figure 31 a, Camera lucida drawing of an intracellularly labeled neuron in the central amygdala of *Bombina orientalis* with an ascending projection to the ventral pallidum (open arrowheads) and a descending projection to the medulla oblongata (black arrowhead). Broken line indicates the border between gray and white matter. The site of soma is indicated in (b) by a white star. DP, dorsal pallium; LP, lateral pallium; MP, medial pallium; VP, ventral pallium; SPTA, striatopallial transition area; COA, cortical (olfactory) amygdala; LA, lateral (vomeronasal) amygdala; NDB, nucleus of the diagonal band of Broca; MS, medial septum; DPAL, dorsal pallidum; BNST, bed nucleus of the stria terminalis; VPAL, ventral pallidum; CA, central amygdala; MA, medial amygdala. Scale bar: 100 μm. Modified from Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35–61.

degree of pedomorphosis (Roth *et al.*, 1993). A similar situation is found in lungfishes, which as a group appear likewise to be pedomorphic; here, the Australian lungfish *Neoceratodus* is less pedomorphic and the lepidosirenid lungfishes profoundly pedomorphic (Northcutt, 1987; Roth *et al.*, 1993).

Among salamanders, the family Plethodontidae is the most specious one (comprising two-thirds of all salamanders in the world), and here the tribe Bolitoglossini (comprising half of salamander species in the world) exhibits many uniquely derived characteristics and has undergone a spectacular radiation in the Neotropics (Wake, 1966, 1987; Wake and Lynch, 1976). Bolitoglossines have developed a spectacular feeding mechanism, i.e., a projectile tongue, which is accompanied by the evolution of specialized characters of the visual system, e.g., in the context of depth perception (overview in Roth, 1987). At the same time, the brain, including the visual system, of plethodontid salamanders and bolitoglossines in particular has the simplest morphology. This includes a greatly reduced auditory system and nonexistent lateral-line system, a small number of large neurons in the sense organs and brain, a small proportion of myelinated fibers in the optic nerve, and a very low degree of cell migration throughout the brain and particularly in the tectum, consisting essentially of a periventricular cellular layer and a superficial fiber layer (Roth, 1987).

A phylogenetic analysis by Roth *et al.* (1993) based on 23 characteristics of the brain and sense organs of all groups of vertebrates came to the conclusion that 19 characters found in the salamander brains and sense organs, including the small number of types of RGCs, the low degree of myelinated fibers and the low degree of cell migration in the tectum, diencephalon, cerebellum, torus semicircularis, medulla oblongata, and spinal cord are most parsimoniously interpreted as secondarily simplified, while only one character appeared to be primitively simple (i.e., cell migration in the medial pallium) and in two cases (i.e., a low number of types of RGCs and a low degree of myelination of the nerve) the question of primitive versus simplified could not be decided. Of all salamander taxa, the Bolitoglossini, believed to be the most derived group, exhibit the most simplified brain and sense organs.

Reductions of brain and sense organs also appear to have occurred within gymnophionans and anurans. As a group, caecilians show reduction in 15 of 23 neuronal characters (Roth *et al.*, 1993). For example, whereas the so-called primitive caecilian *Epicrionops* possesses a multilaminated tectum, tectal lamination is greatly reduced in the derived taxon *Typhlonectes* (Himstedt and Manteuffel, 1985). Most frogs exhibit a multilaminated tectum, but in *B. orientalis* and the Australian frog, *Arenophryne rotunda*, both believed to be pedomorphic species, tectal lamination is substantially reduced (Roth *et al.*, 1994).

The hypothesis presented two decades ago by Roth and Wake (1985b) and now widely accepted is that secondary simplification in the salamander nervous system is related to enlarged genome and cell size (for a recent discussion, see Roth and Wake, 2000; Gregory, 2002a, 2002b). Genome size varies enormously among vertebrates. The smallest genome is found in teleost fishes, with less than 1 pg DNA per haploid nucleus. Some salamanders and all lungfishes have haploid genome sizes between 70 and 90 pg, which are the largest genomes found in any animals (Olmo, 1983). In salamanders, the smallest genome (13.7 pg) is found in the plethodontid *Desmognathus wrighti* (Hally *et al.*, 1986; Sessions and Larson, 1987) and the largest (83 pg) in the neotenic (perennibranchiate) *Necturus maculosus* (Olmo, 1983). The plethodontid salamander *H. italicus* (77 pg) has the largest genome of any terrestrial animal, although several tropical bolitoglossine plethodontids (e.g., *B. subpalmata*, 64 pg) approach this value (Sessions and Larson, 1987). Species of the Bolitoglossini, on average, have larger genome sizes than other plethodontids and than other salamander families, except for the perennibranchiate species (Olmo, 1983; Sessions and Larson, 1987). Caecilians also have relatively large genomes, but the largest known caecilian genome (13.9 pg per haploid nucleus) is equal to the smallest found in salamanders. Among anurans, the mean genome size reported by Olmo (1983) is 3.3 pg. The smallest (approximately 1 pg) is found in *Limnodynastes ornatus*, and the largest is found in *Arenophryne* (19 pg).

There is no universal agreement on the origin and significance of increased genome size in vertebrates (cf. Gregory, 2002a, 2002b). Apparently, genome size tends to increase until the tendency is halted by countervailing selection (Orgel and Crick, 1980). Among plethodontid salamanders, genome size appears to have increased many times independently, especially in the tribes Plethodontini and Bolitoglossini (Sessions and Larson, 1987). However, a phylogenetic analysis of the correlation between genome size and developmental rate concludes that several terrestrial plethodontid species have undergone a secondary reduction of genome size, which counteracts the general increase in genome size seen in terrestrial plethodontids. In the highly miniaturized *Thorius*, for example, the decrease is about 27% from the postulated ancestral bolitoglossine genome size of 34.5 pg (Sessions and Larson, 1987).

An increase in genome size has many important morphological consequences, including: (1) an

increase in cell size, (2) a decrease in cell metabolic rate, (3) a decrease in cell division rate, and (4) a decrease in cell differentiation rate (Sessions and Larson, 1987). Compared to other vertebrates, salamanders in general and bolitoglossines in particular have large to very large cells, very low metabolic rates (Feder, 1983), and slow to extremely slow developmental rates (Sessions and Larson, 1987). The ova of plethodontine and bolitoglossine salamanders are large to very large (up to 9 mm in diameter), and they develop very slowly; *Bolitoglossa* may take 10 or more months to hatch (Hanken, 1979; Houck, 1982; Collazo, 1988).

While in amphibians the correlation between genome and cell size on the one hand and metabolic rate on the other is significant only at certain temperatures (cf. Licht and Lowcock, 1991; Gregory, 2002a, 2002b), increased genome and cell size is significantly negatively correlated with anatomical complexity of the brain and sense organs. Species with small genomes have more and smaller nerve cells per volume of gray matter, their neurons are more differentiated morphologically, and the number of migrated nuclei and the degree of lamination (e.g., inside the tectum) is higher than in species with larger genomes (Roth *et al.*, 1988a, 1990, 1994). As a consequence, anurans – with genome and cell sizes much smaller than salamanders (see above) – generally have more differentiated brains than salamanders. Among anurans and salamanders, taxa with large genomes and cells such as *Bombina* and *Arenophryne* or bolitoglossine salamanders have simpler brains than those with smaller genomes, if we disregard miniaturized taxa (Roth *et al.*, 1994). (Miniaturization is a process that independently leads to secondary simplification; Roth *et al.*, 1990.) The same holds for lungfishes; here *Neoceratodus* has a much smaller genome size and a more complex brain anatomy than lepidosirenids (cf. Roth *et al.*, 1993).

An increase in genome and cell size leads to profound retardation of brain development, but not all developmental processes are retarded to the same degree. As a rule, processes appearing late in ontogeny are more affected than those appearing early. Accordingly, the cerebellum – a structure that develops very late – is deeply affected by retardation. In frogs, the cerebellum is small and simple, in nonbolitoglossine salamanders it is even simpler, and the simplest is found in bolitoglossines. The same holds true for the ontogenetically late cell migration processes in the spinal cord, brainstem, torus semicircularis, tectum, and thalamus as well as the formation of anatomically distinct nuclei all over the brain, which are increasingly retarded and even truncated in parallel to the increase in genome and cell size. However, the degree to which the morphology of the amphibian telencephalon is primitive or secondarily simplified remains undecided. This topic is discussed further below.

A phylogenetic comparison of the amphibian CNS with that of other vertebrates is hindered by several facts. It is generally assumed that modern amphibians are closest to the ancestors of all tetrapod vertebrates, but the sister group of tetrapods and that of amphibians is not precisely known. Most presumably, these are extinct members of sarcopterygians, and within this group forms that are most closely related to the extant dipnoans. However, the brains of the majority of lungfish species, i.e., the African *Protopterus* and the South American *Lepidosiren*, are most probably secondarily simplified (Northcutt, 1987).

One of the most interesting aspects is that secondary simplification of sense organs and brain regions at a morphological level has not, or at least not obviously, affected their functions. This is most apparent in the case of bolitoglossine salamanders, which, on the one hand, exhibit the simplest sense organs and brains at a gross morphological level, and on the other hand the most refined prey-catching apparatus and associated neuronal control system (Roth and Wake, 2000).

2.04.4.2 Comparative Aspects

Differences between amphibians and lungfishes on the one hand and amniotes on the other are minor with respect to the spinal cord, medulla oblongata, midbrain, and the preoptic-hypothalamic diencephalic region (see Evolution of the Nervous System in Fishes). Major differences between amphibians and amniotes are found with respect to (1) the thalamopallial system, (2) the visual system, (3) pallial regions, (4) striatopallidum, and (5) the amygdaloid complex.

2.04.4.2.1 The thalamopallial system In a series of seminal papers, Butler (1994a, 1994b, 1995) argued that the dorsal thalamus of all jawed vertebrates consists of two divisions, i.e., a collothalamic and a lemnothalamic one. The 'collothalamic' division is characterized by a pathway that originates predominantly from the midbrain (tectum mesencephali, colliculi superiores, and colliculi inferiores in mammals) and projects to dorsal thalamic nuclei such as the nucleus rotundus of reptiles and birds and the posterior dorsal thalamic and intralaminar nuclei of mammals, which in turn send projections to the striatum via the lateral forebrain bundle. The 'lemnothalamic' division is characterized by a pathway that includes predominantly sensory

(lemniscal) afferents and projects to dorsal thalamic nuclei such as the mammalian LGN and the lateral geniculate nucleus of reptiles, which in turn project to the medial and dorsal pallium/cortex via the medial forebrain bundle. One characteristic of the lemnothalamus is a direct sensory (mostly retinal) input to the dorsal thalamic nuclei. Butler (1995) argues that both divisions of the dorsal thalamus were elaborated to some degree during amniote evolution. While diapsid and anapsid amniotes mainly developed the collothalamus (with some further specialization of the lemnothalamus in birds), the evolution of the mammalian brain was characterized by an enormous evolution of the lemnothalamus.

Along this concept, Puelles and collaborators (Puelles *et al.*, 2000), based on a series of experiments in the developing chick, as well as Puelles (2001), developed the idea that the dorsal thalamus of tetrapods is organized in three tiers, i.e., a dorsal tier characterized by the lemnothalamic visual LGN (with direct retinal input), an intermediate tier characterized by collothalamic nuclei such as the nucleus rotundus of birds and reptiles and the intra- and paralaminar nuclei in mammals, and a ventral tier containing the auditory medial geniculate nucleus of mammals and the nucleus ovoidalis complex of birds. The dorsal tier receives direct retinal input and projects to the medial and dorsal pallium/cortex, the intermediate tier receives predominantly tectal/superior collicular input and projects to the striatum and ventral pallium (and its derivatives), and the ventral tier receives input from the torus semicircularis/inferior colliculus and projects to specific auditory regions inside the anterior dorsal ventricular ridge (aDVR) in reptiles and birds and to the auditory cortex in mammals.

The dorsal thalamus of amphibians contains a central nucleus, which in some respects resembles the collothalamus *sensu* Butler, the ventral tier and intermediate tier *sensu* Puelles, and an anterior nucleus resembling the lemnothalamus and the dorsal tier. However, there are major inconsistencies regarding its lemnothalamic nature. The anterior dorsal thalamic nucleus of amphibians projects in a lemnothalamic, or first tier, fashion to the medial and dorsal pallium, but it receives only indirect retinal input via ventral thalamus, tectum, and central dorsal thalamic nucleus. It also sends collaterals to the medial amygdala and lateral septum, which is atypical for a lemnothalamic pathway. It is, therefore, safe to conclude that in amphibians a lemnothalamus in a strict sense does not exist.

The central dorsal nucleus of amphibians projects to the caudal ventral pallium (sparsely) and striatum (massively); also, it receives a projection from the torus semicircularis. Therefore, this nucleus can be regarded as a combined collothalamic nucleus of the intermediate and ventral tier in the sense of Puelles and is probably homologous to the nucleus rotundus of reptiles and birds.

In turtles (Hall and Ebner, 1970; Hall *et al.*, 1977; Zhu *et al.*, 2005) and lizards (Desfilis *et al.*, 2002), three dorsal thalamic nuclei exist that project to pallial–cortical structures and constitute the thalamocortical system of these taxa:

1. the dorsomedial anterior nucleus, which projects to the small-celled medial cortex;
2. the dorsolateral anterior nucleus, which projects to the large-celled dorsal (ventral) medial cortex; and
3. the LGN, which is the main retinorecipient nucleus and projects to the dorsal cortex via the medial forebrain bundle and to the pallial thickening via the lateral forebrain bundle (Zhu *et al.*, 2005).

The dorsomedial and dorsolateral anterior nuclei are multimodal, including visual afferents, but apparently without direct retinal input (Pritz, 1995). The three nuclei mentioned surround the large nucleus rotundus, which does not receive direct retinal but rather visual afferents from the tectum and projects to the anterolateral portion of the dorsal ventricular ridge, but not to the cortex.

It is still controversial whether the cortical areas mentioned project back to the dorsal thalamic nuclei from which they receive afferents (for a discussion see Zhu *et al.*, 2005), as is the case in mammals. The small-celled medial and the large-celled dorsomedial cortices are considered to be homologous to the mammalian hippocampus (possibly Ammon's horn region and dentate gyrus or subiculum), whereas the dorsal cortex is considered homologous to the mammalian isocortex. Auditory information from the torus semicircularis is relayed to the nucleus reuniens, and this nucleus projects to the ventral aDVR, but not to the cortex.

While the projection of the LGN to the dorsal cortex most probably represents a lemnothalamic pathway and the projection of the nucleus rotundus to the anterior dorsal ventricular ridge certainly a collothalamic pathway *sensu* Butler (1994b), it is unclear what kind of pathway originates from the dorsomedial and dorsolateral nucleus (Zhu *et al.*, 2005). Given that in reptiles these two nuclei apparently do not receive direct retinal input, they can only be considered to carry multimodal and limbic information and are not lemnothalamic in a strict sense, but closely resemble the anterior dorsal thalamic nucleus of amphibians.

2.04.4.2.2 Pallium The pallium of amphibians is unlaminated, despite extensive cell migration in the medial and to a lesser degree in the dorsal and lateral pallium. In dipnoans, the pallium is relatively small and occupies the dorsolateral telencephalon. Laterally, it is divided from the striatum by the sulcus limitans pallii. In *Neoceratodus* and *Protopterus*, there is a thick periventricular layer and a thin layer of migrated cells. In *Neoceratodus*, a dorsal hippocampal pallium, an intermediate general pallium, and a piriform ventral pallium are distinguished, but there are no clear subdivisions (Nieuwenhuys, 1998). Unfortunately, no modern tracer studies exist on the afferents and efferents of pallial neurons in dipnoans. The reptilian pallium consists of two parts, the cerebral cortex and the dorsal ventricular ridge, unique to reptiles and birds. The cortex is divided into a medial, dorsomedial, dorsal, and lateral cortex plus a pallial thickening. The lateral cortex is the main olfactory cortex (ten Donkelaar, 1998). The medial and dorsomedial cortex receive olfactory information via the lateral cortex plus multisensory and limbic information via the dorsomedial and dorsolateral thalamic nuclei.

The situation found in reptiles and amphibians regarding pallial regions is similar: the small-celled medial and the large-celled mediodorsal cortices of reptiles are largely homologous to the small-celled ventral and the large-celled dorsal portion of the medial pallium of amphibians, and the dorsal cortex of reptiles is largely comparable to the dorsal pallium of amphibians. These regions receive multimodal sensory and limbic afferents from the anterior dorsal thalamus, which in reptiles comprise the dorsomedial and dorsolateral nuclei. The only major difference consists in the existence of a lemnothalamic sensory relay nucleus in the dorsal thalamus of reptiles, the LGN, which receives direct visual input and projects in parallel to the dorsal cortex and to the pallial thickening; such a lemnothalamic nucleus does not exist in amphibians. However, a strict homologization of the LGN of reptiles and the LGN of mammals is problematic, because in the medial, dorsomedial, and dorsal cortex of reptiles, as in the medial and dorsal pallium of amphibians, no precise topographic, unimodal sensory maps have been found to date (see overview in ten Donkelaar, 1998).

The dorsal ventricular ridge (DVR, Johnston, 1923) is a structure uniquely found in reptiles and birds. It is divided into an anterior and a posterior part (aDVR, pDVR) separated by the anterior commissure (ten Donkelaar, 1998). The aDVR is divided into three longitudinal zones as main targets of ascending sensory pathways. Visual information reaches the lateral part, somatosensory information terminates in the central part of the aDVR, and auditory information in the medial part of the aDVR. The pDVR receives nontopographically organized multisensory limbic afferents from the dorsal thalamus. The nucleus sphericus receives the main olfactory and vomeronasal afferents and projects to the ventromedial hypothalamus and to the AOB.

At present, it is debated to which structure of the mammalian telencephalon the aDVR should be considered homologous. Bruce and Neary (1995) regarded the aDVR as homologous to the mammalian basolateral amygdala, whereas Striedter (1997) homologized it with the mammalian endopiriform nucleus/claustrum, and Puelles *et al.* (2000) argued that the ventral pallium gives rise to the endopiriform nucleus and lateral amygdala nucleus in mammals and to the sensory-recipient part of the aDVR in reptiles and birds. More recently, Molnár and Butler (2002) argued that a strict homologization between these structures in the mammalian and sauropsid brain is impossible, but that a field homology can be postulated between the aDVR of sauropsids and the claustrum-endopiriform nucleus plus the basolateral amygdala of mammals, both developing from the collothalamic lateral–ventral pallium.

Amphibians, like mammals, lack a dorsal ventricular ridge. A strict homology of the anterior ventral pallium of amphibians with the aDVR is unlikely, because the ventral pallium receives no substantial visual, auditory, and somatosensory input from the dorsal thalamus, but its dominant input comes from the AOB. Also, it lacks connections with the dorsal pallium. However, one could envision an evolutionary process during the transition from amphibian to reptilian ancestors, in which sensory afferents from the dorsal thalamus extend further rostrally into the anterior ventral pallium replacing the olfactory and vomeronasal input.

In summary, the amphibian pallium is likely to represent a situation prior the divergent evolution of the mammalian cortex and the reptilian–avian cortex aDVR. However, the precise functions of the amphibian pallial regions are unclear. First, inside the medial, dorsal, and lateral pallium of frogs and salamanders, only multimodal responses can be recorded (F. Laberge, unpublished data). Second, the large dorsal and lateral pallium have no extra-telencephalic projections, and the only extra-pallial projection reaches the septum. The extra-telencephalic projections of the medial pallium, which is assumed to be homologous to the mammalian hippocampus, mostly reach the ventral thalamus and the dorsal and ventral hypothalamus, whereas projections to the dorsal thalamus and the brainstem are weak.

The major output regions of the amphibian telencephalon are the nucleus accumbens, septal region, the centromedial amygdala, and the caudal striatopallidum. Whereas nucleus accumbens, septum, and centromedial amygdala have reciprocal connections with pallial regions, the striatopallidum does not. It remains to be investigated how in amphibians the pallium influences the striatopallidal motor output. Only the rostral and ventral pallium exhibit some projections to the striatopallidal complex.

An unsolved question is whether the amphibian pallial regions are primitive or secondarily simplified (cf. Northcutt and Kicliter, 1980). This question can be answered only by a phylogenetic analysis. Such an analysis is hindered by the fact that the dipnoans have pallial regions that exhibit a simple morphology and closely resemble those of amphibians, but themselves are secondarily simplified. The pallium of *Latimeria*, representing the sister group of dipnoans, is assumed not to be secondarily simplified, but likewise gives a primitive appearance (cf. Nieuwenhuys, 1998). This would suggest that the pallium of amphibians is primitive and not secondarily simplified or pedomorphic. However, even in the medial and dorsal pallium of dipnoans, we find an incipient lamination, which is likewise found in turtles and lizards, but completely absent in amphibians. Therefore, it is safe to assume that some lamination in the medial and dorsal pallium is a plesiomorphic feature of sarcopterygians and all tetrapods and was lost in amphibians.

2.04.4.2.3 The visual system In amphibians, like in all anamniote vertebrates as well as in lizards and turtles and partly in birds, the tectum is the major brain center for visual perception and visuomotor functions. In the amphibian tectum, localization and recognition of objects and depth perception take place, and separate pathways descend to premotor and motor centers in the brainstem and cervical spinal cord involved in the guidance of visual behavior. Ascending pathways run bilaterally to the dorsal and ventral thalamus. Unlike other jawed vertebrates, the amphibian tectum has no saccadic system, because eye movements do not exist in adult amphibians, but this probably is due to a secondary loss, because eye movements are present during ontogeny of amphibians with an aquatic or semiaquatic lifestyle. Also, directionally selective neurons are absent in the amphibian tectum, but exist in all amniotes.

On the basis of recent tract tracing and intracellular and extracellular recording experiments in frogs and salamanders presented above, it appears that the amphibian visual system is organized in essentially the same way as that of amniotes in the sense that object recognition is based on population coding and occurs in a parallel-distributed fashion simultaneously and subsequently at several to many visual centers. Interaction and modulation between these centers occurs to a larger extent, because they are interconnected by several feedback loops, and top-down influences are most likely (Roth *et al.*, 1998; Schuelert and Dicke, 2005). This pattern of interaction is paralleled by a complex chemoarchitecture.

In amphibians as well as in all other vertebrates studied, three separate retinotectal subsystems for object recognition exist, which process information about (1) size and shape, (2) velocity and movement pattern, and (3) changes in ambient illumination (such as that caused by large moving objects). These kinds of information are processed at the level of different types of RGCs and tectal neurons, as described above, in close interaction with neurons in other visual centers such as the nucleus isthmi or the thalamus. Accordingly, different types of tectal neurons receiving different retinal input give rise to separate ascending pathways to thalamic and eventually telencephalic associative and limbic centers and to separate descending pathways to different premotor and motor centers in the medulla oblongata and rostral spinal cord, where they meet other descending pathways from diencephalic and telencephalic centers such as the central amygdala, septum, and striatopallidum.

At least three major streams of information meet at premotor and motor levels in order to elicit the various steps of visually guided behavior:

1. Information about certain properties of the object perceived concerning size, contrast, color, shape, velocity, movement pattern, etc.
2. Information about the precise location of that object. Pathways (1) and (2) need to interact in order to fully identify visual objects, including their absolute size.
3. Information about the level of motivation, most probably coming from limbic telencephalic regions (amygdala, nucleus accumbens/ventral striatopallidum) and the hypothalamus. How these latter influences are mediated to the main visual center is under investigation.

These tectal pathways found in the amphibian visual system strongly resemble those found in amniote vertebrates. However, there are remarkable differences between amphibians and mammals. One of them is the absence of a visual relay nucleus in the dorsal thalamus in amphibians that receives direct retinal input and projects monosynaptically to the cortex, and another is the presence or absence of

primary and topographically organized visual areas in the cortex (the striate cortex). Birds have evolved, apparently independently, a similar system, i.e., a pathway from the retina to the nucleus geniculatus lateralis pars dorsalis, which in turn projects to the visual Wulst. The situation in turtles and reptiles is somewhat intermediate, because there is the LGN, which receives direct retinal input and projects both to the dorsal cortex and the pallial thickening. However, in neither area are retinotopically arranged visual areas found (for details see ten Donkelaar, 1998; Dubbeldam, 1998; Voogd *et al.*, 1998; see Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?, Visual Cortex of Turtles).

2.04.4.2.4 Amygdaloid complex According to a recent concept developed by Laberge and Roth (cf. Laberge *et al.*, 2006), the amphibian amygdaloid complex is composed of an autonomic-visceral component equivalent to the mammalian extended central amygdala including the BNST and to the striatoamygdaloid transition (SAT) area of reptiles (Russchen and Jonker, 1988; Bruce and Neary, 1995; ten Donkelaar, 1998). There is a vomeronasal amygdala of pallial origin in the ventral caudolateral telencephalon including the caudal SPTA and homologous to the mammalian posteromedial cortical amygdala and to the nucleus sphericus and medial amygdala of reptiles. Possibly, in amphibians there is a ventromedially situated subpallial vomeronasal amygdala comparable to the mammalian medial amygdala (F. Laberge, unpublished observation). Also, there is an olfactory amygdala in the caudal lateral pallium homologous to the anterior and posterolateral cortical amygdala of mammals and the external and ventral anterior amygdala in reptiles (Lanuza and Halpern, 1998).

An unsolved problem is which part of the amphibian telencephalon is homologous or at least equivalent to the mammalian basolateral amygdala. Bruce and Neary (1995) as well as Marín *et al.* (1998) and Moreno and González (2003, 2006) assume that this is the case for the ventral pallium. Based on studies of gene expression pattern and topological position, it has been suggested that in vertebrates the ventral pallium is homologous (at least in the sense of a field homology) to the ventral part of the anterior dorsal ventricular ridge in birds and part of the claustrum and lateral amygdala in mammals (Brox *et al.*, 2002; Molnár and Butler, 2002). However, there are problems with considering the ventral pallium of amphibians homologous with the mammalian basolateral amygdala. The latter is characterized by strong reciprocal connections with the hippocampal formation and sensory, associative, and limbic cortical areas as well as receiving collothalamic input from the posterior dorsal thalamus (Alheid *et al.*, 1995; Pitkänen, 2000). In amphibians, there are no primary visual, auditory, and somatosensory pallial regions; furthermore, the anterior ventral pallium receives no or only very weak sensory input from the anterior dorsal thalamic nucleus and only sparse input from the central dorsal thalamic nucleus. Also, this part does not project back to the medial pallium considered homologous to the hippocampal formation and has no connections with the dorsal pallium. Therefore, it cannot exert multisensory integration in close interaction with cortical/pallial and hippocampal regions. The fact that it receives strong olfactory and vomeronasal input and that its efferents join the stria terminalis on its way to the hypothalamus is atypical of the mammalian basolateral amygdala. However, this does not exclude the possibility that during the evolution of the amniote brain this area eventually developed into a mammalian basolateral amygdala.

A region that at least partially fulfills these connectional criteria is the ventromedial portion of the ventral caudal telencephalon of anurans including the ventral-most portion of the lateral septum, traditionally called medial amygdala. This region receives multimodal sensory and limbic input from the anterior dorsal thalamus and projects heavily to the septum; the septum in turn projects to the medial and dorsal pallium. This medial amygdala also contains neurons that project directly to the medial pallium, from where it receives substantial input. On the other hand, it is entirely of subpallial and not of pallial origin, as is the case for the basolateral amygdala of mammals. Therefore, it appears that the medial amygdala of amphibians is not homologous but homoplastic to the basolateral amygdala, i.e., it is of different origin, while serving similar functions (Laberge *et al.*, 2006).

Thus, the evolution of a portion of the amygdala of pallial origin with strong reciprocal connections to sensory, associative, and limbic pallial–cortical areas appears to be a major step in the evolution of the amniote telencephalon, enabling the formation of new and more complex types of emotional learning. The amygdaloid complex found in amphibians with a vomeronasal, olfactory, and a mixed autonomic-visceral and associative amygdala certainly represents the ancestral tetrapod and perhaps vertebrate condition. In this context, new findings demonstrate that in mammals not only the basolateral amygdala (as was assumed for a long time; cf. LeDoux, 2000), but also the central nucleus are the site of emotional

conditioning, albeit in a simpler fashion (Everitt *et al.*, 2003; Paré *et al.*, 2004). This would be consistent with the view that the presence of a mixed autonomic-visceral-associative amygdala enables amphibians to develop simple forms of affects and emotions, while the formation of more complex emotions would be based on the evolution of a basolateral amygdaloid complex (Everitt *et al.*, 2003).

2.04.4.2.5 Striatopallidum The amphibian dorsal striatopallidal complex is divided into a rostral portion corresponding to the dorsal striatum of mammals, a caudal portion corresponding to the dorsal pallidum of mammals, and an intermediate portion with properties shared by both structures. Enkephalin-ir and substance P-ir neurons are mainly found in the rostral and intermediate part of the dorsal striatopallidal complex, while Leu-enkephalin-ir and serotonin-ir fibers are most abundant in the intermediate and caudal parts. Furthermore, there is a distinct dopaminergic input from the posterior tubercle, which is believed to be homologous to the substantia nigra pars compacta of mammals (Marín *et al.*, 1995). All this characterizes the amniote including mammalian dorsal striatopallidum (Mori *et al.*, 1985; Graybiel, 1990; Reiner *et al.*, 1998).

Major differences between the amphibian and mammalian dorsal striatopallidum consist in the following:

1. A low number of GABAergic neurons in the amphibian striatum (H. Endepols, unpublished data), while in mammals nearly all striatal output cells are GABAergic.
2. A lack of segregation into histochemically different patches and matrix typical of the mammalian striatum; however, in amphibians histochemically different layers can be observed.
3. The complete or nearly complete absence of cholinergic neurons, which in mammals are concentrated in the so-called matrix.
4. Only weak input from the rostral and ventral pallium in amphibians, while there is a strong cortical input to the striatum in mammals.
5. No or only a weak projection of the caudal striatopallidal complex to the dorsal thalamus.

As a consequence, the main motor output of the amphibian striatopallidum is the projection of the pallidum to brainstem and spinal cord motor regions (which, of course, is also present in mammals), rather than the corticospinal tract in mammals. Furthermore, a modulation of sensory and executive functions does not take place in the pallium/cortex, because a projection of the pallidum back to the cortex via ventral thalamic nuclei is lacking. Such a modulation appears to occur either via a projection of the pallidum to the ventral thalamus and from there to the tectum or to the tegmentum (equivalent of the substantia nigra pars reticulata) and from there to the tectum. The tectum, then, projects to the premotor and motor regions in the brainstem and rostral spinal cord.

The striatopallidum of reptiles appears to represent an intermediate evolutionary stage between amphibians and mammals (cf. Reiner *et al.*, 1998; Marín *et al.*, 1998). First, the reptilian striatum contains both GABAergic output neurons and cholinergic interneurons, both of which are either small in number or absent in amphibians. However, the cholinergic neurons are not arranged in a distributed island typical of the mammalian striatum. Second, the striatum of reptiles, like that of amphibians and unlike that of mammals, receives very little input from the cortex. Third, it is presently unclear whether reptiles possess thalamic motor nuclei comparable to the ventral anterior and ventrolateral relay nuclei of mammals, which receive projections from the dorsal pallidum and project to cortical regions with connections with the striatum. Thus, it appears that neither amphibians nor reptiles posses a re-entrant circuitry (dorsal loop) between pallium/cortex, striatopallidum, and thalamus.

2.04.5 Summary and Conclusions

In zoology and evolutionary biology, amphibians have always played a problematic role. Although modern amphibians, the *Lissamphibia*, are highly derived vertebrates with very little resemblance to the paleozoic ancestors of tetrapods, they usually are considered primitive vertebrates. This misunderstanding results at least in part from many features of sense organs and brains of amphibians appearing to be much simpler than those of nearly all other vertebrates. As discussed above, many of these features have undergone secondary simplification, especially affecting processes of morphological differentiation, formation of laminae, and anatomically distinct nuclei in the brain. Other features, especially those concerning the thalamus and telencephalon, appear to be primitive. Thus, sense organs and brains of amphibians represent a mixture of primitive and secondarily simplified traits.

In recent years, a large amount of new data on the morphology of the brains of frogs and salamanders using modern neuroanatomical methods has accumulated. These studies demonstrated that the brains of frogs and salamanders possess nearly all the properties characteristic of the brains of amniotes. One main conclusion that can be drawn from these new

insights is that in many aspects the brains of turtles and lizards are closer to that of amphibians than to mammals and birds. Major differences between amphibians on the one hand and mammals and birds on the other are the following:

1. Visual object recognition and visual guidance of behavior is mostly exerted by the retinotectopretectal system; a unimodal visual thalamotelencephalic system characteristic of mammals and birds is absent in amphibians and poorly developed in reptiles. Thalamic and telencephalic centers appear to exert a modulatory role.
2. The amphibian medial pallium is partly homologous to the mammalian hippocampal formation, but a dentate gyrus appears to be missing; the precise homologization of the medial and dorsomedial cortex of reptiles to the mammalian hippocampus is likewise unclear.
3. The amphibian dorsal pallium possesses no unimodal, topographically organized areas as found in the mammalian isocortex and the avian hyper-, meso-, and nidopallium (cf. Reiner *et al.*, 2004), and is most probably homologous to the limbic-associative cortex of mammals. The situation found in turtles and lizards is unclear, but the presence of unimodal and topographically organized areas has not been demonstrated in the dorsal cortex.
4. The amphibian striatopallidum receives input from the rostral and ventral pallium and projects to ventral thalamic nuclei, which however do not project to pallial–cortical areas connected with the dorsal striatum (the ventrolateral and ventral anterior thalamic nuclei of mammals). Such a dorsal loop appears to be missing in reptiles as well.
5. Pallial premotor and motor areas are likewise missing in amphibians as well as in lizards and turtles. The main motor output of the telencephalon in amphibians, lizards, and turtles is the projection of the dorsal pallidum to the pretectum and to the mesencephalic tegmentum and from there to the tectum mesencephali.
6. In the anterior striatopallidum of amphibians corresponding to the mammalian dorsal striatum, GABAergic projection neurons and cholinergic interneurons, characteristic of the mammalian dorsal striatum, are largely or completely missing. The striatum of turtles and lizards, in contrast, appears to possess such types of neurons, although not in a quantity comparable to the mammalian situation.
7. The amphibian amygdaloid complex consists of a vomeronasal amygdala of subpallial and pallial origin homologous to the mammalian posteromedial cortical amygdala, a pallial olfactory amygdala homologous to the anterior and posterolateral cortical amygdala of mammals and the external and ventral anterior amygdala in reptile amygdala, and an extended central amygdala homologous to the mammalian BNST, medial amygdala, and central amygdala. An amygdala of pallial origin homologous to the mammalian basolateral amygdala appears to be missing in amphibians, but probably has – at least in part – a functional equivalent in the mediocentral amygdala.

In summary, the differences mentioned between amphibians on the one hand and mammals and birds on the other concern mostly the connection between thalamus and pallium/cortex, the intra-telencephalic connections of the pallium/cortex, predominantly to the striatopallidum, and the further differentiation, enlargement, and specialization of pallial/cortical structures. This further differentiation of the thalamocortical system apparently has occurred independently in mammals and birds originating from different reptilian ancestors.

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2.05 Evolution of the Nervous System in Reptiles

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Glossary

homologue	Traits that are derived from a common ancestral region and formed by similar developmental processes.
pallium	The dorsal part of the telencephalon that arises from the rostral, dorsal neural folds, including hippocampus, cortex, and part of the amygdala.
telencephalon	The rostral expansion of the brain including pallium and subpallium.
tetrapods	Vertebrates with four feet, generally including amphibians, reptiles, birds, and mammals.

2.05.1 Introduction

Living reptiles are traditionally classified as either anapsids or diapsids based largely on the morphology of a single key character, the temporal fenestrae (e.g., Williston, 1917). These fenestrae are presumed to provide better jaw muscle attachment in the diapsid rather than the anapsid condition. Anapsids lack temporal fenestrae. Diapsids have two fenestrae on each side and evolved from ancestors that had none. Snakes, lizards, crocodiles, and dinosaurs are diapsids. Testudamorpha (turtles and tortoises), as well as many Paleozoic reptiles, are anapsids. The absence of fenestrae is considered a primitive state and the presence of fenestrae is considered a derived state. In traditional interpretations of the phylogeny of extant reptiles, Testudamorpha are considered basal to other reptiles (lizards, snakes, tuatara, crocodiles, and birds) (Figure 1a). Testudamorpha are typically classified as living representatives of the more extinct anapsid reptiles, and placed with the early fossil reptiles. This has led many researchers, including comparative neurobiologists, to consider turtles as the reptile of choice for evolutionary studies.

A growing body of evidence from molecular and osteological analyses suggests that Testudamorpha should be reclassified as specialized diapsids. Cladistic analyses of 168 osteological characters in 14 living and fossil reptilian taxa led Rieppel and De Braga (1996) to conclude that Testudamorpha are nested within the clade Diapsida as the sister group to birds and crocodiles (Figure 1b).

The earliest molecular approach (Fitch and Margoliash, 1967), based on analyses of the cytochrome *c* gene amino acid sequences in vertebrates and invertebrates, resulted in a phylogenetic scheme that was largely similar to the traditional scheme with one notable exception – turtles were more closely associated with birds than with the other living reptiles. Fitch and Margoliash noted this departure from conventional interpretations, but at the time considered it to be an anomaly.

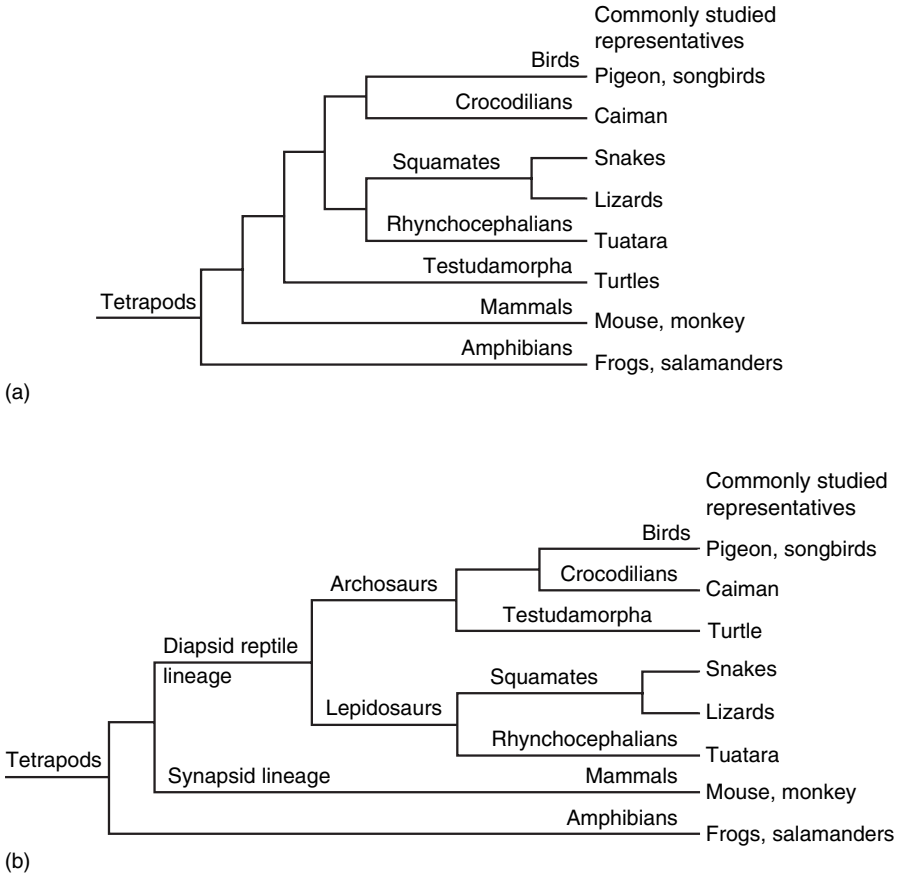

Figure 1 Relationships among the major groups of living tetrapods. a, The traditional phylogeny places Testudamorpha at a basal position relative to other reptiles. b, The consensus phylogenetic tree based on molecular and morphological analyses places Testudamorpha as a sister group to birds and crocodiles. Modified from Rieppel, O. 1999. Turtle origins. *Science* 283, 945–946 and Platz, J. E. and Conlon, J. M. 1997. . . . and turn back again. *Nature* 389, 246.

Platz and Conlon (1997) analyzed the molecular changes of pancreatic polypeptide, utilizing seven amphibians (representing all three orders) as the outgroup to examine living reptile phylogeny (snake, turtle, and alligator, and three birds). Their results were consistent with the earlier analyses of cytochrome *c*. Thus, turtles nest within the diapsids above snakes and are the sister group to birds and crocodiles. More recently, analyses of nuclear genes and mitochondrial genomes (Hedges and Poling, 1999; Janke *et al*., 2001) have provided further support for this tree.

In summary, five independent data sets, one morphological and four molecular, all support testudamorphs as diapsids. Because rhynchocephalians are presently endangered, lizards are the best alternative model to compare with other lepidosaurs, and to identify traits present in the common ancestors of birds and mammals. The evolutionary history of snakes remains controversial and requires further study, although the prevailing view is that they are derived from lizards. For studies of avian evolution, crocodilians (alligators, caiman, and crocodiles) are the most closely related living group. Testudamorpha represent a basal member of the living archosaurian diapsids, and therefore provide insights into crocodilian and avian evolution.

2.05.2 How Do Differences in Brain Organization Evolve?

The term 'homology' was coined by Owen (1848) to recognize organs of common tissue origin, regardless of form and function in different species. Applied to the brain, 'homology' typically refers to neural units that are formed by similar developmental processes.

The brains of extant animals are studied to identify presumed homologies. This involves examination and demarcation of neural regions that were derived from a common ancestral region (Campbell and Hodos, 1970; Northcutt, 1984). To do this we

must recognize and eliminate nonhomologous regions, in particular those that have arisen by parallel or convergent evolution; meaning those similarities that are not derived from common ancestry. If we can identify homologies, then mapping neuronal traits onto a well-established tree will allow one to differentiate between shared, derived, or primitive states, and those that arose by convergence.

Comparative neuroanatomists, like all scientists, are limited by the tools available to them as well as by assumptions made as to how evolution 'should work'. In the early part of the twentieth century, neuroanatomists made comparisons based on analyses of size, gross morphology, and cytoarchitecture, and followed the principle that brain regions evolved from 'simple' to 'complex' (e.g., Edinger, 1908; Ariëns Kappers *et al*., 1936). They relied on Nissl, myelin, and Golgi stains, and brain dissections, which provided information about neuronal morphology, topology, and some axonal pathways. Though some of their conclusions have been disproved, many are still valid today and others are still being debated. Continued searches for homologies are essential to resolving these issues.

There are well-accepted approaches for studying homology: topology, connections, neurochemical expression patterns, genetic expression patterns, cell morphology, and neurophysiological characteristics. Each approach has strengths and weaknesses. Therefore, evidence for or against a particular homology should be based on multiple approaches, and use multiple techniques. Again, if one has a good molecular-based phylogeny, one can 'map' neural states on to it.

2.05.2.1 Topology

Using topology as a criteria assumes that the relative position of a given brain region is determined by interactions with adjacent regions. This is useful because the topological fate of a neuronal group and its connections is determined by multiple genes that are expressed in specific spatial and temporal patterns. A weakness of this approach is that neuronal groups may migrate away from their ventricular site of origin. Therefore, topological analyses alone may be misleading. A classic example of a mistaken topology-based homology is Edinger's (1908) comparison of the avian nidopallium (previously named neostriatum; Reiner *et al*., 2004) and reptilian dorsal ventricular ridge (DVR) to part of the mammalian striatum, because they all form a large ridge in the lateral ventricle. Karten (1969) subsequently used histochemical analyses to show that these are pallial, and not striatal, structures.

2.05.2.2 Connections

The formation of neuronal pathways is regulated by multiple families of genes at both the sites of origin and termination. Identification of similar connections is thus another indication of homology. However, the weakness of this approach is that the same region (e.g., thalamus) may give rise to more than one projection to a target (e.g., telencephalon), or may invade new regions. Thus, a single connection may not be a reliable indicator of homology. The acquisition of novel connections reflects changes in genetic regulation, implying evolutionary change. If, for example, this change took place in a bird, but was not seen in crocodilians or turtles (basal group of the three; Figure 1b), one could tentatively assign the changed state in birds to a derived condition.

2.05.2.3 Neurochemical Expression Patterns

Comparisons of expression patterns have proven very useful for identifying homologies. Neuronal traits regulated solely by local genetic environment are best for identifying retained evolutionary features. Genes or peptides that are expressed during early developmental stages and maintained into maturity are especially useful for interspecies comparisons. For example, the expression of factors that regulate dopamine, noradrenaline, and serotonin are highly conserved in nuclei among different vertebrate classes (Smeets and Reiner, 1994), and are useful for identifying homologous nuclei and pathways. Care must be taken because some expression patterns can be sculpted by peripheral or environmental influences. For example, expression of calcium-sequestering peptides can be altered by the presence or absence of neuronal input (Britto *et al*., 1994; Diaz de Barboza *et al*., 2003); thus, their presence or absence may not correlate with neuronal ancestry. Finally, if the same antibody is used in two distant taxa, it is uncertain whether it is binding to the homologous peptide.

2.05.2.4 Gene Expression

Genetic analyses reveal expression patterns at earlier developmental stages than most neurochemical markers. The ontogenetic history of a neural group can be followed from ventricular origin, through proliferation, specification, and migration. A comparison of such embryological fate maps allows the identification of conserved neural domains. For example, analyses of gene-expression patterns

revealed the presence of a ventral pallial domain in tetrapods (Brox *et al*., 2004). However, limitations are that genes may be up- and/or downregulated during development. For example, heterochrony (expression of genes at different stages of development) may result in a positive expression in animal A but not in animal B at one stage, and the opposite expression a few days later. Thus, the stage at which two species are compared must be carefully selected.

2.05.2.5 Morphology

Neuronal size, shape, and packing density are variable, and are usually not regarded as strong indicators of homology. For example, the laminated optic tectum of reptiles and the nucleated superior colliculus of mammals are homologous although structurally different.

2.05.2.6 Physiological Characteristics

A neuron's function is determined by a complex interaction of local and nonlocal genetic factors within the brain, as well as influences from peripheral organs and the environment. Thus, comparative analyses of cellular response characteristics are more likely to reveal diverse evolutionary adaptations than retained homologous features. For example, comparisons of cellular responses at each level of the auditory pathway reveal that amphibians, reptiles, birds, and mammals have all evolved unique adaptations for processing auditory information (Grothe *et al*., 2004).

2.05.2.7 Summary

In summary, every neural region contains both evolutionarily conserved (primitive) and derived states. While recognizing the difficulty in identifying homologous features, using the total evidence from the approaches discussed above should allow us to reach a consensus with regard to homology. Mapping changed character states in the brain to a cladistically derived phylogenetic tree should reveal shared evolved patterns (synapomorphies) and at the same time identify instances of convergent evolution (a form of homoplasy), as well as derived traits.

2.05.3 How Can We Recognize Brain Areas That Have Evolved from a Common Ancestor?

Each brain region can be recognized by a unique set of traits, including gene-expression patterns, embryology, neurochemistry, and connections. Our null hypothesis is that a region does not change in the evolution from one species to another. Furthermore, we assume that homologous structures develop from topologically equivalent precursors (Braford, 1995). By searching for homologies, one should identify the neural regions that have retained most of their characteristics, and in the process recognize those that have changed.

The greater the number of traits two structures in divergent species have in common, the greater the likelihood that they are homologous. Thus, making a comparison based on a single pathway (e.g., the retino-tecto-thalamo-pallial projection), or a single gene is a weaker argument than a comparison based on multiple connections or characteristics. Controversies over homologues often occur because different investigators rely on or ignore different traits to reach their conclusions.

Evolution happens: brains clearly differ among the vertebrate classes, and the components have evolved to varying degrees. For example, evolutionary geneticists can identify a gene as 90% homologous with a similar gene from another species. Comparative neurology cannot achieve such quantification, yet we know that the homologues of some nuclei are easier to identify than others. As in gene evolution, we expect some neuronal traits to be more variable, while others are more constrained. Our current hypotheses about homologies will continue to be tested, and hopefully confirmed, as more and more traits are identified.

2.05.4 Sensory Pathways

The first mammalian studies of auditory, visual, and somatosensory pathways to the telencephalon recognized the main components of the sensory pathways, and found that sensory information was relayed through the midbrain and thalamus to the primary sensory areas of the cortex. The sensory pathways of amphibians, reptiles, and birds were compared to these pathways. With the introduction of new techniques, we have since learned that the components and connections of these sensory systems are more numerous and complicated, requiring re-evaluation of proposed homologues.

2.05.4.1 Auditory System

2.05.4.1.1 Reptiles In reptiles, peripheral sound is conveyed through a tympanic middle ear to the basilar papilla. The basilar papilla projects centrally to two subdivisions of the cochlear nucleus, nucleus magnocellularis and nucleus angularis (Carr and Code, 2000). A third brainstem target, nucleus laminaris, also receives direct peripheral auditory input in lizards and crocodiles (DeFina and Webster,

1974; Barbas-Henry and Lohman, 1988). Nucleus laminaris also has features comparable to the superior olive: it receives input from the cochlear nuclei and projects to the midbrain torus semicircularis. It is usually poorly developed in turtles and lizards, reflecting its role in all low-frequency processing (Miller, 1975; Miller and Kasahara, 1979; Barbas-Henry and Lohman, 1988b). In crocodiles and birds it is a larger, distinctly monolayer structure (Rubel and Parks, 1975). Further studies of the reptilian nucleus laminaris are needed to elucidate its evolutionary status. The next level of the vertebrate auditory system is the lateral lemniscus. Within the rostrolateral hindbrain of reptiles there is a region called the lateral lemniscus, but little is known of its connections or other characteristics.

The reptilian auditory midbrain is located in the medial torus semicircularis. It has similar structure, connections, and embryonic origin among tetrapods, and is thus considered a homologue (Wilczynski, 1988; McCormick, 1999). A tonotopic organization is present in crocodiles (Manley, 1971). In reptiles, as in other vertebrates, there is a core area that is the main target of ascending projections from the brainstem, and a belt or laminar area that appears to receive auditory input from the core or from nonbrainstem auditory areas. In lizards and crocodiles the auditory core of the torus projects to the thalamus (Pritz, 1974a; Foster and Hall, 1978). The laminar area projects to thalamus and spinal cord, and parts of it are correlated with vocalizations and social communication behaviors (Kennedy, 1975; Distel, 1978; Butler and Bruce, 1981; Hoogland, 1982).

The auditory midbrain projects to two thalamic areas in reptiles: nucleus medialis (known as nucleus reuniens in turtles and crocodiles), and the dorsolateralis anterior (Pritz, 1974a; Foster and Hall, 1978; Hoogland, 1982). In addition, auditory information from the superior olive projects to an area immediately lateral to nucleus rotundus (Hoogland, 1982). Thus, there may be as many as three distinct auditory thalamic areas, although nucleus medialis/reuniens is the main one. Further studies are needed are needed to resolve this issue.

Nucleus medialis/reuniens projects to the striatum and the medial part of the DVR, and the nucleus dorsolateralis anterior is reciprocally connected with the cortex (Pritz, 1974b; Foster and Hall, 1978; Lohman and van Woerden-Verkley, 1978; ten Donkelaar and de Boer-Van Huizen, 1981; Bruce and Butler, 1984a, 1984b).

2.05.4.1.2 Amphibian to reptile transition Amphibians have two sensory organs that detect airborne sound, the amphibian papilla and basilar papilla, but only the basilar papilla appears to be homologous to the acoustic-sensing organs of other tetrapods (Fritzsch and Neary, 1998; Fritzsch *et al.*, 2002). The amphibian cochlear nucleus, the dorsolateral nucleus, has features unique to amphibians, and its homology with the amniote cochlear nuclei remains unclear (McCormick, 1999). However, cells within the dorsolateral nucleus are morphologically and physiologically similar to those in the cochlear nuclei of other tetrapods. The brainstem auditory nuclei are extensively interconnected (see Fritzsch and Neary, 1998), so only the main connections will be described here (Figure 2a). The amphibian superior olive receives projections from the dorsolateral nucleus, and projects to the torus semicircularis in the midbrain.

The auditory part of the torus semicircularis has similar characteristics among tetrapods, although differences in local circuitry and sound processing are apparent. In amphibians it projects strongly to the central thalamic nucleus and more weakly to the lateral and anterior thalamic nuclei (Hall and Feng, 1987; Neary, 1990). Auditory information from the central and lateral thalamic nuclei is conveyed primarily to the striatum and sparsely to the ventral part of the lateral pallium; that from the anterior thalamic nucleus is conveyed primarily to the medial pallium (Mudry and Capranica, 1980; Wilczynski and Northcutt, 1983a; Hall and Feng, 1987; Neary, 1990; Allison and Wilczynski, 1991; Feng and Lin, 1991).

The basic pattern of connections can be recognized in amphibians and reptiles. Similar thalamic zones can be recognized, although there is increased size and specificity in the reptilian thalamus. The greatest change in the auditory pathway occurred in the telencephalon. Assuming that the common tetrapod ancestor had a forebrain similar to that of extant amphibians, then the ventral part of the lateral pallium in amphibians underwent extensive elaboration to give rise to the reptilian DVR with its distinct sensory regions. The amphibian medial pallium also underwent considerable change, including the development of separate hippocampal and cortical regions. See Sections 2.05.5.2 and 2.05.5.3 for further discussion of the evolution of the thalamus and pallium.

2.05.4.1.3 Reptile to bird transition During the transition from ancestral reptiles to crocodiles and birds, the cochlear nucleus laminaris appears to have enlarged and become distinctly laminated (Rubel and Parks, 1975). The auditory midbrain, torus semicircularis, appears to be generally homologous between reptiles and birds (Figure 2c). Its main projection in

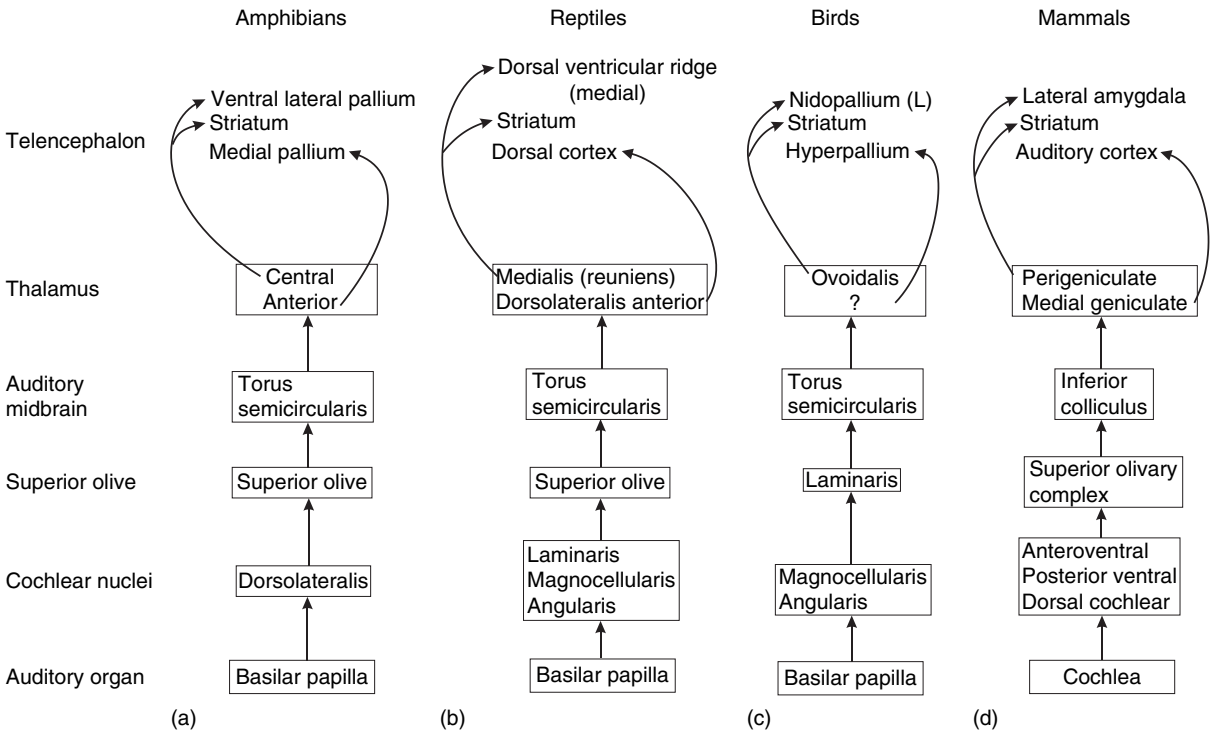

Figure 2 Comparison of the major ascending auditory pathways in tetrapods.

birds is to the auditory thalamic nucleus, ovoidalis (Arthur, 2005). Nucleus ovoidalis projects to the striatum and to Field L in the DVR (Wild *et al.*, 1993). A toral projection to a thalamic nucleus that projects to the hyperpallium has not been described, although Adamo and King (1967) recorded acoustic responses in the medial cortex. The auditory region of the DVR is more elaborate in birds than reptiles, and appears to be correlated with the evolution of vocal behaviors. Many of the connections from the auditory midbrain to circuits involving vocalization appear to be present among tetrapods, although the descending connections like those from the avian arcopallium (formerly archistriatum) to the midbrain have not been reported in reptiles.

2.05.4.1.4 Reptile to mammal transition The evolution of the mammalian auditory system is correlated with the appearance of more complex cell types and connections (Grothe *et al.*, 2004). The mammalian cochlear nucleus contains intrinsic connections and nonprimary inputs that have no known homologue in nonmammals, and which may be associated with the evolution of a high frequency hearing range, and of mobile pinnae that increased sound-localization cues (Grothe *et al.*, 2004). The correspondence of the three subdivisions of the mammalian cochlear nucleus with those of the reptilian nucleus remains uncertain. Nonetheless, the same basic pattern of connectivity seen in other tetrapods is also present in mammals (Figure 2d). The auditory midbrain, inferior colliculus, projects to two thalamic groups: the medial geniculate and a perigeniculate group. The medial geniculate projects to auditory areas in the temporal cortex, whereas the perigeniculate group, including the medial division of the medial geniculate nucleus, the posterior intralaminar nucleus, and the suprageniculate nucleus, projects to the lateral amygdalar nucleus (Doron and LeDoux, 1999, 2000). The mammalian thalamic nuclei and telencephalic regions devoted to audition and vocalization appear to have undergone considerable expansion and parcellation during the transition to mammals. They show considerable variation from the nonmammalian condition, and identification of their homologues is very controversial and will be dealt with separately (Sections 2.05.5.2 and 2.05.5.3).

2.05.4.1.5 Summary The general features and synaptic levels of the auditory pathways are present in all tetrapods, suggesting a conserved Bauplan. However, within each of these levels there is considerable anatomic and physiologic diversity among the vertebrate taxa. Another trend is the increase in size and complexity of the auditory thalamic and cortical regions, particularly in the reptile to mammal transition. Hypotheses about the homologues of thalamic

and pallial regions in amphibian reptiles, and birds are in general agreement, but comparisons with the mammalian auditory regions have proved more difficult and more controversial (see Shared Features of the Auditory System of Birds and Mammals).

2.05.4.2 Visual System

The targets of primary retinal projections will first be presented, followed by the visual pathways to the telencephalon.

2.05.4.2.1 Reptiles *Primary retinal projections.* The primary visual system has been studied in many reptiles, allowing the comparison of a general pattern of retinal projections. The retinal ganglion cells project bilaterally with a contralateral dominance in most lizards, but are predominantly contralateral in crocodiles, and entirely contralateral in *Chameleo* and *Uromastix* lizards (Bennis *et al*., 1994; Derobert *et al*., 1999). Retinal fibers terminate in six general targets within the diencephalon and midbrain of all tetrapods studied with modern experimental techniques (lizards: Northcutt and Butler, 1974; Cruce and Cruce, 1975, 1978; Bruce and Butler, 1984b; Reperant *et al*., 1978; de la Calle *et al*., 1986; Kenigfest, *et al*., 1997; Casini, *et al*., 1993; Bennis, *et al*., 1994; snakes: Repérant, and Rio, 1976; Schroeder, 1981; Dacey and Ulinski, 1986; turtles: Hall *et al*., 1977; Bass and Northcutt, 1981a, 1981b; Kunzle and Schnyder, 1983; Sjöström and Ulinski, 1985; Ulinski and Nautiyal, 1988; Hergueta *et al*., 1992, 1995; crocodiles: Derobert *et al*., 1999). These studies are summarized in the following paragraphs. A variety of nomenclatures have been used for these retinal-recipient nuclei, so we here follow that of Repérant *et al*., 1992 except as noted.

1. *Hypothalamus.* Retinal fibers terminate in the suprachiasmatic nucleus. The retinohypothalamic projection helps to synchronize endogenous rhythms with seasonal changes in the diurnal cycle (Underwood and Groos, 1982; Pickard, 1982). In chameleons and crocodiles there is an additional retinal projection to periventricular hypothalamic area, nucleus opticus periventricularis hypothalami posterior (Bennis *et al*., 1994; Derobert *et al*., 1999).
2. *Ventral thalamus.* The ventral thalamus is distinguished from the dorsal thalamus by its lack of projections to the telencephalon. Visual projections terminate in nucleus geniculatus lateralis pars ventralis, in a medially adjacent group, nucleus ventrolateralis, and in a dorsal group called either nucleus ovoidalis or geniculatus lateralis pars dorsalis (GLd). The dorsal surface of the retino-recipient ventral thalamic area is capped by a sheet of neuropeptide Y (NPY)-like immunoreactive cells, above which is dorsal thalamus (Medina *et al*., 1992).
3. *Dorsal thalamus.* Dorsomedial to the ventral thalamic area is a small cell group that receives retinal projections and projects to the telencephalon. In turtles, this nucleus is called the lateral geniculate nucleus (Hall and Ebner, 1970a, 1970b). In lizards, it is called intercalatus (Bruce and Butler, 1984a) or the lateral part of the dorsolateralis anterior (Bennis *et al*., 1994). It appears to be smaller in lizards than in turtles.
4. *Pretectal nuclei.* There are four visual pretectal nuclei. Nucleus lentiformis mesencephalicus is largest in snakes and some lizards, and poorly developed in most turtles (Reperant *et al*., 1992). Nucleus griseus tectalis is better developed in lizards than in snakes. Nucleus geniculatus pretectalis and nucleus posterodorsalis are similar features amongst reptiles.
5. *Optic tectum.* The retina terminates in the superficial optic tectum in a retinotopic organization.
6. *Mesencephalic tegmentum.* Nucleus opticus tegmenti is present in all reptiles, but is particularly large in chameleons.

Visual pathways to the telencephalon. Two visual pathways to the telencephalon are usually recognized in the reptilian visual system (see Repérant, *et al*., 1992, for a historical review). A primary pathway ascends from the retina to the thalamus to the telencephalic cortex. A secondary pathway ascends from the retina to the optic tectum (superior colliculus), to the thalamus, and then to a visual area within the DVR. The primary visual pathway is sometimes referred to as the lemnothalamic pathway, and the pathway through the tectum may be called the collothalamic pathway (Butler and Hodos, 1996). There is, however, a third distinct route through which visual information reaches the telencephalon, raising the question of which pathways are homologous among tetrapods (Figure 3b).

A retino-thalamo-telencephalic projection has been described in turtles (Hall and Ebner, 1970a, 1970b; Hall *et al*., 1977) and lizards (Bruce and Butler, 1984a; Kenigfest *et al*., 1997). In turtles it projects to the anterolateral parts of the dorsal cortex (Hall and Ebner, 1970a; Desan, 1988; Zhu *et al*., 2005). In lizards it projects to or near a rostral telencephalic nucleus, the pallial thickening (Bruce and Butler, 1984a). Further studies are needed to identify the specific telencephalic target in lizards and crocodiles.

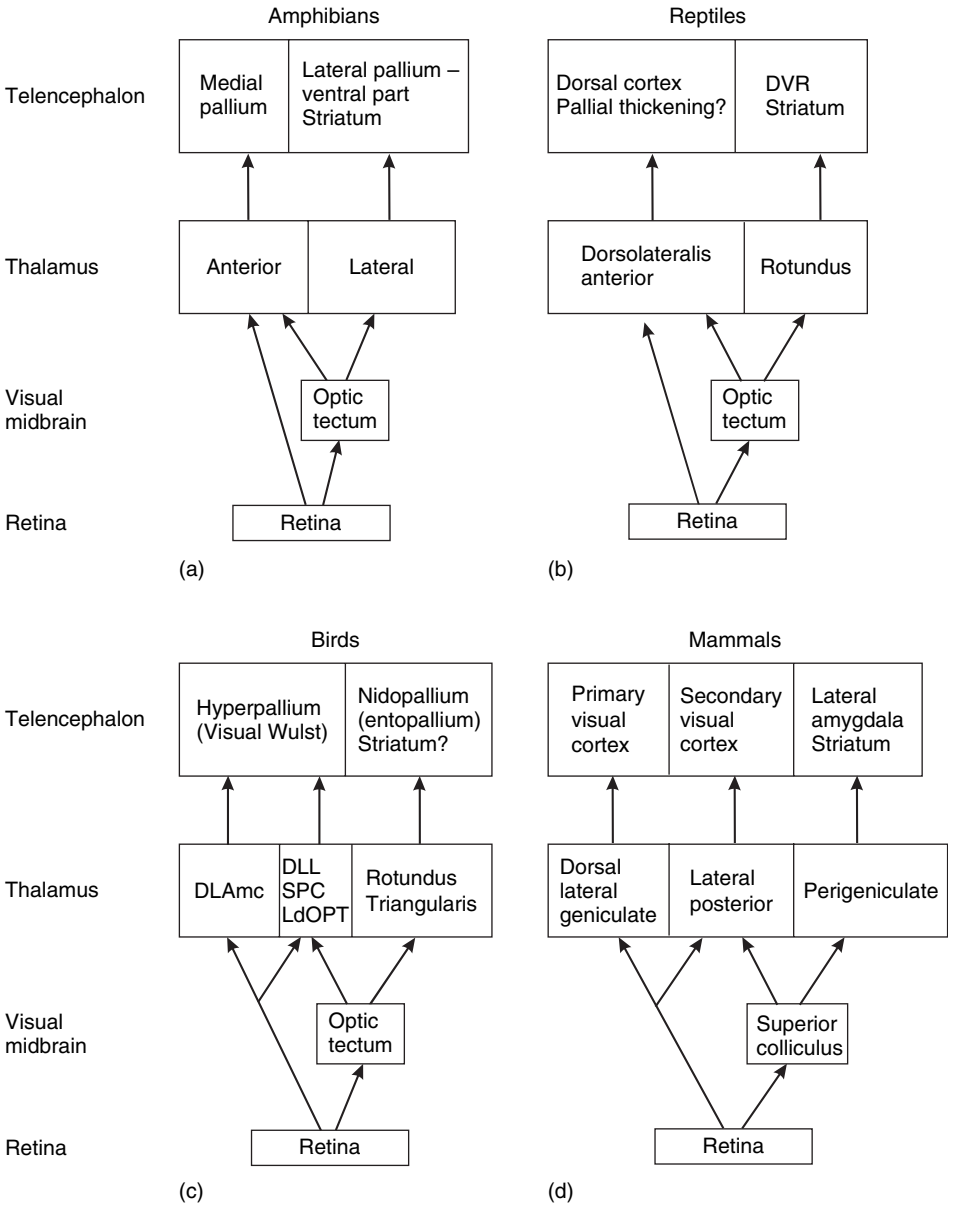

Figure 3 Comparison of the major ascending visual pathways to the telencephalon in tetrapods. Refer to text for abbreviations.

Retino-tecto-thalamo-telencephalic projections have been the focus of a number of studies (lizards: Butler and Northcutt, 1971; Bruce and Butler, 1984b; Guirado *et al.*, 2000; turtles: Hall and Ebner, 1970a, 1970b; Balaban and Ulinski, 1981; Rainey and Ulinski, 1982; Foster and Hall, 1975; Hoogland, 1982; Desfilis *et al.*, 2002; Belekhova *et al.*, 2003; crocodiles: Braford, 1972; Pritz, 1975). Neurons in the optic tectum that extend dendrites into the visual-recipient layers project to rotundus and, at least in lizards, to the dorsolateralis anterior. Nucleus rotundus then projects to the striatum and DVR, and the dorsolateralis anterior projects to the dorsal cortex. Thus, visual information reaching the optic tectum is conveyed to two different thalamic regions, and then each projects to a separate pallial region, either the dorsal cortex or the DVR. Visual responses have been recorded from the rostrolateral dorsal cortex, the lateral part of the medial cortex (Andry and Northcutt, 1976), and from the visual DVR (Peterson and Rowe, 1976; Manger *et al.*, 2002). These two regions develop from embryologically distinct pallial domains, dorsomedial and ventromedial, respectively (Fernandez *et al.*, 1998).

2.05.4.2.2 Amphibian to reptile transition The basic pattern of retinal projections seen in reptiles

is also present in amphibians, indicating that it was present in the common ancestor (Figure 3a). Visual projections terminate in the hypothalamus, ventral thalamus, dorsal thalamus, pretectum, mesencephalic tectum, and mesencephalic tegmentum (Scalia and Gregory, 1970; Scalia, 1976; Roth *et al*., 1998; Wye-Dvorak *et al*., 1992).

A retino-thalamo-telencephalic pathway is present in amphibians, as in reptiles. Retinal axons terminate on the dendrites of neurons in the anterior nucleus, within a cell-poor terminal region called the neuropil of Bellonci (Scalia and Gregory, 1970). The anterior thalamic nucleus then projects to the medial, dorsal, and lateral pallia (Neary, 1990; Northcutt and Ronan, 1992).

Two retino-tecto-thalamo-telencephalic pathways are present and comparable to those in reptiles. The optic tectum projects to the lateral and anterior thalamic nuclei. The anterior thalamic nucleus projects to the pallial cortices, especially the medial pallium, whereas the lateral thalamic nucleus projects heavily to the striatum and sparsely to the ventral part of the lateral pallium (Wilczynski and Northcutt, 1977, 1983a; Neary, 1990; Montgomery and Fite, 1991). These two pallial targets develop from embryologically distinct domains, dorsomedial and ventrolateral pallia, respectively (Brox *et al*., 2004).

2.05.4.2.3 Reptile to bird transition The avian visual system has been the subject of numerous studies, and a great deal more is known about it than the reptilian visual system, so here we focus on comparable studies (Figure 3c). As in other tetrapods the avian retina projects to targets in the hypothalamus, ventral thalamus, dorsal thalamus, pretectum, midbrain tectum, and midbrain tegmentum (Gamlin and Cohen, 1988; Norgren and Silver, 1989a, 1989b). Within the dorsal thalamus the retinorecipient nuclei that project to the hyperpallium are the dorsolateralis anterior thalami, pars lateralis (DLL), dorsolateralis anterior thalami, pars magnocellularis (DLAmc), lateralis dorsalis nuclei optici principalis thalami (LdOPT), and the suprarotundus (SpRt) (Güntürkün *et al*., 1993). This group is sometimes called the nucleus geniculatus lateralis, pars dorsalis. These nuclei project bilaterally to the hyperpallium (visual Wulst), except the SpRt, which projects ipsilaterally.

Thalamic nuclei that receive visual input via the optic tectum include rotundus, triangularis, superficialis parvocellularis (SPC), part of the DLL, and the LdOPT (Karten and Revzin, 1966; Sugita *et al*., 1996). One group of nuclei (SPC, DLL, and LdOPT) projects to the visual Wulst in the hyperpallium (Güntürkün *et al*., 1993). The SPC projects to additional telencephalic targets including the somatosensory Wulst and the area parahippocampalis. The other nuclei (rotundus and triangularis) project to the entopallium, a visual region within the nidopallium (Hellmann and Güntürkün, 2001). A projection from rotundus to the striatum apparently has not been documented, although rotundus axons clearly pass through the striatum enroute to the entopallium. Thus, visual information from the optic tectum projects through two separate thalamic regions and then to the hyperpallium and the nidopallium. The nidopallium, like the reptilian DVR is embryologically derived from the ventrolateral pallium; the hyperpallium, like the dorsal cortex is derived from the mediodorsal pallium (Fernandez *et al*., 1998; Puelles, 2000; Brox *et al*., 2004)

The avian visual system follows the same basic plan seen in reptiles, although the retino- and tectorecipient thalamic nuclei and the visual hyperpallium appear to have enlarged and segregated further during the reptile to bird transition. This enhanced ability to process visual cues may be correlated with the evolution of flight.

2.05.4.2.4 Reptile to mammal transition As in other tetrapods, ganglion cells in the mammalian retina project to targets within the hypothalamus, ventral thalamus, dorsal thalamus, pretectum, midbrain tectum, and midbrain tegmentum. These are, respectively, the suprachiasmatic nucleus, ventral lateral geniculate, dorsal lateral geniculate, several pretectal nuclei, superior colliculus, and medial terminal nucleus (Figure 3d; Sefton and Dreher, 1985).

The flow of information from the retina to the telencephalon is often regarded as a 'dual visual system', but in fact visual information reaches the telencephalon through at least three distinct pathways. One pathway is from retina to thalamus (dorsal lateral geniculate) to primary visual cortex. The second and third pathways are relayed through the superior colliculus to the thalamus. However, the superior colliculus projects to multiple thalamic groups, including perigeniculate, midline, and intralaminar nuclei, in addition to the well-known lateral posterior/pulvinar nuclei (Holstege and Collewijn, 1982; Linke, 1999; Linke *et al*., 1999). Thus, the second pathway is from the retina to the superior colliculus (midbrain tectum) to the lateral posterior (or pulvinar in primates) thalamic nuclei to secondary visual cortical areas (Diamond, 1976). The third pathway is from the retina to the superior colliculus to the visual perigeniculate thalamus (particularly the suprageniculate nucleus), which projects to the striatum and lateral amygdala (Linke *et al*., 1999, 2000; Doron and LeDoux, 1999). Embryological studies

show that the lateral amygdala is derived from the ventrolateral pallium, whereas the visual cortex arises from the dorsomedial pallium (Fernandez *et al*., 1998; Puelles *et al*., 2000; Brox *et al*., 2004). The midline and intralaminar nuclei have widespread, nonspecific projections to cortical and striatal regions. Which of these colliculo-thalamo-telencephalic targets are comparable among the vertebrate classes is currently under considerable debate. However, connectional and embryological data indicate that the visual areas of the reptilian DVR and mammalian lateral amygdala are comparable, and those of the reptilian dorsal cortex and mammalian visual cortices are comparable (see Section 2.05.5.3).

2.05.4.2.5 Summary In all tetrapods the retina projects to the hypothalamus, ventral thalamus, dorsal thalamus, pretectum, mesencephalic tectum, and mesencephalic tegmentum. Thus, this pattern was present in a common ancestor. Furthermore, in all tetrapods retinal information is conveyed to the telencephalon by at least three distinct pathways: (1) a thalamocortical pathway that runs from the retina directly to a cell group in the thalamus, and then to a dorsomedial pallial region; (2) a tecto-thalamo-cortical pathway that travels from the retina to the midbrain tectum to a cell group in thalamus and then to a dorsomedial pallial region. This dorsomedial pallial region corresponds to visual parts of the medial pallium in amphibians, dorsal cortex in reptiles, hyperpallium in birds, and isocortex in mammals; and (3) a tecto-thalamo-amygdalar pathway that runs from the retina to the optic tectum to a different cell group in the thalamus, and then to both the striatum and a nucleated region within the ventrolateral pallium. This ventrolateral pallium corresponds to a visual area of the ventral lateral pallium in amphibians, DVR in reptiles, nidopallium in birds, and lateral amygdala in mammals.

Another noteworthy trend is the increase in size and complexity of the visual thalamic and cortical regions, which occurred during each transition, but was especially remarkable in the reptile to mammal transition. Identification of homologues between amphibians, reptiles, and birds has been relatively straightforward, but comparisons with the mammalian visual system have proven more difficult to make and more controversial.

2.05.4.3 Somatosensory System

2.05.4.3.1 Reptiles Somatosensory information about the body reaches the thalamus from the spinal cord and dorsal column nuclei (Figure 4b). Information about the head reaches the thalamus from the trigeminal nuclei. In addition, the spinothalamic, dorsal column and trigeminal regions project to a somatosensory midbrain area, which then projects to the thalamus (snakes: Ebbesson, 1969; lizards: Ebbesson, 1967; Bruce and Butler, 1984b; Ebbesson, 1978; turtles: Ebbesson, 1969; Siemen and Kunzle, 1994; Kunzle and Schnyder, 1983; Kunzle and Woodson, 1982; crocodiles: Pritz and Northcutt, 1980; Ebbesson and Goodman, 1981; Pritz and Stritzel, 1994).

Spinal and dorsal column somatosensory information appears to terminate in three thalamic regions in reptiles: (1) a posterior thalamic group called medialis posterior and posterocentralis nuclei in lizards, called the medialis complex in crocodilians, and called the lateral part of nucleus reuniens in turtles; (2) the dorsolateralis anterior nucleus; and (3) the ventral thalamic nuclei (Pritz and Northcutt, 1980; Ebbesson and Goodman, 1981; Bruce and Butler, 1984b; Belekhova *et al*., 1985; Siemen and Kunzle, 1994). Trigeminal nuclei have a similar projection pattern, terminating in the dorsolateralis anterior nucleus, in a region near medialis posterior, and in the ventral thalamus (Hoogland, 1982; Desfilis *et al*., 2002).

These three somatosensory-recipient thalamic areas have different ascending projections. The posterior thalamic group projects to posterior regions of the striatum and to the caudal part of the anterior DVR; the caudal DVR may project back to the somatosensory thalamus. Nucleus dorsolateralis anterior has reciprocal projections to the dorsal, medial, and lateral cortices, although the main cortical somatosensory target is believed to be the dorsal cortex. The ventral thalamic nuclei lack telencephalic connections (Bruce and Butler, 1984a, 1984b; Pritz and Northcutt, 1980; Balaban and Ulinski, 1981; Gonzalez *et al*., 1990; Pritz and Stritzel, 1994; Lohman and van Woerden-Verkley, 1978; Voneida and Sligar, 1979).

2.05.4.3.2 Amphibian to reptile transition Two spinal somatosensory pathways to the telencephalon are present in amphibians, and thus are presumed to be present in the common tetrapod ancestor (Figure 4a). In amphibia the spinal cord projects directly to the thalamus, or indirectly via the torus semicircularis in the midbrain. There are three spinal and midbrain thalamic targets: a massive projection to the ventral nuclei, a moderate projection to the central nucleus, and a sparse projection to the anterior nucleus (Munoz *et al*., 1997). A homologue of the dorsal column nuclei appears to project only to the ventral thalamic nuclei (Munoz

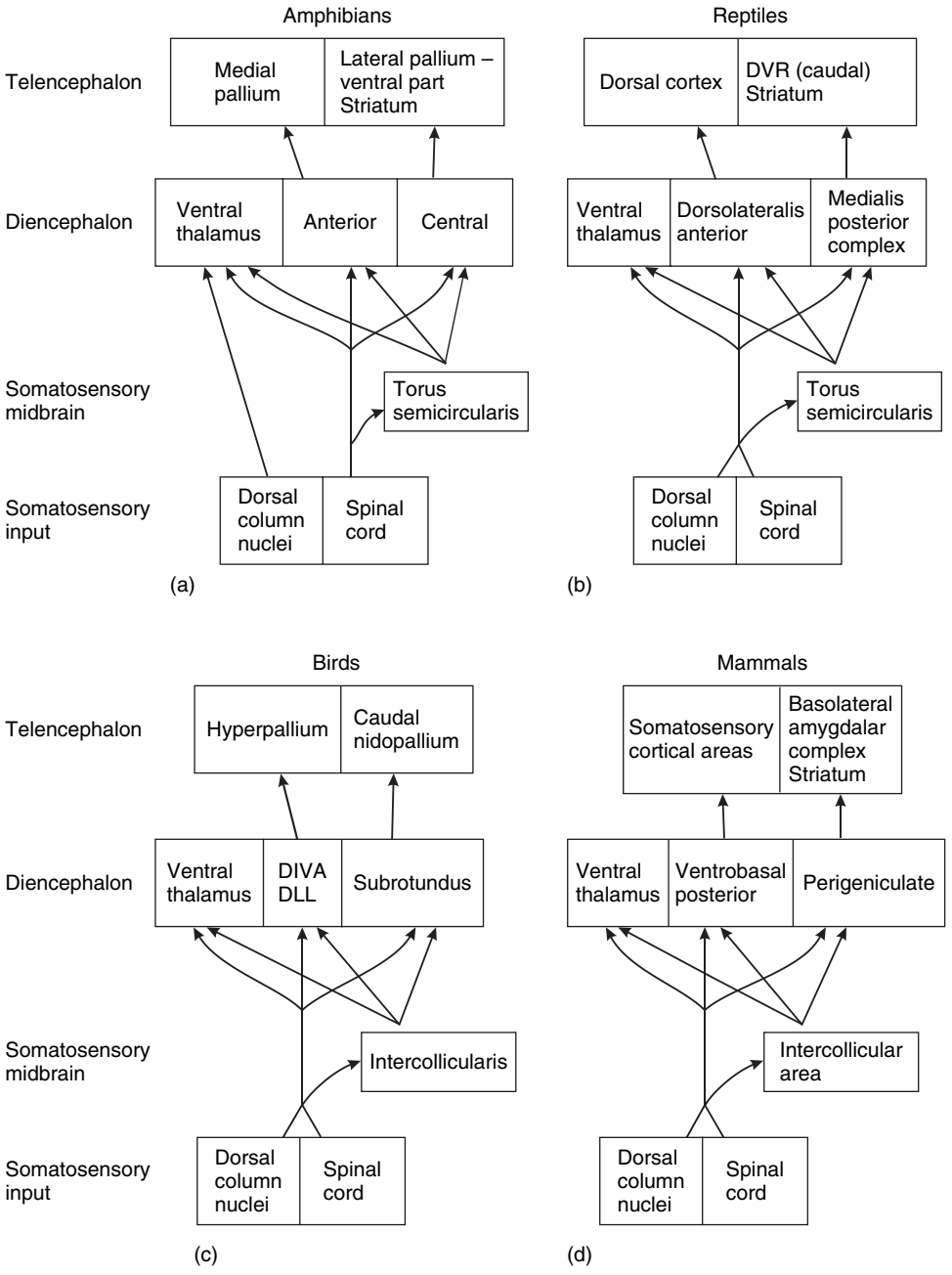

Figure 4 Comparison of the major ascending somatosensory pathways to the telencephalon in tetrapods. Refer to text for abbreviations.

et al., 1996) and thus more extensive brainstem-thalamic projections appear to have evolved in reptiles.

Three somatosensory-recipient thalamic groups in amphibians appear to correspond to those in reptiles: (1) the central thalamic nucleus, which projects heavily to the striatum and sparsely to the ventral part of the lateral pallium; (2) the anterior thalamic nucleus, which projects heavily to the medial pallium and sparsely to the dorsal and lateral pallia. Evoked potential studies suggest that the medial pallium is the target of polysensory ascending sensory information from the anterior thalamus (Karamian *et al*., 1966; Northcutt, 1970; Vesselkin *et al*., 1971; Mudry and Capranica, 1980); and (3) a ventral thalamic group lacks telencephalic projections (Neary, 1990). Thus, the central and anterior nuclei appear to be comparable to the reptilian posterior somatosensory group (medialis posterior and posterocentralis nuclei), and to the dorsolateralis anterior, respectively. Projections from the dorsal column nuclei to the dorsal thalamus appear to be

absent in amphibians, but present in reptiles, suggesting that the axons may have invaded new territory in a reptilian ancestor, or that the projection was present in the common tetrapod ancestor, but was lost in extant amphibians.

2.05.4.3.3 Reptile to bird transition The dorsal column nuclei, spinal cord, and trigeminal nuclei project to the midbrain (nucleus intercollicularis; Karten, 1963; Arends *et al*., 1984; Necker, 1989; Wild, 1997). They also have extensive thalamic projections, including: (1) three ventral thalamic nuclei, intercalatus, ventrolateral, and reticular nuclei; (2) nuclei that project to the hyperpallium, dorsointermedius ventralis anterior (DIVA), and DLL; and (3) a nucleus that projects to the caudal nidopallium, subrotundus. In addition, sensory information from the body and face reaches several intralaminar-like nuclei in birds (dorsolateralis posterior, and dorsolateralis anterior, pars medialis), but a comparable projection has not been reported in reptiles (Figure 4c; Karten, 1963; Schneider and Necker, 1989; Delius and Bennetto, 1972; Arends *et al*., 1984; Wild, 1989, 1997; Korzeniewska and Güntürkün, 1990; Veenman *et al*., 1997; Kroner and Güntürkün, 1999). There is a projection from the principle trigeminal nucleus directly to the nucleus basalis in the telencephalon, which is devoted to the bill and beak cavity sensation, and appears to be unique to birds (Cohen and Karten, 1974; Dubbeldam *et al*., 1981).

Thus, the basic pattern of most somatosensory connections was conserved during the evolution from reptiles to birds, although the thalamic targets were greatly elaborated in birds. A direct trigeminal projection to the telencephalon appears to be a unique avian feature. It may have evolved by the invasion of primary trigeminal fibers into the nearby parabrachial nucleus, which projects to the telencephalon in reptiles and mammals.

2.05.4.3.4 Reptile to mammal transition In mammals, spinothalamic, dorsal columnar, and trigeminal nucleus projections terminate in the mesencephalon (intercollicular area) and in four thalamic areas including: ventral thalamus (zona incerta); the ventrobasal and posterior thalamic nuclei; a perigeniculate area at the ventromedial edge of the medial geniculate; and the intralaminar nuclei, particularly the central lateral nucleus (Giesler *et al*., 1981; 1988; Cliffer *et al*., 1991; Willis and Coggeshall, 1991; LeDoux *et al*., 1987).

These four thalamic nuclei can be classified based on their additional connections: (1) a ventral thalamic nucleus that lacks projections to the telencephalon; (2) nuclei that project to the somatosensory cortex: the ventrobasal and posterior nuclei; (3) a limbic thalamic area that projects to the striatum and a ventrolateral pallial derivative, the basolateral amygdaloid complex, (LeDoux *et al*., 1987; Turner and Herkenham, 1991; Bordi and LeDoux, 1994; Price, 1995; Linke *et al*., 2000); and (4) intralaminar nuclei which project to the striatum and frontal motor cortex (Figure 4d).

2.05.4.3.5 Summary Somatosensory information from the spinal cord reaches the forebrain through similar pathways in all tetrapods, and thus appears to be a phylogenetically ancient feature. Somatosensory information from dorsal column nuclei appears to reach the telencephalon only in amniotes, suggesting that modifications of this pathway may have occurred in the common amniote ancestor. Three somatosensory recipient thalamic nuclei are common to all tetrapods including: (1) ventral thalamic nuclei; (2) nuclei that project to a cortical target; and (3) nuclei that project to a striatal and ventrolateral pallial target. An intralaminar-like somatosensory thalamic region has been identified in birds and mammals, and further studies are needed in amphibian and reptiles to determine its evolutionary origins (see The Evolution of the Dorsal Thalamus in Mammals, The Dual Elaboration Hypothesis of the Evolution of the Dorsal Thalamus).

This scheme suggests that the thalamic nuclei that project to the amphibian ventral lateral pallium, reptilian DVR, avian nidopallium, and mammalian pallial amygdala are homologous. This comparison is considered further in Section 2.05.5.3 (see Evolution of the Somatosensory System – Clues from Specialized Species, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?).

2.05.4.4 Olfactory System

In most tetrapods olfaction is detected in two peripheral organs, the main olfactory organ, which is sensitive to lighter, airborne molecules and the vomeronasal organ, which is sensitive to heavy odor molecules (Johnson and Leon, 2000). The vomeronasal system is unique to tetrapods and is involved in both foraging and reproductive behaviors, and in some pheromonally mediated behaviors (Eisthen, 1992; Halpern and Martinez-Marcos, 2003). The sensory neurons in the main olfactory organ and vomeronasal organ project to the main olfactory and accessory olfactory bulbs, respectively. This section compares the projections of the main and accessory olfactory bulbs.

2.05.4.4.1 Reptiles A vomeronasal organ is present in rhynchocephalians (tuatara), lizards, snakes, and turtles, but is absent in crocodilians and birds (Figure 5b). The central projections have been studied in four species of lizards, two snakes, a turtle, and caiman (Heimer, 1969; Scalia *et al.*, 1969; Halpern, 1976; Ulinski and Peterson, 1981; Reiner and Karten, 1985; Martinez-Garcia *et al.*, 1991; Lohman and Smeets, 1993; Lanuza and Halpern, 1998). The later studies that used more sensitive techniques provide greater details, and are the basis for the following summary.

The main olfactory bulb projections travel in a superficial sheet over the telencephalon to targets that include the olfactory tubercle, lateral cortex, rostral septum, nucleus of the diagonal band, and several cortical amygdalar nuclei. Olfactory fibers continue caudally in the stria medullaris, cross in the habenular commissure, and then innervate similar targets in the contralateral hemisphere.

The accessory olfactory bulb projects caudally, terminating in the medial amygdala and its medial extension (the bed nucleus of stria terminalis), in the nucleus sphericus in both lizards and snakes, and in

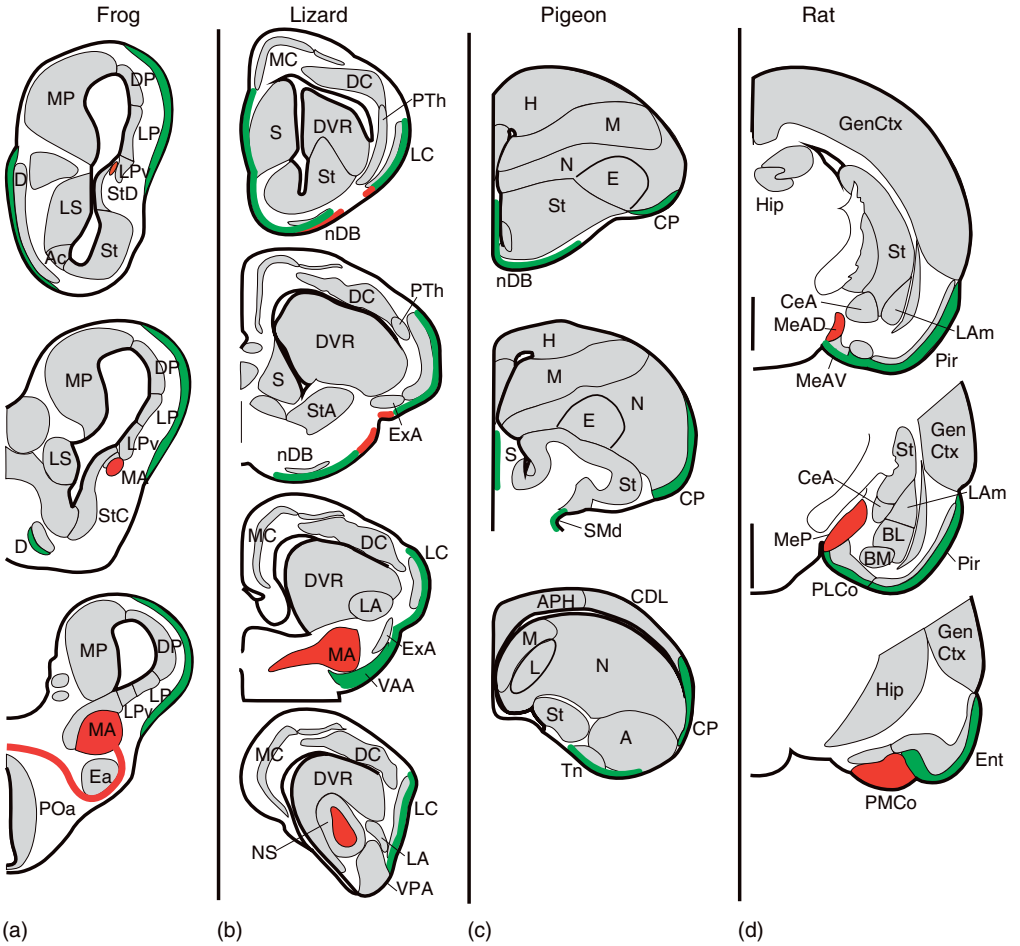

Figure 5 Comparison of the pathways from main olfactory bulb (green) and accessory olfactory bulb (red) in (a), amphibians; (b), reptiles; (c), birds; and (d), mammals. Sections are through the right hemisphere, with rostral sections at the top. Abbreviations: Frog – Ac, accumbens; D, nucleus of the diagonal band; DP, dorsal pallium; Ea, anterior entopeduncular nucleus; LP, lateral pallium; LPv, ventral part of the lateral pallium; LS, lateral septal nucleus; MA, medial amygdala; MP, medial pallium; POa, anterior preoptie area; St, striatum; StC, caudal striatum; StD, dorsal striatum. Lizard – DC, dorsal cortex; DVR, dorsal ventricular ridge; ExA, external amygdalar nucleus; LA, lateral amygdala; LC, lateral cortex; MA, medial amygdala; MC, medial cortex; nDB, nucleus of the diagonal band; NS, nucleus sphericus; PTh, pallial thickening; S, septal nuclei; St, striatum; StA, striatoamygdalar area; VAA, ventral anterior amygdala; VPA, ventral posterior amygdala. Pigeon – A, arcopallium; APH, area parahippocampalis; CDL, dorsal lateral cortex; CP, cortex piriformis; E, entopallium; H, hyperpallium; L, Field L; M, mesopallium; nDB, nucleus of the diagonal band; N, nidopallium; S, septal nuclei; St, striatum; SMd, stria medullaris tract, dorsal part; Tn, nucleus Taeniae. Rat – BL, basolateral amygdala; BM, basomedal amygdala; CeA, central amygdala; Ent, entorhinal cortex; GenCtx, general (nonolfactory, nonhippocampal) cortex; Hip, hippocampus; LAm, lateral amygdala; MeAD, anterodorsal division of medial amygdala; MeAV, anteroventral division of medial amygdala; MeP, medial pallium; Pir, piriform cortex; PLCo, posterior lateral cortical amygdala; PMCo, posterior medial cortical amygdala; St, striatum. Modified from Bruce, L. L. and Neary, T. J. 1995c. The limbic system of tetrapods: A comparative analysis of cortical and amygdalar populations. *Brain Behav. Evol.* 46, 224–234.

the ventral posterior amygdala in at least one lizard (Martinez-Garcia *et al*., 1991). Nucleus sphericus is present in most squamate reptiles, and is correlated with the importance of chemosensory function in their ecology (Halpern and Martinez-Marcos, 2003). Although a vomeronasal system is present in Testudamorpha, it is uncertain whether or not Reiner and Karten included it in their olfactory bulb injections (Reiner and Karten, 1985).

2.05.4.4.2 Amphibian to reptile transition Olfactory pathways have been studied in tiger salamanders and three species of ranid frogs (Northcutt and Royce, 1975; Kokoros and Northcutt, 1977; Kemali and Guglielmotti, 1987; Scalia *et al*., 1991; Moreno *et al*., 2005). The targets of the main olfactory bulb are very similar to those in reptiles, and include the postolfactory eminence, lateral and dorsal pallia, rostral septum, nucleus of the diagonal band, and ventral part of the lateral pallium (LPv; sometimes named the lateral amygdala); and a rostral part of the medial amygdala. The lateral and dorsal pallia are comparable to the reptilian lateral cortex, and the LPv and rostral medial amygdala may be comparable in part to the reptilian olfactory cortical amygdala (Bruce and Neary, 1995c; Moreno *et al*., 2005). A fascicle of fibers decussates in the habenular commissure, comparable to that in reptiles (Figure 5a).

A vomeronasal system is present in most aquatic and terrestrial amphibians, but is absent in some aquatic salamanders (Eisthen, 2000). The accessory olfactory bulb projects to the medial amygdaloid nucleus (formerly called the lateral amygdala) and to part of the caudal LPv. Accessory fibers cross in both the anterior commissure and the habenular commissure, unlike the ipsilateral reptilian condition. Thus, most accessory olfactory targets receive bilateral input. The amphibian medial amygdala thus appears comparable to the reptilian medial amygdala, and part of the caudal LPv appears comparable to the reptilian accessory recipient cortical nucleus. Furthermore, the reptilian nucleus sphericus appears to be a specialization of the accessory olfactory amygdala, reflecting the importance of vomeronasal information in the ecology of squamate reptiles.

2.05.4.4.3 Reptile to bird transition Birds, like crocodilians, have a main olfactory system but lack a vomeronasal system (Figure 5c). Olfactory bulb projections have been reported in pigeons and ducks (Reiner and Karten, 1985; Ebinger *et al*., 1992). The targets are remarkably similar to those seen in reptiles. Targets of the olfactory bulb include the olfactory tubercle, cortex pyriformis, rostral septum, nucleus of the diagonal band, and cortical amygdalar nuclei in the caudal telencephalon, including nucleus Taeniae. Axons cross in the habenular commissure. Unlike reptiles, the olfactory bulb does not project to most of the caudal telencephalic pole (the arcopallium).

2.05.4.4.4 Reptile to mammal transition In mammals, the main and accessory olfactory bulb projections are entirely ipsilateral (Figure 5d). Main olfactory bulb targets include the olfactory tubercle, olfactory cortex (pyriform and entorhinal), nucleus of the diagonal band, several cortical amygdalar areas (anterior amygdala, anterior cortical, and posterolateral cortical amygdalar nuclei), and the ventral anterior part of the medial amygdala (de Olmos *et al*., 1978; Switzer *et al*., 1985). Accessory olfactory bulb projections include most of the medial amygdala, the medial bed nucleus of the stria terminalis, and the posteromedial cortical amygdalar nucleus. Thus, the main and accessory olfactory targets of mammals are very similar to those of reptiles, except that mammals lack contralateral main olfactory projections.

2.05.4.4.5 Summary The main olfactory bulb targets are similar in all tetrapods, suggesting they were present in a common ancestor. A contralateral projection through the habenular commissure is present in amphibians, reptiles, and birds, but not mammals, suggesting it was lost in a mammalian ancestor.

Likewise, the accessory olfactory bulb targets are similar among most tetrapods, suggesting they were established in a common ancestor. However, contralateral projections are present only in amphibians, suggesting they were lost in ancestral reptiles. In squamate reptiles, the vomeronasal targets include nucleus sphericus, which is probably derived from the vomeronasal amygdala (Bruce and Neary, 1995c; Lanuza and Halpern, 1998). The absence of the vomeronasal system in crocodilians and birds, cetaceans, and in some primates suggests that it was lost independently in several taxa (see Evolution of Vertebrate Olfactory Subsystems, The Evolution of the Vomeronasal System).

2.05.5 Brain Regions

2.05.5.1 Hindbrain: Cranial Motor Nuclei

There are a number of extensive reports and reviews on the development and evolution of the tetrapod hindbrain (Barbas-Henry and Lohman, 1984, 1988a, 1988b; Roth *et al*., 1988; Szekely and Matesz, 1988; Bruce *et al*., 1997; Fritzsch, 1998; Gilland and Baker, 2005). These are summarized in the following description of cranial nerve motor nuclei (Figure 6).

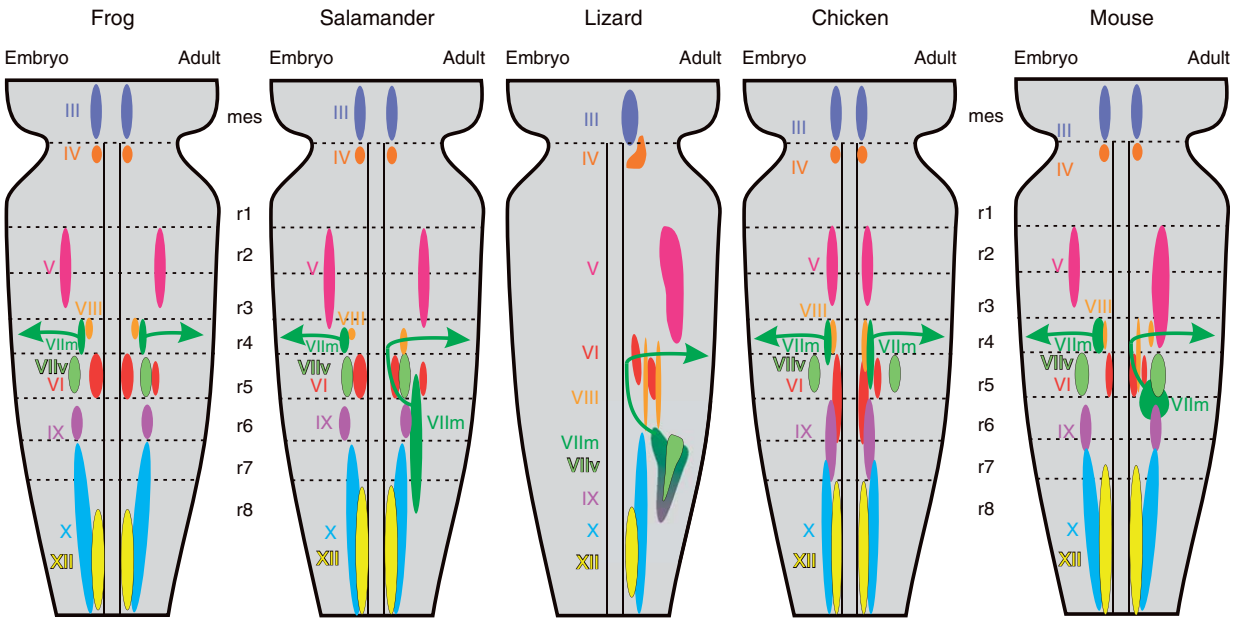

Figure 6 Comparison of the embryonic (left side) rhombomeric origin (r1–r8) and adult (right side) locations of cranial nerve motor nuclei in frog, salamander, lizard (adult only), chicken, and rat. Green arrows show the trajectory of VIIm axons. Note that the facial genu is formed by the caudal migration of VIIm neurons. Modified from Gilland, E. and Baker, R. 2005. Evolutionary patterns of cranial nerve efferent nuclei in vertebrates. *Brain Behav. Evol.* 66, 234–254 and Fritzsch, B. 1998b. Ontogenetic and evolutionary evidence for the motoneuron nature of vestibular and cochlear efferents. In: The Efferent Auditory System: Basic Science and Clinical Applications (*ed.* C. I. Berlin), pp. 31–60. Singular.

Hindbrain motor neurons can generally be divided into three major groups: somatic (cranial nerves III, IV, VI, and XII), branchiomotor (V, VII, IX, X, and XI), and visceral (VII, IX, and X). Somatic and branchiomotor neurons innervate striated muscle, and visceromotor neurons innervate parasympathetic ganglia. The octavolateral efferents innervate the auditory and vestibular periphery of tetrapods, as well as mechanosensory hair cells in the lateral line of amphibians, and are closely related to the branchiomotor neurons of VII.

The cranial nerve nuclei of all vertebrates are born near the midline floorplate and are derived from similar rostrocaudal segments, or rhombomeres, and from similar dorsoventral partitions within the hindbrain. A variety of genes regulate rhombomeric (e.g., Hox and Fgf gene families) and dorsoventral organization (e.g., Shh). Variations in gene expression are correlated with variations in the origin of the cranial nerve nuclei, and are beyond the scope of this review. The hindbrain is divided into eight rhombomeres (r1–8), although in mammals r8 is indistinct and usually included in r7. The development of hindbrain motor neurons has not been adequately studied in reptiles, but they are believed to follow the avian pattern.

2.05.5.1.1 Oculomotor nuclei (III) In all tetrapods the oculomotor motor neurons are born in the somatic column in the caudal mesencephalon, and remain near their origin. Their axons project mainly ipsilaterally, with contributions from a few cells in the contralateral caudal pole.

2.05.5.1.2 Trochlear nuclei (IV) Trochlear motor neurons of tetrapods derive from the contralateral somatic column in the rostral part of r1, and remain there. Their axons take a unique dorsal trajectory, decussating at the dorsal midline and then exiting the brain. A few ipsilateral trochlear neurons are present in mice.

2.05.5.1.3 Trigeminal nuclei (V) In all tetrapods the trigeminal motor nuclei are derived from r2 and the rostral two-thirds of r3. They remain close to their origin. Motor neurons derived from r2 appear to innervate muscles homologous to jaw adductors; those from r3 innervate the jaw openers (Song and Boord, 1993).

2.05.5.1.4 Abducens nuclei (VI) The origin of the abducens nucleus is variable, arising from r5 alone (frogs, salamanders, and mammals) or from both r5and r6 (chickens). In all tetrapods the abducens gives rise to the principal abducens, which remains adjacent to the medial longitudinal fascicle and innervates the lateral rectus muscle of the eye, and to the accessory abducens, which migrates laterally and innervates the ocular retractor muscles.

2.05.5.1.5 Facial motor nuclei (VIIm) In salamanders the facial motor nuclei are born in the caudal half of r4; in frogs, chickens, and mammals they originate from the full extent of r4. The facial motor nucleus migrates caudally but variably in salamanders, chickens, mice, but not in frogs. In salamanders it migrates to r5 and further caudally so that it overlaps the entire glossopharyngeal and parts of the vagus and hypoglossal nuclei. In chickens it extends from r4 to r5, and in mice from r5 to r6. In adult lizards the facial motor nucleus is located caudal to the abducens, suggesting that this is also a migrated population. Adult lizards have a flexure in their caudal brainstem, making relationships to other tetrapods imprecise. In animals with a migrated facial population, an internal facial genu is formed by the axons that remain in the migratory route. The facial motor nucleus innervates muscles derived from the second branchial arch.

2.05.5.1.6 Octavolateral nuclei (VIII) The octavolateral nucleus derives from the same ventricular area of r4 as the facial motor nucleus. In all tetrapods the octavolateral nuclei remain in r4, but in lizards and mice some of the neurons migrate laterally and away from the ventricle and form two clusters. In chickens a contralateral octavolateral population forms by neuronal migration across the midline; in mice it forms by the growth of axons to the contralateral side. Lizards also have a contralateral projection, although its origin is unknown. The octavolateral efferents innervate hair cells in the auditory and vestibular epithelia. In amphibians a group of octavolateral efferents arising from r4 (with the facial motor) and r6 (with the glossopharyngeal) innervate mechanoreceptors of the lateral line.

2.05.5.1.7 Facial visceral nuclei (VIIv) Also known as the superior salvitory nucleus, the facial visceral nucleus arises from r5 in all tetrapods, and remains there in adults. Although in reptiles the facial motor, facial visceral, glossopharyngeal, and vagal nuclei appear to overlap in dorsal view because of the flexure in this region, they are largely distinct nuclei (Figure 6). The facial visceral nucleus innervates postganglionic neurons to the Haderian gland.

2.05.5.1.8 Glossopharyngeal nuclei (IX) The glossopharyngeal nuclei include both a branchiomotor and a visceral group. In frogs and mammals the glossopharyngeal nuclei originate from r6, but in chickens they originate from both r6 and r7. The neurons remain in their rhombomere of origin. In adult salamanders and lizards the glossopharyngeal nuclei are intermingled with the facial motor neurons. The glossopharyngeal nucleus innervates muscles derived from the third branchial arch.

2.05.5.1.9 Vagal nuclei (X) The vagal nuclei of frogs and mice originate from r7 and r8, whereas those of chickens originate from r8. In adults the vagal nuclei may extend into the spinal cord, but it is not clear if this is due to neuronal migration.

2.05.5.1.10 Spinal accessory nuclei (XI) In tetrapods the spinal accessory nuclei originate in the rostral spinal cord. Its rostral extent often blends with the caudal extent of the vagal nuclei.

2.05.5.1.11 Hypoglossal nuclei (XII) Hypoglossal nuclei are present in all tetrapods with tongues. The rostral pole originates from progressively more rostral levels in frogs (caudal border of r8; called the first spinal nerve nucleus), chickens (caudal border of r7; called the supraspinal nucleus), and mice (middle of r7). The hypoglossal nucleus innervates the tongue musculature.

2.05.5.1.12 Summary The comparative development of the tetrapod hindbrain motor nuclei is fairly sparse, and requires analysis of more taxa, particularly from reptiles. The rostral poles of the motor nuclei have similar rhombomeric limits in all tetrapods, and thus appears to be a highly conserved feature. In contrast, the caudal pole may vary by as much as one rhombomere, particularly in chickens, and thus appears to be under fewer genetic constraints. Motor nuclei V, VIIm, and VIII migrate caudally from their ventricular origins in some taxa. In frogs there is no caudal migration, in salamanders only the facial motor nucleus migrates, in chickens the facial motor and octavolateralis nuclei migrate, and in mice the facial motor, octavolateralis, and trigeminal nuclei migrate caudally. Thus, caudal migration appears to be most prominent in mammals and least prominent in amphibians. The genu of the facial nerve forms as a result of the caudal neuronal migration, and is prominent in salamanders, lizards, and mammals. Thus, the facial genu may have been present in the common ancestor of amphibians, mammals, and reptiles, but lost in the anuran lineage.

2.05.5.2 Dorsal Thalamus

The reptilian diencephalon consists of the epithalamus, dorsal thalamus, ventral thalamus, and the hypothalamic regions. The organization of the dorsal thalamus is the best-studied region, yet some of its mammalian homologues are controversial. Thus,

the functional connections will be described and compared in this section.

2.05.5.2.1 Reptiles The reptilian dorsal thalamus consists of nuclei that project to and often receive projections from the telencephalon. It can be subdivided into at least four groups on the basis of connectional and functional characteristics (Table 1).

1. *Nuclei that receive specific sensory projections and project to the cortex*. In squamates this group includes the dorsolateralis anterior (DLA) and a retino-recipient nucleus, called either lateral geniculate, intercalatus, or the lateral part of the dorsolateralis anterior (Hall and Ebner, 1970a, 1970b; Cruce and Cruce, 1975; Hall *et al*., 1977; Hoogland, 1982; Bruce and Butler, 1984a, 1984b; Bennis *et al*,. 1994; Desfilis *et al*., 2002; Guirado and Davila, 2002). The DLA receives spinal, septal, and hypothalamic projections and a small projection from the auditory/vocal area of torus semicircularis in the midbrain. In some lizards the DLA is further subdivided into the pars magnocellularis (DLAm), which projects bilaterally to the lateral and medial cortices, and the pars parvocellularis (DLAp), which projects ipsilaterally to the dorsal cortex and pallial thickening. In Testudamorpha these subdivisions correspond to the dorsomedial anterior and the dorsolateral anterior, respectively (Desan, 1988; Zhu *et al*., 2005).
2. *Nuclei that receive specific sensory brainstem projections and project to specific regions of the striatum and DVR*. These include rotundus, medialis (reuniens in turtles and crocodilians), and medialis posterior (caudalis in turtles; medialis complex in crocodilians), which convey visual, auditory, and somatosensory information, respectively (see Section 2.05.4).
3. *A nonspecific midline area that receives widespread input and projects to cortex, DVR, central amygdala, accumbens and striatum* (Hoogland, 1982; Gonzalez *et al*., 1990; Desfilis *et al*., 2002; Heredia *et al*., 2002). In lizards the dorsomedial nucleus projects to the accumbens, striatum, and receives projections from the septum, preoptic area, ventromedial hypothalamus, ventral thalamus, and locus coeruleus.
4. *Calcitonin gene-related peptide (CGRP)-positive neurons that project to the pallium (cortex and DVR)*. In lizards these cells lie at the posterior ventral pole of the thalamus (Martinez-Garcia *et al*., 2002a).

Table 1 Comparisons of dorsal thalamic nuclei in tetrapods

	Reptile				*Mammal (rodent)*	
Amphibian	*Lizard*	*Crocodilian*	*Turtle*	*Birds*	*Revised*	*Traditional*
	DM		DM	DLM, DMA, DMP, SHL, DIP, DLP	Paraventricular, MD, CL, CM, PC, PF, IMD	Paraventricular, MD, CL, CM, PC, PF, IMD
Anterior	DLAm and DLAp		Dorsomedial anterior and Dorsolateral anterior	Superficialis parvocellularis, DLA	Anterior, reuniens, VPM, VPL, MGN, LP	Anterior, reuniens
Anterior	Intercalatus (lateral DLA)		Dorsal lateral geniculate	Dorsal optic complex	Dorsal lateral geniculate	Dorsal lateral geniculate
Lateral	Rotundus	Rotundus	Rotundus	Rotundus	Perigeniculate: Supragenículate	Lateral posterior (pulvinar)
Central	Medialis	Reuniens	Reuniens	Ovoidalis	Perigeniculate: MGm	Medial geniculate
Central	Medialis Posterior and posterocentral	Medialis complex	Caudalis	Subrotundus	Perigeniculate: Posterior intralaminar	VPM, VPL
				Ventral intermediate area	VA, VL	VA, VL

CM, central medial thalamic nucleus; CL, centrolateral thalamic nucleus; DIP, nucleus dorsointermedius posterior thalami; DM, nucleus dorsomedialis; DLA, dorsolateral anterior thalamic nucleus; DLAm, dorsolateral anterior thalamic nucleus, magnocellular part; DLAp, dorsolateral anterior thalamic nucleus, parvocellular part; DLM, nucleus dorsolateralis anterior thalami, pars medialis; DMA, nucleus dorsomedialis anterior thalami; DMP, nucleus dorsomedialis posterior thalami; DLP, nucleus dorsolateralis posterior thalami; IMD, intermediodorsal nucleus; LP, lateral posterior thalamic nucleus; MD, mediodorsal nucleus; MGN, medial geniculate nucleus; PC, paracentral thalamic nucleus; PF, parafascicular thalamic nucleus; SHL, lateral subhabenular nucleus; VA, ventral anterior thalamic nucleus; VL, ventral lateral thalamic nucleus; VPM, ventral posterior medial thalamic nucleus; VPL, ventral posterior lateral thalamic nucleus.

2.05.5.2.2 Amphibian to reptile transition The amphibian dorsal thalamus consists of the anterior, central, and lateral thalamic nuclei (Neary and Northcutt, 1983).

1. *Nuclei that receive specific sensory projections and project to the cortex* (Mudry and Capranica, 1980; Wilczynski and Northcutt, 1983a; Neary, 1984, 1988, 1990; Allison and Wilczynski, 1991; Montgomery and Fite, 1991; Northcutt and Ronan, 1992). The anterior thalamic nucleus receives visual inputs from the retina, somatosensory inputs from the obex region, spinal cord, and torus semicircularis, and auditory inputs from the lateral torus semicircularis and pretectal grey. It projects heavily to the medial and dorsal pallia and the septum, and more weakly to most parts of the telencephalon and the ventral hypothalamus.
2. *Nuclei that receive specific sensory brainstem projections and project to specific regions of the striatum and DVR*. Both the central and lateral thalamic nuclei receive widespread sensory brainstem projections and project to specific regions within the striatum and ventral part of the lateral pallium (Wilczynski and Northcutt, 1983a, 1983b; Hall and Feng, 1987; Neary, 1990, 1995; Allison and Wilczynski, 1991; Feng and Lin, 1991). The central nucleus receives input from the optic tectum and somatosensory part of the torus semicircularis. The lateral thalamic nucleus receives input from the auditory part of the torus semicircularis. Both the lateral and central nuclei project to the striatum and the ventral part of the lateral pallium.
3. *A nonspecific midline area that receives widespread input and projects to the telencephalon.* Neurons with these characteristics have not been described in amphibians.
4. *CGRP-positive neurons that project to the pallium.* These neurons are scattered within the ventromedial and posterior part of the central thalamic nuclei (Petko and Santa, 1992).
5. Thus, at least three of the four thalamic groups present in reptiles are also recognized in amphibians, suggesting that they were present in the common tetrapod ancestor. A nonspecific midline thalamic area has not been identified in amphibians, and further studies are needed to determine if it exists. Furthermore, the reptilian thalamus appears to have increased in size and become further segregated during the amphibian to reptile transition.

2.05.5.2.3 Reptile to bird transition The following thalamic groups are present in birds:

1. *Nuclei that receive specific sensory projections and project to the cortex*. Visual information from both the retina and optic tectum reaches the dorsolateralis pars lateralis (DLL), SPC, and LdOPT, whereas only the retina projects to the DLAmc. Somatosensory information from the torus semicircularis reaches the DIVA and DLL. All these thalamic nuclei project to visual and somatosensory Wulst areas in the hyperpallium (hyperstriatum).
2. *Nuclei that receive specific sensory brainstem projections and project to specific regions of the striatum and DVR (see Section 2.05.5.3)*. Auditory, visual, and somatosensory information reaches nucleus ovoidalis, nucleus rotundus, and nucleus subrotundus, respectively. Each of these thalamic nuclei then projects to a unique target in the nidopallium (previously called the neostriatum).
3. *A nonspecific midline area that receives widespread input and projects to cortex, nidopallium, accumbens, and striatum*. The dorsomedialis posterior, dorsomedialis anterior, subhabenular nucleus, dorsointermedius posterior nucleus, and dorsolateralis posterior meet these connectional criteria, and have also been compared to mammalian homologues based on neurochemical and mRNA expression patterns (Veenman *et al*., 1997; Bruce *et al*., 2002; Atoji and Wild, 2005).
4. *CGRP-positive neurons that project to the pallium*. A scattered population of CGRP-expressing neurons that projects to the hyperpallium and nidopallium is mainly located in the shell surrounding nucleus ovoidalis and in nucleus dorsolateralis posterior (Brauth and Reiner, 1991; Lanuza *et al*., 2000).
5. *Thalamic nuclei that receive motor input from cerebellar nuclei, substantia nigra, and globus pallidus, and project to the pallium*. In pigeons the ventral intermediate area has these characteristics (Medina *et al*., 1997).

Thus four of the five thalamic groups (1, 2, 3, and 4) described in birds can also be identified in reptiles (Table 2). However, a motor thalamic nucleus (5) has been identified in birds, but not reptiles. Further studies are needed to determine if it has a reptilian homologue. Also noteworthy is the increased size of many of the avian thalamic nuclei, compared to those in reptiles.

Table 2 Comparison of current models of the evolution of the avian hyperpallium, mesopallium, and sensory nidopallium

	Hyperpallium dorsal cortex	*Mesopallium pallial thickening*	*Sensory nidopallium anterior DVR*	*Caudal nidopallium posterior DVR*
Bruce and Neary (1995c), and Bruce (this article)	Isocortex	Claustrum and endopiriform	Lateral amygdala (sensory part)	Basolateral amygdala
Striedter (1997)	Isocortex	Claustrum	Endopiriform	Pallial amygdala
Puelles *et al.* (2000) and Martinez-Garcia *et al.* (2002b)	Isocortex – claustrum	Isocortex – claustrum	Endopiriform	Basal and lateral amygdalar nuclei
Butler and Molnar (2002)	Isocortex	Not mentioned	Isocortex, claustrum, endopiriform, basal and lateral amygdalar nuclei	Isocortex, claustrum, endopiriform, basal and lateral amygdalar nuclei
Karten (1997) and Reiner (2000)	Isocortex	Isocortex	Isocortex	Isocortex

These four avian territories (top row) are compared to their suggested mammalian homologue according to the proposals of various authors (left column).

2.05.5.2.4 Reptile to mammal transition The following thalamic groups are present in mammals:

1. *Nuclei that receive specific sensory projections and project to the cortex*. Auditory information from the inferior colliculus reaches the medial geniculate nucleus. Visual information from the retina reaches the dorsal lateral geniculate, and from the superior colliculus reaches the lateral posterior nucleus in rodents (LP; called pulvinar in primates). Somatosensory information from the spinal cord, dorsal column nuclei, trigeminal nuclei, and intercollicular area reaches the ventrobasal and posterior nuclei. All of these thalamic nuclei project to specific auditory, visual, and somatosensory areas in the isocortex.
2. *Nuclei that receive specific sensory brainstem projections and project to specific regions of the striatum and amygdala (see Section 2.05.5.3)*. Auditory, visual, and somatosensory information reaches the perigeniculate region, sometimes called the posterior intralaminar region. Each of these thalamic nuclei then projects to a different region of the lateral amygdala (Doron, and LeDoux, 1999; LeDoux *et al.*, 1987, 1990; Linke, 1999; Linke *et al.*, 1999, 2000; Linke and Schwegler, 2000).
3. *A nonspecific midline area that receives widespread input and projects to cortex, amygdala, accumbens, and striatum*. The centromedial thalamic nucleus, intermediodorsal, paraventricular, and parafascicular nuclei meet these connectional criteria and also have similar neurochemical expression patterns (Veenman *et al.*, 1997; Bruce *et al.*, 2002).
4. *CGRP-positive neurons that project to the pallium*. CGRP-expressing neurons project to diffuse targets in the cortex, amygdala, and striatum. They are scattered in the peripeduncular nucleus, posterior intralaminar nucleus, subparafascicular nucleus pars lateralis, and subparafascicular nucleus (Brauth and Rener, 1991; Yasui *et al.*, 1991).
5. *Thalamic nuclei that receive motor input from cerebellar nuclei, substantia nigra, and globus pallidus, and project to the pallium*. The ventral lateral and ventral anterior thalamic nuclei meet these criteria (Price, 1995).

The reptile to mammal transition is marked by a considerable increase in size and subdivisions of the thalamic nuclei that project to the cortex. At least three of these thalamic populations are comparable in reptiles and mammals (1, 3, and 4). A motor thalamic nucleus (5) has not yet been identified in reptiles, although one is present in birds. Whether the reptilian thalamic nuclei that project to the DVR should be compared with the mammalian thalamic nuclei that project to the cortex (traditional view) or with those that project to the lateral amygdala (revised view), is an ongoing controversy. These two thalamic populations appear to have few discriminating neurochemical expression patterns, which would resolve the issue. The group they are compared to is currently determined by the homology of their telencephalic target, a controversy addressed in Section 2.05.5.3. Most evidence suggests that the reptilian thalamo-DVR population is comparable to the mammalian thalamo-amygdalar population.

2.05.5.2.5 Summary The transition from amphibians to reptiles, and from reptiles to birds and mammals is marked by an increased hypertrophy and segregation of the thalamic groups with projections to the cortex. Homologues of the reptilian thalamic nuclei that project to the DVR have been identified in amphibians and birds, but the comparable group in mammals is controversial, with some

comparing these nuclei to the sensory nuclei that project to specific cortical areas (traditional view), and others to sensory nuclei that project to the lateral amygdala (revised view). Two additional thalamic groups, sensory nuclei that project to the cortex, and a CGRP-positive group, are present in amphibians, reptiles, birds, and mammals, suggesting that they were present in the common ancestor of tetrapods. A nonspecific midline group with widespread pallial projections is present in reptiles, birds, and mammals, but has not been identified in amphibians, and a motor thalamic group has been identified in birds and mammals, but not in reptiles or amphibians. Further study is needed to determine if these are present, before its evolutionary appearance can be considered.

2.05.5.3 Telencephalon

2.05.5.3.1 Reptiles The reptilian telencephalon contains two major divisions, the pallium and the subpallium. This section focuses on the pallium (telencephalic roof), including the pallial amygdalar nuclei. The pallium includes the medial, dorsal, and lateral cortices, the pallial thickening, DVR, and several amygdaloid nuclei (Figures 7b and 8b). The subpallium includes the striatum, central amygdaloid nucleus, and most septal nuclei. The medial amygdaloid nucleus contains both pallial and subpallial characteristics (Northcutt, 1978; Puelles *et al*., 2000; Brox, *et al*., 2004).

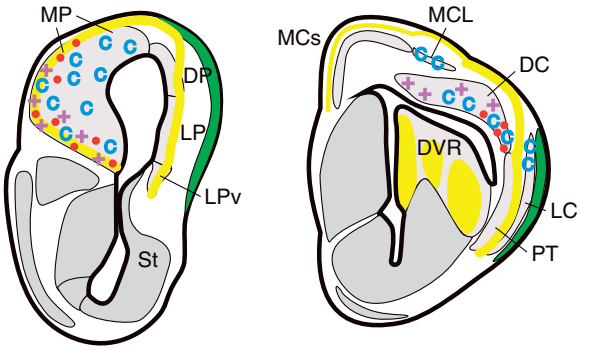

Figure 7 Comparison of pallial connections in frog (a), and lizard (b). Schematic shows the locations of neurons that project to the contralateral pallium (c), to the thalamus (+), and to the hypothalamus (red dots) in sections through the right hemisphere. Terminal areas from neurons in the olfactory bulb (green), and thalamic nuclei (yellow) (anterior nucleus in frogs; dorsolateral and dorsal lateral geniculate in reptiles) and caudal thalamic nuclei (lateral and central in frogs; medialis, medialis posterior, and rotundus in lizards: orange) are also shown. Rostral-most sections are at the top. Note that the connections of the ventral part of the frog medial pallium are most similar to those of the lizard DC. Abbreviations: frog – DP, dorsal pallium; LP, lateral pallium; LPv, ventral part of the lateral pallium; MP, medial pallium; St, striatum. Lizard – DC, dorsal cortex; DVR, dorsal ventricular ridge; LC, lateral cortex; MCL, large-celled part of medial cortex; MCs, small-celled part of medial cortex; PT, pallial thickening.

Cortical regions. The lateral cortex is the primary target of the main olfactory bulb, and reciprocates this connection (Lohman and Mentink, 1972; Martinez-Garcia *et al*., 1991; Lohman and Smeets, 1993; Lanuza and Halpern, 1998). The medial cortex is generally regarded to be a hippocampal region based on connectional and neurochemical staining patterns (e.g., Ariëns Kappers *et al*., 1936; Northcutt, 1969; Belekhova and Kenigfest, 1983; Bruce and Butler, 1984b; Olucha, 1988; Perez-Clausell, 1988; Smeets *et al*., 1989, Butler, 1994), and corresponds to the medial cortex of Testudamorpha (Desan, 1988; Zhu, 2005). The dorsal cortex appears to be a general cortical area (e.g., nonhippocampal, nonolfactory cortex). It is reciprocally connected with sensory thalamic nuclei and with the contralateral hemisphere (Northcutt, 1969, 1981; Hall and Ebner, 1970a; Bruce and Butler, 1984a; Desan, 1988; Hoogland and Vermeulen-Vanderzee, 1989; Reiner, 1991; Butler, 1994; Bruce and Neary, 1995c).

Pallial thickening. In turtles part of the pallial thickening lies deep to the olfactory-recipient lateral cortex, but the rest (the primary visual thalamic target) extends superficially and becomes continuous with the dorsal cortex (Desan, 1988; Zhu, 2005). The deep part of the pallial thickening lies over the sensory-recipient DVR, and has mainly intrinsic telencephalic connections. In lizards the pallial thickening receives a projection from the dorsolateral anterior nucleus and from the retinothalamic nucleus (intercalatus or dorsal lateral geniculate) (Bruce and Butler, 1984a; Kenigfest *et al*., 1997; Desfilis *et al*., 2002). Further studies are needed to determine if these projections are segregated into two regions of the pallial thickening, as in turtles. The pallial thickening appears to be mainly a lateral pallial derivative (Fernandez *et al*., 1998).

Dorsal ventricular ridge (DVR). The anterior DVR contains three sensory-recipient regions (auditory, visual, and somatosensory). The posterior DVR contains a caudomedial region that projects to the ventromedial hypothalamus, and a caudodorsal region that projects to the lateral hypothalamus (Bruce and Neary, 1995c). Based on Testudamorpha and avian data, the anterior DVR appears to be a ventral pallial derivative and the posterior DVR appears to include both ventral and lateral pallial territories (Fernandez *et al*., 1998; Puelles *et al*., 2000; Martinez-Garcia *et al*., 2002b).

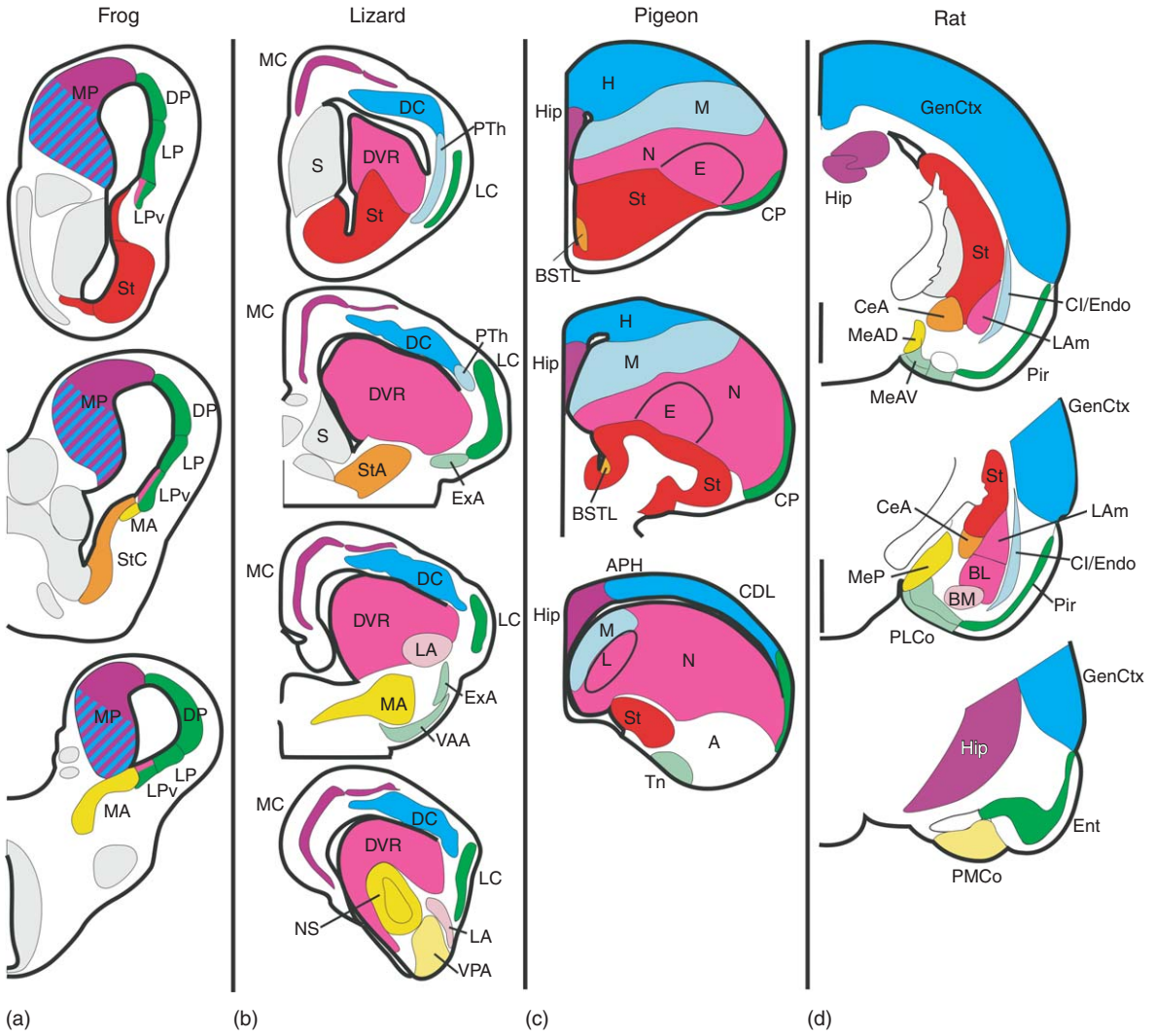

Figure 8 Summary of homologous telencephalic areas in a, amphibians; b, reptiles; c, birds; and d, mammals. Homologous areas are identified by the same color. Abbreviations: frog – DP, dorsal pallium; LP, lateral pallium; LPv, ventral part of the lateral pallium; MA, medial amygdala; MP, medial pallium; St, striatum; StC, caudal striatum. Lizard – DC, dorsal cortex; DVR, dorsal ventricular ridge; ExA, external amygdala; LA, lateral amygdalar nucleus; LC, lateral cortex; MA, medial amygdala; MC, medial cortex; NS, nucleus sphericus; PTh, pallial thickening; S, septal nuclei; St, striatum; StA, striatoamygdalar area; VAA, ventral anterior amygdala; VPA, ventral posterior amygdala. Pigeon – A, arcopallium; APH, area parahippocampalis; BSTL, lateral bed nucleus of stria terminalis; CDL, dorsolateral cortex; CP, cortex piriformis; E, entopallium; H, hyperpallium; Hip, hippocampus; L, Field L; M, mesopallium; N, nidopallium; St, striatum; Tn, nucleus Taeniae. Rat – BL, basolateral amygdala; BM, basomedal amygdala; CeA, central amygdala; cl/Endo, claustrum and endopiriform; Ent, entorhinal cortex; GenCtx, general (nonolfactory, nonhippocampal) cortex; Hip, hippocampus; LAm, lateral amygdala; MeAD, anterodorsal division of medial amygdala; MeAV, anteroventral division of medial amygdala; MeP, medial pallium; Pir, piriform cortex; PLCo, posterior lateral cortical amygdala; PMCo, posterior medial cortical amygdala; St, striatum. Adapted from Bruce, L. L. and Neary, T. J. 1995c. The limbic system of tetrapods: A comparative analysis of cortical and amygdalar populations. *Brain Behav. Evol.* 46, 224–234, with permission from Karger, Basel.

Amygdalar groups. The medial amygdaloid nucleus and nucleus sphericus receive input from the accessory olfactory bulb. The medial amygdaloid nucleus projects to the ventromedial and lateral hypothalamus (Bruce and Neary, 1995a, 1995b). The lateral amygdaloid nucleus receives sensory information from the anterior DVR and projects to the core of the ventromedial hypothalamus (Voneida and Sligar, 1979; Bruce and Neary, 1995a). The dorsolateral amygdaloid nucleus (DLA) receives sensory input from nucleus medialis posterior in the thalamus and from the anterior DVR. It receives dopaminergic input from the ventral tegmental area and cholinergic input from the basal forebrain. Its main output is to the striatum and accumbens (Lanuza *et al.*, 1998). The ventral anterior amygdaloid nucleus lies superficial to the medial amygdala. It receives olfactory input and projects to the

ventromedial hypothalamus. The ventral posterior amygdaloid nucleus lies superficial to nucleus sphericus, receives vomeronasal input, and projects to the lateral hypothalamic area. Further studies are needed to determine if these groups are derived from the ventral or lateral pallium.

The central amygdaloid nucleus has a subpallial origin, and is the only subpallial region with long descending projections to both the hypothalamus and brainstem (Russchen and Jonker, 1988; Bruce and Neary, 1995b).

2.05.5.3.2 Amphibian to reptile transition *Lateral, dorsal, and medial pallia*. The pallium of amphibians is divided into lateral, dorsal, and medial pallial fields (Northcutt, 1981), which were traditionally compared to the lateral, dorsal, and medial cortices of reptiles. However, a re-analysis of the amphibian pallial connections led Bruce and Neary (1995c) to conclude that both the dorsal and lateral pallia are comparable to those of the reptilian lateral cortex, whereas the medial pallium is comparable to both the reptilian medial and dorsal cortices (Figures 7a, 7b, and 8a). The connections of the reptilian lateral cortex and the amphibian lateral and dorsal pallia are similar: they receive a substantial projection from the main olfactory bulb, moderate input from the rostral thalamus, lack commissural connections, and do not project outside the hemisphere (Northcutt and Royce, 1975; Neary, 1990; Scalia *et al*., 1991). The amphibian medial pallium is topographically and connectionally similar to the reptilian medial cortex, but it also has connections in common with the reptilian dorsal cortex. These include projections to the olfactory bulb, hypothalamus, thalamus, and midbrain, contralateral projections, and the lack of direct olfactory input (Bruce and Butler, 1984a; Hoogland and Vermeulen-Vanderzee, 1989; Neary, 1990; Northcutt and Ronan, 1992).

Ventral part of the lateral pallium (LPv). The LPv is an embryologically and connectionally unique subdivision of the lateral pallium that is comparable to the DVR and overlying olfactory cortex in reptiles (Bruce and Neary, 1995c; Brox *et al*., 2004; Moreno and Gonzalez, 2004). Both the anterior LPv and the anterior DVR are ventral pallial derivatives, whereas the posterior LPv and part of the posterior DVR are lateral pallial derivatives (Fernandez *et al*., 1998; Martinez-Garcia *et al*., 2002b; Brox *et al*., 2004). The caudal LPv is the only pallial region that receives input from the main olfactory bulb and the hypothalamus, and projects to the medial hypothalamus.

Medial amygdala. Traditionally known as the pars lateralis of the amygdala (Northcutt and Kicliter, 1980), the medial amygdala receives input from the accessory olfactory bulb and projects to the hypothalamus (Northcutt and Royce, 1975; Wilczynski and Allison, 1989; Scalia *et al*., 1991; Neary, 1995), and thus appears to be homologous to the reptilian medial amygdaloid nucleus, nucleus sphericus, and the ventral posterior amygdalar nucleus (Bruce and Neary, 1995c).

2.05.5.3.3 Reptile to bird transition *Cortical areas*. There is general agreement about the homologues between the reptilian and avian cortical regions (Figures 7c and 8c). The squamate medial cortex is comparable to the avian hippocampus (dentate and Ammon's horn). The reptilian dorsal cortex is comparable to the avian parahippocampal area, dorsolateral cortex, and hyperpallium. The reptilian lateral cortex is comparable to the avian cortex piriformis (e.g., Ariëns Kappers *et al*., 1936; Bruce and Neary, 1995c; Striedter, 1997; Reiner, 2000).

Nidopallium and arcopallium. The reptilian dorsal ventricular ridge has been compared to the avian nidopallium, mesopallium, and arcopallium (Striedter, 1997), or to only the nidopallium (Bruce and Neary, 1995c). These homologues are evaluated in detail below. Several connections of the arcopallium have not been identified in reptiles (e.g., afferents to the optic tectum), and further studies are needed to identify its homologue with certainty.

Mesopallium. Although the mesopallium (formerly named hyperstriatum ventrale) and nidopallium together form a ventricular ridge like the reptilian DVR, the connections of the mesopallium compare best to those of the reptilian pallial thickening, rather than the DVR. The mesopallium is reciprocally connected with the nidopallium, hyperpallium and striatum, and receives input from the posterior amygdala, substantia nigra, and locus coeruleus (Bradley *et al*., 1985; Alpar and Tombol, 1998; Metzger *et al*., 1998; Csillag *et al*., 1994). Comparable connections are associated with the pallial thickening of lizards (Bruce and Butler, 1984a).

Amygdalar groups. In birds the posterior amygdala and olfactory recipient nucleus Taeniae are the only regions of the avian brain consistently agreed to be amygdalar. Thus, a great deal more work is needed to identify avian amygdalar groups. A homologue of the posterior amygdala has not been identified, but nucleus Taeniae is homologous in part with olfactory-recipient amygdalar groups of reptiles.

2.05.5.3.4 Reptile to mammal transition *Cortical areas*. The lateral, dorsal, and medial cortices are generally compared to the piriform, general (i.e., nonolfactory, nonhippocampal), and hippocampal

cortices of mammals (Figures 7d, 8b, and 8d). There is little controversy over these comparisons as they occupy similar positions in the hemisphere and have similar connections (Ariëns Kappers *et al*., 1936; Northcutt, 1969; Bruce and Neary, 1995c). In addition to the massive increase in the size of the mammalian cortex relative to the reptilian dorsal cortex, the major difference is that the mammalian general cortex develops with an 'inside-out' migration pattern (Goffinet *et al*, 1986).

Claustrum, endopiriform, and the basolateral amygdalar complex. The homologues of these regions are highly controversial and will be addressed in detail below.

Basomedial amygdala. The reptilian lateral amygdalar nucleus and basomedial amygdalar nucleus of mammals have similar connections, including a projection to the ventromedial hypothalamus, and appear to be homologues (Bruce and Neary, 1995c; Martinez-Garcia *et al*., 2002b).

Vomeronasal and olfactory amygdaloid nuclei. Amygdaloid nuclei receiving vomeronasal and olfactory input have been identified in reptiles and mammals, and appear to be homologuous, in part (see Section 2.05.4.4).

Central amygdala. Both the mammalian central amygdala and reptilian striatoamygdalar area have long descending projections to the brainstem and appear to be homologues (Russchen and Jonker, 1988; Bruce and Neary, 1995c; Martinez-Garcia *et al*., 2002b).

2.05.5.3.5 Homologues of the DVR and pallial thickening The identification of mammalian homologues of the reptilian DVR and pallial thickening, and the avian nidopallium and mesopallium has been a long-standing controversy (Table 2). The avian nidopallium (neostriatum) and reptilian DVR are often compared to parts of the mammalian neocortex (Karten, 1969, 1997; Reiner, 2000). However, a number of laboratories have recognized similarities between the avian nidopallium, the reptilian DVR, and the mammalian basolateral amygdalar group. Several of these have focused on the comparison between the sensory nidopallium and its mammalian homologue. Striedter (1997) compared the mesopallium and nidopallium to the claustrum and endopiriform areas, respectively. Puelles *et al*. (2000) and Martinez-Garcia *et al*. (2002b) suggested that the nidopallium/DVR may contain cells comparable to the endopiriform area and the basolateral amygdalar complex. Butler and Molnar (2002) compared the nidopallium/DVR to the mammalian basolateral amygdalar complex plus temporal isocortex and the claustrum-endopiriform area. Bruce and Neary (1995c) compared only the basolateral amygdalar group to the nidopallium, and specifically compared the rostral sensory nidopallium to the mammalian lateral amygdala (also a sensory thalamic target).

To determine which evolutionary model fits the current data best, the expression patterns of various early genetic markers, receptors, and ligands in reptiles, birds, and mammals will be summarized, as well as developmental and connectional data (Table 3).

Emx-1. In turtles Emx-1 expression appears in the cortices and pallial thickening, but little or none is expressed in the anterior (sensory) DVR; in mammals it is expressed in the claustrum and cortex, but not in the lateral amygdala (sensory amygdala) or the endopiriform nucleus; in birds Emx-1 is expressed in the hyperpallium (Wulst) and mesopallium, but not in the sensory nidopallium (Fernandez *et al*., 1998; Puelles *et al*., 2000). Thus, the mammalian cortex and claustrum are in the same Emx-1

Table 3 Comparison of various traits

	Mammalian structures				*Avian structures*		
	Iso	*Cl*	*E*	*LA*	*H*	*M*	*Ns*
Emx-1 gene	+++	+++			+++	+++	
a-adrenergic receptor	++	+++	+++	+	++	+++	+
Opiate delta receptor	+++	++++	++++	+++	+/++	++	+/++
Opiate kappa receptor	++	++++	++++	+++	+++	++++	++
VIP	+++	+++	+++	++	+/++	+++	+
NT receptor	+	+	+++	+/++	+	+/+++	+
Development	Dorsal	Lateral	Lateral	Ventral	Dorsal	Lateral	Ventral & lateral
Sensory thalamic afferents	Yes	No	No	Yes	Yes	No	Yes
Sensory thalamic efferents	Yes	No	No	No	Yes	No	No

Cl, claustrum; E, endopiriform nucleus; H, hyperpallium; Iso, isocortex; LA, lateral amygdala; M, mesopallium; Ns, sensory nidopallium; NT, neurotensin; VIP, vasoactive intestinal protein.
+ – density of expression from low (+) to very dense (++++).

positive pallial domain as the avian hyperpallium and mesopallium, and the reptilian cortex and pallial thickening. The mammalian endopiriform nucleus and lateral amygdalar nucleus, the avian sensory nidopallium, and most, if not all, of the reptilian anterior DVR belong to an Emx-1 negative region.

Alpha 2 adrenergic receptor. In quails the hyperpallium, cortex dorsolateralis, and mesopallium contain high levels of expression, in contrast to the adjacent nidopallium, which expresses low to moderate levels (Ball *et al*., 1989; Ball, 1994; Fernandez-Lopez *et al*., 1990). In rats the alpha 2A adrenoreceptor subtype is expressed only in cortical layers 1–4 and in the claustrum and endopiriform nuclei, but not within the amygdala, and the alpha 2C-adrenoreceptor is expressed in the striatum but not the pallium (Uhlen *et al*, 1997). These data indicate that the alpha 2 adrenoreceptor differentiates the mammalian cortico-claustro-endopiriform areas and the avian cortico-hyperpallial-mesopallial areas from the mammalian lateral amygdala and avian neostriatum.

Opiate receptors. In turtles the delta opiate receptor is expressed more densely in the pallial thickening than surrounding areas (figures 2 and 4 of Xia and Haddad, 2001). In pigeons the delta and kappa opiate receptors are more abundant in the hyperstriatum ventrale relative to the neostriatum, and this is especially true for the kappa opiate receptor (Reiner *et al*., 1989). In rats the endopiriform-claustrum exhibits very dense binding with delta and kappa opiate receptors (Mansour *et al*., 1987). The lateral and basolateral amygdalar nuclei exhibit moderate to dense delta and kappa labeling. The density of labeling in the lateral amygdala appears to be considerably less than in the endopiriform-claustrum. Thus, the delta and kappa opiate receptors are expressed throughout the pallium of mammals, birds, and turtles, but are expressed more strongly in the mammalian claustro-endopiriform, avian mesopallium, and turtle pallial thickening than in adjacent areas.

Vasoactive intestinal protein (VIP). In birds VIP-like immunoreactivity and mRNA expression is present in most, but not all, of the hyperpallium and mesopallium, whereas little or none is expressed throughout the nidopallium (Shimizu and Karten, 1990; Hof *et al*., 1991; Kuenzel *et al*., 1997). In rats VIP is expressed in neurons and terminals in the claustrum and endopiriform, but in the lateral amygdala cell bodies but not terminal fibers express VIP (Sims *et al*., 1980; Kowianski *et al*., 2001). Thus, VIP expression is higher in the mammalian claustro-endopiriform area and avian mesopallium than in adjacent cortical and amygdalar/neostriatal areas.

Neurotensin. In turtles neurotensin immunoreactive terminals are particularly dense in the molecular layer of the dorsal cortex and lateral to the pallial thickening (Reiner, 1992). In lizards it is expressed in the lateral (olfactory) cortex, including a region that appears to correspond to the pallial thickening (Bello *et al*., 1994). In mammals they are scattered in the piriform cortex and have a dense accumulation in the claustrum and a small part of the lateral amygdalar nucleus (Jennes *et al*., 1982).

Neurotensin receptors. In pigeons neurotensin receptors are distributed densely in the hyperpallium and mesopallium, although a small region within the lateral mesopallium is conspicuously lightly labeled (Brauth *et al*., 1986). The nidopallium is less intensely labeled except for a group of cells surrounding Field L that are densely labeled. In rats, very high densities of neurotensin receptors occur in the superior rhinal cortex and in the endopiriform cortex, whereas the core of the claustrum has a very low density. Labeling in the lateral amygdala has a lower density of expression than in the adjacent endopiriform area (Young and Kuhar, 1981; Quirion *et al*., 1982).

Development. Developmental studies have concluded that the nidopallium develops from a precursor region that in mammals gives rise to olfactory cortex, the endopiriform, claustrum, and the basolateral amygdaloid complex (Bayer and Altman, 1991; Fernandez *et al*., 1998; Striedter *et al*., 1998; Puelles *et al*., 2000; Reblet *et al*., 2002; Brox *et al*, 2004).

Connections. The connections of the avian hyperpallium and nidopallium are similar to those of the reptilian dorsal cortex and DVR, and of the mammalian isocortex and lateral amygdala, respectively (see Bruce and Neary, 1995c). In mammals the endopiriform projects principally to olfactory cortical areas including amygdalocortical nuclei, and sparsely to all the deep amygdalar nuclei except the accessory basal nucleus to which it projects densely (Behan and Haberly, 1999). The claustrum is extensively interconnected with cortical areas, receives projections from the nucleus centralis thalami, and probably from the locus coeruleus and the lateral hypothalamus (LeVay and Sherk, 1981). The avian mesopallium receives projections from the hyperpallium accessorium, caudal and intermediate parts of the nidopallium. Thalamic projections to mesopallium arise from the nucleus dorsolateralis posterior thalami (Funke, 1989; Gamlin and Cohen, 1986; Metzger *et al*., 1998; Leutgeb *et al*., 1996; Lanuza *et al*., 2000), which is comparable to a

group of mammalian thalamic nuclei that includes the central lateral nucleus (Veenman, 1997; Bruce *et al*., 2002). Efferents from the mesopallium include a projection to the nidopallium (Wild *et al*., 1993). Most studies indicate that the mesopallium projects principally within the telencephalon, including a projection to the dorsal arcopallium (Karten, 1969; Nauta and Karten, 1970; Metzger *et al*., 1998). The connections of the mammalian endopiriform and claustrum, avian mesopallium, and reptilian pallial thickening are similar and thus are consistent with a possible homology. However, none of the connections are unique to claustrum-endopiriform, mesopallium, or pallial thickening, and so cannot be used as strong indicators of a homology.

Together the data indicate that:

1. The best comparison of the mammalian general cortex (including isocortex) is with the avian hyperpallium and dorsolateral cortex and with reptilian dorsal cortex. This is consistent with all models.
2. The best comparison of the mammalian claustrum and endopiriform areas is with the avian mesopallium and reptilian pallial thickening. This is consistent with the models of Bruce and Neary (1995c), Bruce (this article), Striedter (1997), and Puelles *et al*. (2000).
3. The best comparison of the mammalian sensory lateral amygdala is with the avian sensory nidopallium and reptilian DVR. This is consistent with the models of Bruce and Neary (1995), Puelles *et al*. (2000), and Butler and Molnar (2002).
4. The hypotheses that the avian sensory nidopallium is comprised of overlapping territories corresponding to the mammalian claustrum, endopiriform nucleus, and lateral amygdala (Butler and Molnar, 2002), or the mammalian endopiriform nucleus and lateral amygdala (Striedter, 1997; Puelles *et al*., 2000) are weakly supported by this data.
5. The hypothesis that the avian sensory nidopallium is comparable to parts of the mammalian neocortex (Karten, 1997; Reiner, 2000) is also poorly supported by this data.

2.05.5.3.6 Summary If we assume that the forebrain of the common tetrapod ancestor was similar to that of extant amphibians, then the amphibian–reptile transition was marked by segregation of pallial fields (Figure 8). The medial pallium gave rise to the medial and dorsal cortices of reptiles, the dorsal and lateral pallia gave rise to the pallial thickening and most of the lateral cortex, and the ventral part of the lateral pallium gave rise to the DVR and overlying lateral and amygdalar cortices.

The changes during the transition from the reptilian to avian forebrain were not dramatic, since recognition of homologous regions is fairly straightforward. However, the identification of a homologue of the avian arcopallial region is uncertain. In reptiles the vomeronasal system projects to a topographically similar region as the arcopallium. Thus, the appearance of the arcopallium may result from an adaptation to the loss of the vomeronasal input.

Connectional as well as genetic and neurochemical expression patterns indicate that the reptilian DVR, avian nidopallium, and mammalian basolateral amygdalar complex are homologous. The reptile to mammal transition is marked by a massive hypertrophy of the dorsal (general) cortex, which may be associated with a more caudal location of the amygdaloid groups in mammals, compared to reptiles or birds (see The Origin of Neocortex: Lessons from Comparative Embryology, What Fossils Tell Us about the Evolution of the Neocortex, Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?, Evolution of the Amygdala in Vertebrates).

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2.06 The Evolution of Dome Pressure Receptors in Crocodiles

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Glossary

crocodilian sensory skin	Skin with concentrations of sensory receptors, the dome pressure receptors.
tactile	Relating to the sense of touch.

2.06.1 Introduction

2.06.1.1 Crocodilian Phylogeny

Presently, three primary families are currently recognized within the Crocodilia: the Alligatoridae (which includes the genera *Alligator, Caiman, Melanosuchus*, and *Paleosuchus*), the Crocodylidae (*Crocodylus, Osteolaemus,* and *Tomistoma*), and the Gavialidae, which includes only one species, *Gavialis gangeticus* (Figure 1). The dominant terrestrial vertebrates during the Mesozoic (225–65 Mya) were reptilians of the subclass Archosauria. During the Triassic (250–205 Mya) the order Thecodontia, the basal group of Archosaurs, radiated to produce the orders Saurichia and Ornitischia (together commonly known as dinosaurs); the Pterosaurs, fliers with wings made of skin, and the Crocodilia. Crocodilia is a crown-group name based on the last common ancestor of *Gavialis, Alligator, Paleosuchus, Caiman, Melanosuchus, Tomistoma, Osteolaemus,* and *Crocodylus,* and all of its descendants (Benton and Clark, 1988; Clark, 1994; Brochu, 1997, 1999). Crocodilians are the only surviving archosaurian reptiles (Pough *et al*., 1998) and have been successful freshwater predators for the past 200 My. Extinct crocodilians outnumber their living relatives by a wide margin and three major forms have existed: (1) completely terrestrial, (2) completely aquatic, and (3) semi-aquatic animals. Extant crocodilians are all semi-aquatic and constitute 23 living species within this order. Fossils of extant species begin to appear in the Cretaceous era and the emergence of the eight genera present in the order Crocodilia begins in the Tertiary of Europe with Crocodylus (true crocodiles) (Densmore and Owen, 1989). Gavials emerged between the Oligocene and

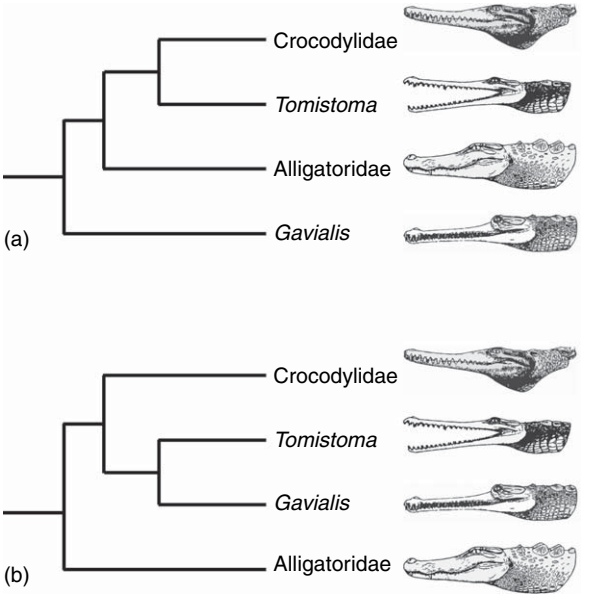

Figure 1 Schematic view of crocodilian relationships based on: a, morphological data and b, molecular data. Modified from Janke, A., Gullberg, A., Hughes, S., Aggarwal, R. K., and Arnason, U. 2005. Mitogenomic analyses place the gharial (*Gavialis gangeticus*) on the crocodile tree and provide pre-K/T divergence times for most crocodilians. *J. Mol. Evol.* 61, 620–626.

Miocene periods in South America and India, while the *Caiman* (true caimans) and Paleosuchus (smooth-fronted caimans) emerged in the Pliocene period. Alligators emerged in the Miocene period of North America. Alligators and Paleosuchus are both monophyletic phyla and are sister taxa. Within alligators, the American alligator and the Chinese alligator (*Alligator sinensis*) are sister taxa, possessing the same most recent ancestor. Although the intergeneric and interspecific relationships within the order Crocodilia has been largely determined utilizing nontraditional DNA analysis, advanced research in this respected analysis as well as new methods of research are required to finalize and complete the true phylogenetic tree (see Evolution of the Amphibian Nervous System).

2.06.1.2 Crocodilian Skin

Most species of crocodilians occur in freshwater, but some invade brackish or salt-water environments, tightly constraining the qualities in the skin (Davis *et al.*, 1980; Dunson and Mazzotti, 1988). Briefly, the crocodilian skin is hard, although its stiffness is different in different regions of the body. The skin is harder and thicker in dorsal parts where it often contains osteoderms (bony plates), and less in ventral and lateral parts (Brazaitis, 1987). The toughness results from both the strongly collagenous dermis (Alibardi and Thompson, 2000) and the presence in the epidermal layers of a hard form of keratin (Alibardi and Thompson, 2001). The epidermis of the Crocodilia closely resembles that of birds and mammals and has a stratified epithelium with a layer of cornified cells which varies in thickness and shape across the scale depending on function.

2.06.2 Dome Pressure Receptors

2.06.2.1 Anatomy

The skin of crocodilians is covered with small pigmented domes (Figure 2). Initially, before the function of these organs was known, these organs were named integumentary sensory organs (von During, 1973) but were renamed dome pressure receptors (DPRs) (Soares, 2002) once their sensory modality was discovered. The distribution of DPRs is different in the families of the Crocodilia: in the Alligatoridae DPRs are segregated to the face and mouth while in the Crocodylidae DPRs are seen all over the body, being present on the caudal portion of most scales (dorsal and ventral), including those on the belly, the legs, the tail, and even around the cloaca. In the gavial, DPRs are also present all over the body but in less density. The significance of this differential distribution is still elusive since all crocodilians have populated similar niches. One exception may be that the gavial, although sharing many of the characteristics of a crocodilian lifestyle, feeds almost solely on fish. Their physiognomy is adapted for this diet and their long thin snouts give little resistance to the water during swiping motions that snap up fish.

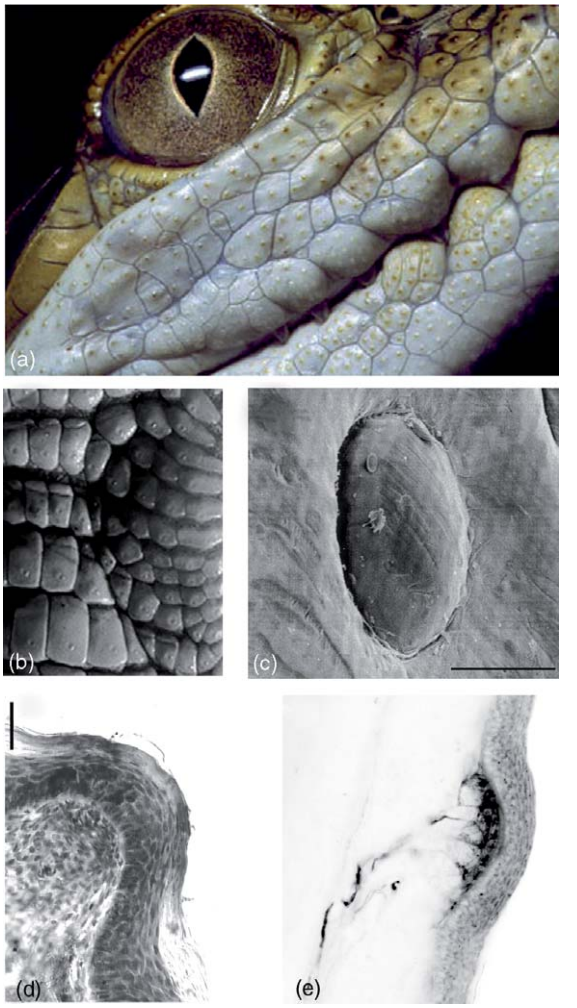

Figure 2 Anatomy of DPRs. a, DPRs are present on the faces of alligators but (b) all over the bodies of crocodiles and gavials. Photograph showing the distribution of DPRs on the ventral portion near the cloaca of a crocodile. Note a single DPR per scale. c, Scanning electron micrograph of a single DPR of an alligator. d, Cresyl violet-stained transverse section of single DPR. e, Cross section of DPR showing trigeminal innervation in the dermis region. Scale bar : 100 μm. a, Courtesy of Adam Britton.

DPRs are round dome-like structures that lack pores or protruding hairs. The epidermis is thinner immediately above the DPRs while the keratin layer is approximately 60% finer and compact. The dermal evagination where each organ is located contains highly branched nerve bundles where

secondary and tertiary branching occurs immediately under the epidermis. Electron microscopy studies show that at least in alligatorids unmyelinated branches rise from larger myelinated branches of axons. In the American alligator, these bundles are of trigeminal origin and somata of innervating neurons are located in the trigeminal ganglion lateral of the brainstem. The trigeminal nerve is the largest of all the cranial nerves in the alligator. Within the brainstem itself, fibers terminate in sensory nucleus V. This brainstem nucleus is hypethrophied and can be seen as large lateral lobes of the brainstem during gross observations of the brain. It is not surprising that the trigeminal system innervates DPRs, for this pathway innervates many specialized organs in vertebrates, such as the electrosensory organs in the platypus (Gregory *et al*., 1987), infrared detectors in snakes (Molenaar, 1974), gustatory organs in the catfish (Finger, 1976), and mechanosensory vibrissa in rats (Gibson and Welker, 1983). The source of innervation of DPRs located on the bodies of crocodiles and gavials is still unknown.

2.06.2.2 Behavior

2.06.2.2.1 Hunting Although crocodilians in general are known to have diverse diets, all species are believed to be opportunistic feeders and lay-and-wait hunters. Additionally, crocodilians will engage in necrophagy. Studies on stomach contents of crocodiles and alligators have yielded an understanding of food choices of animals during development. Subadult alligators for example, mostly eat invertebrates, such as giant water bugs (*Belostoma* spp.), apple snails (*Pomacea paludosa*), crayfish (*Procambarus penninsulatus*), round-tailed muskrats (*Neofiber alleni*), and marsh rabbits (*Sylvilagus palustris*). Adults primarily consume red-bellied turtles (*Pseudemys nelsoni*), peninsular cooters (*P. floridana*), stinkpots (*Sternotherus odoratus*), gizzard shad (*Dorosoma cepedianum*), and gars (*lepisosteus platyrhincus*) (Delany and Abercrombie, 1986).

Only one behavioral experiment has been done to date to elucidate the role of DPRs in feeding (Soares, 2002). During these behavioral trials, half-submerged subadult alligators (*Alligator mississippiensis*) responded to disturbances of the water interface by orienting themselves to the source of the stimulus. In this study the stimulus used was a droplet of water on to a flat body of water that contained a half-submerged alligator. This orienting behavior was robust and consisted of either head-turning or a full body lunge toward the source in a predatory manner and often included biting. Interestingly, this behavior only occurred when the alligators' faces were located on the air–water interface, and not when the animals were completely submerged or had their heads entirely out of the water. Errors when determining source location were very small. In one instance (unpublished), the same paradigm was used but a background surface wave noise was introduced. Alligators were still able to detect the source of the stimulus, albeit they were slower to reach the target and made a few more errors in precision. When all DPRs were covered by a plastic elastomer this behavior was extinguished. Animals did not orient to the source of the stimulus and many did not move at all. Removal of the plastic elastomer allowed for the behavior to be regained. Popular reports (personal communication) suggest that adult animals (American alligators) perhaps display the same type of orienting behavior and are attracted to animals that disturb the air–water interface either to drink or to submerge (such as turtles).

The importance of DPRs in hunting is likely to change according to the animal's size and may be correlated with prey choice. The distribution of DPRs changes during the life span of the animal, where hatchlings start with a very compacted array of DPRs and large adults have up to 2–3 cm of skin in between DPRs. This difference may be related to the prey choice, since, when juveniles, crocodilians tend to prefer smaller prey such as insects which will create smaller, higher-frequency surface waves and will avoid larger surface waves (such as those created by larger animals). So it is therefore possible that DPRs are tuned to different frequencies at different stages of life.

2.06.2.2.2 Mating Although no studies have been done on the importance of DPRs in other behaviors, it is likely that these sensory organs may play a role in the courtship of crocodilians. In the American alligator, initiation of breeding patterns begins with courtship activities. One notable courtship activity that may be especially suited for DPR detection is bellowing (Figure 3a). Bellowing occurs in shallow water, where the animal has its caudal part submerged and sometimes its rostrum on land. Bellowing serves as a sexual attractant (Vliet, 1989) between male and female alligators and this particular courtship activity requires various changes in body posturing of the male. Bellowing begins with inhalation, where the alligator raises its head above the water and performs gulping motions. Second, the alligator raises its head 30–40° above water and arches its tail. Then the

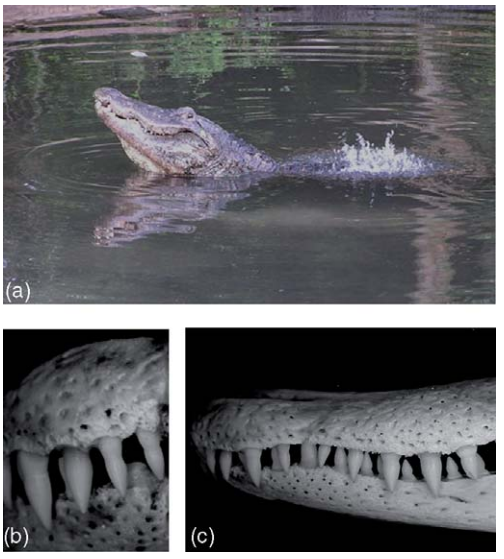

Figure 3 Behavior and osteology. a, Photograph of a bellowing alligator. b, and c, Foramina from which trigeminal innervation arises toward DPRs. Similar distribution of foramina can be seen in extinct forms of crocodilians that have a semi-aquatic lifestyle. Scale bar: 3 cm. a, Reproduced by permission of Glenn Baker.

alligator produces very low frequency vibrations with its throat and body, visibly tensing its muscles and inducing an upward motion of water on its torso. During this portion of the behavior many surface waves can be seen surrounding the animal's body, and the water has been described as on a 'frying pan' about the alligator's torso. Lastly, the alligator performs an audible bellow. Bellowing occurs in both the male and female and provides information about the size, sex, location, and social position of the active bellower.

In addition to bellowing, alligators display other forms of courtship behavior that may be sensed by DPRs, such as head-slapping and snout- and head-rubbing (Vliet, 1989). Male and female alligators (Davis *et al*., 2001) respond differently to these courtship activities. The male alligator portrays its size and strength and continues the courtship activities of bellowing and head-slapping in the presence of a bellowing female, while the female alligator migrates toward the strong bellowing and head-slapping activities of a male. Differential participation of DPR input can perhaps be involved in the differences in behavior of the two sexes.

2.06.2.3 Evolution

The phylogenetic history of DPRs can be traced because their innervation leaves markings in the mandibulary and maxillary bones in the form of foramina (Figure 3). These foramina allow for the passage of trigeminal nerve branches from the ganglia near the brain to the DPRs on the skin. The spatial distribution of these foramina is distinct in the skull bones of living crocolilians. It appears as a beehive pattern with many spatially distributed foramina. One study examined the distribution of foramina in extant and extinct crocodilians and found that all living specimens have both foramina and DPRs (Alligatoridae: *Alligator mississippiensis, A. sinensis, Caiman crocodilus, C. c. apaporiensis, C. c. fuscus, C. latirostris, C. yacare, Melanosuchus niger, Paleosuchus palpebrosus,* and *P. trigonatus*; Crocodylidae: *Crocodylus acutus, C. cataphractus, C. intermedius, C. johnstoni, C. mindorensis, C. moreletii, C. niloticus, C. novaeguineae, C. palustris, C. porosus, C. rhombifer, C. siamensis, Osteolaemus tetraspis*, and *Tomistoma schlegeli;* Gavialidae: *Gavialis gangeticus:* data not shown). Only extinct crocodilian forms that are believed to have had a semi-aquatic lifestyle also show this pattern of foramina (all specimens examined: Triassic: *Protosuchus richardsori, Eutretauranosuchus*; Jurassic: *Metriorhynchus, Mystriosaurus,* and *Goniopholis lucastii;* Creteceous: *Borrealosuchus [leidyoduchus]-stenobergii, Branchychampsa montana, Prodiplocynodon longi, Halops obsceris, Hypsaur rogersii, Deinosuchus rugosus, Teleorhinus robustus,* and *Lleidyosuchus gelmorei*; Paleocene: *Allognathosuchus mooki, Navajosuchus novemexicanus, Lleidyosuchus multidentatus,* and *Brachyuranochampsa zangeli;* Eocene: *Pristichampsa uorax, Asiatosuchus mongoliensis, Brachyuranochampsa zangeli,* and *Sebeceus caeorhinus*; Oligocene: *Diplocynodon ratelii;* Pliocene: *Porossaurus brasiliensis*; Plesitocene: *Crocodilus rhombifer*). Fully terrestrial crocodilians (such as *Sebecus*) do not show this pattern of foramina and the most ancient crocodilian specimen available (*Protosucus*) shows a pattern that is more lizard-like. All lizard groups display a linear pattern of foramina on top and bottom of the teeth line and none shows the crocodilian beehive pattern, including the marine iguana (*Amblyrhynchus cristatus*), which takes advantage of land and water environments. The emergence of DPRs is likely to have occurred during the early Jurassic and as a specialization of simpler reptilian cutaneous sensory organs. Since these organs are tuned to the air–water interface, ancestral, extinct crocodilians that were not semi-aquatic do not appear to have evolved or lost DPRs.

2.06.2.4 Physiology

To date, only one study has been done examining how DPRs encode sensory information (Soares, 2002). In this study, multicellular and unitary

extracellular recordings from the trigeminal ganglion of juvenile American alligators showed that DPRs are especially sensitive to surface waves (Figure 4). Surface waves evoked a single spike phase locked to the stimulus frequency and increasing the wave amplitude increased the probability of spike firing. Responses saturated at one spike per cycle of the surface wave stimulus. DPRs appeared to be tuned to frequencies up to tens of hertz (unpublished) and higher frequencies led to greater spike failure. With continuous stimulation, DPRs adapted and responses decreased with 5–10 min.

Similar coding strategies were seen when individually stimulating a single DPR with a small rod.

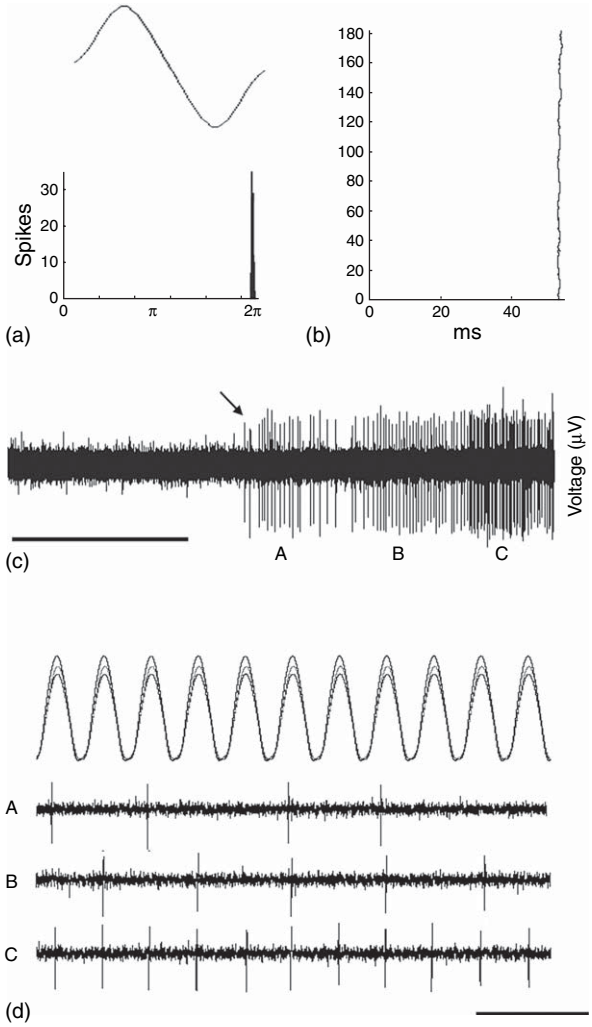

Figure 4 Physiological responses of DPRs. a, Representation of spike timing of trigeminal responses in relation to phase of stimulus. b, Raster plot showing the same date as in (a). c, Multiunit extracellular recording of trigeminal somata in the trigeminal ganglion; responses increase with increase of amplitude of surface wave stimulus (ABC). d, Close-up of ABC in c showing saturation of response. Scale bar: 200 ms.

No responses were noted when the skin between DPRs was pressed using the same pressure which evoked responses in the DPRs. This observation supports the notion that the DPRs themselves are sufficient and necessary for the trigeminal response that is associated with water interface disruptions. Although the transduction mechanism is yet to be discovered, it is likely that DPRs are sensitive to pressure differences, and not particle motion, although responses to shear cannot be ruled out.

2.06.2.5 Comparisons with Other Reptilians

There are four main groups within the reptilia: (1) turtles and tortoises, (2) lizards and snakes, (3) crocodiles and alligators, and (4) the tuatara (single species of the sphenodontia), making this a paraphyletic group. DPRs belong to a general class of sensory organs found in all reptilians. This class of tactile sensory organs (also called touch papilla) is well developed and occurs in all reptilian orders (von During and Miller, 1978). Touch papillae of lizards have bristles and forms and always indicate sensitive areas of the skin. Different receptor terminals such as discoid terminals, Merkel cells, and naked free nerve endings in dermal layers as well as laminated receptors are present in tactile sensory organs. Another common characteristic between all touch papillae are a modified keratin layer, where a more pliable region is located above the organ itself. DPRs appear to be the most complex arrangement of different receptor types while pythons and veranus show mainly discoid-like receptors and branched nerve receptors in the connective tissue papillae. These discoid receptors lie just deep of the modified keratinous layer and the connective tissue papillae contain columns of connective tissue cells that contact nerve ending fibers of other receptor types. Adaptation-driven selection appears to have worked on an ancestral form of a crocodilian touch papilla to give rise to DRPs. Modifications include hypertrophy of skin receptors in the periphery, in the central nervous system, and an increase in sensitivity. The most similar tactile sensory organ of a noncrocodilian to DRPs are the touch papillae of the iguana (personal observation), especially the marine iguana of the Galapagos, which takes advantage of an aquatic environment. No cutaneous comparative studies of turtles, tortoises, and the tuatara have been made at this date to determine any similarities to DPRs. Osteologically, foramina associated with tactile sensory organs of reptiles are spatially distributed differently than in the extant Crocodilia (personal observation). When present,

there are fewer foramina arranged in a line above and below the teeth line and not in the crocodilian beehive pattern.

2.06.3 Conclusions

In summary, crocodilians have evolved an armored body shield while maintaining tactile sensitivity to its environment. All living crocodilians that have taken advantage of a semi-aquatic niche either have DPRs or, in case of extinct species, bone markings suggesting the presence of DPRs. Ancient terrestrially bound crocodilians did not have ostealogical indications of DPRs. Crocodilians are able to detect surface waves based on the information from the DPRs and prey detection seems to be one of the roles for these receptors but other behavioral roles are possible since crocodilians use the water–air interface for communication and courtship. The transduction mechanism for DPRs is yet to be determined.

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2.07 Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?

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Glossary

amniote Group of vertebrates that develop an amniotic membrane around the embryo; includes reptiles, birds, and mammals.

arcopallium A caudal (or posterior) subdivision of the dorsal ventricular ridge in birds.

cortex A laminar brain structure consisting of cells arranged in layers parallel to the ventricular/pial surfaces and generally orthogonal (perpendicular) to radial glial fibers.

developmental regulatory gene A gene encoding a transcription factor (or a cofactor) or a signaling protein that is expressed during development in specific patterns, and is able to control expression of other genes and regulate patterning and morphogenesis of specific body parts.

DVR Dorsal ventricular ridge: a large region of the ventrolateral pallium of the telencephalon of birds and reptiles.

hippocampal formation Derivative of the medial pallium in different vertebrates. In mammals it includes the dentate gyrus, the hippocampus proper (Ammon's fields), and the subiculum. In birds it includes the hippocampus and the area parahippocampalis, whereas in reptiles it includes the medial and dorsomedial cortices.

homologous Having the same relative position (topological position), embryonic origin, and common ancestor; exhibiting biological homology.

homologue The same organ in different animals under every variety of form and function (Owen's definition, in 1843; see A History of Ideas in Evolutionary Neuroscience and Field Homologies on this concept).

homology A similarity attributed to common evolutionary origin (see 'homologous').

hyperpallium A dorsal region of the avian telencephalon that develops from the dorsal pallium. It typically forms a bulge on the dorsal surface of the telencephalon. It was often called Wulst.

M1 Primary motor area of the mammalian neocortex.

mesopallium A dorsal subdivision of the DVR in birds.

neocortex Derivative of the dorsal pallium in mammals, that typically shows a six-layered organization. It is also known as isocortex.

nidopallium A ventral subdivision of the DVR in birds.

pallial thickening A lateral expansion of the dorsal cortex of reptiles, showing a non-cortical organization. It is generally considered a lateral part of the dorsal cortex. However, only part of it may be a dorsal pallial derivative, and more comparative and developmental studies of this structure are needed before reaching any conclusion.

pallium A major dorsocaudal division of the telencephalon in all vertebrates, which in mammals gives rise to the cortical regions, claustrum, and part of the amygdala (including the cortical areas plus the basolateral complex). It is subdivided into four parts, called medial, dorsal, lateral, and ventral pallia.

piriform cortex Olfactory cortex of different vertebrates. It derives from the ventrolateral pallium. In reptiles, it is also known as lateral cortex.

S1 Primary somatosensory area of the mammalian neocortex.

sauropsid Group of vertebrates that includes reptiles and birds.

subpallium A major ventrorostral (or basal) division of the telencephalon in all vertebrates, that in mammals gives rise to most of the septum, the basal ganglia, part of the amygdala (including the intercalated and centromedial nuclei), and other cell groups of the basal telencephalon, such as the cholinergic corticopetal groups. It is subdivided into striatal, pallidal, and anterior entopeduncular parts.

telencephalon Bilateral evaginations of the rostral forebrain. It shows two major divisions in all vertebrates: pallium and subpallium.

tetrapod Group of vertebrates having two pairs of limbs, that includes amphibians, reptiles, birds, and mammals.

thalamus Forebrain structure that derives from the alar plate of the diencephalon (in particular, from prosomeres 2 to 3). It is subdivided into a ventral thalamus (also called prethalamus, which derives from prosomere 3), and a dorsal thalamus (or simply thalamus, which derives from prosomere 2). The dorsal thalamus contains cell groups that typically relay sensory information to the subpallium and pallium in vertebrates. In amniotic vertebrates, the dorsal thalamus contains specific cell groups that relay unimodal sensory and/or motor information to specific areas of the dorsal pallium.

topology Geometric configuration of any given structure (such as the brain) according to internal coordinates, which remain unaltered independent of deformations or differential growth of subdivisions that occur during development. According to this, the topological position of any subdivision within the structure, and its relation to neighbors, remains the same throughout ontogeny. Further, in organisms sharing the same configuration and basic organization plan (for example, vertebrates), the topological position of homologous subdivisions should be the same across species.

V1 Primary visual area of the mammalian neocortex.

Wulst German term previously employed to name the hyperpallium. It literally means bulge, making reference to the swollen or protuberant appearance of this subdivision of the avian telencephalon.

2.07.1 Introduction

One of the most challenging questions in brain evolution is to ascertain the origin of neocortex and to know whether a comparable cortical (pallial) region is present in extant birds and reptiles, which would

mean that a primordium of this structure was already present in stem amniotes. This question has been addressed by researchers since the end of the nineteenth century and continues to be discussed nowadays (for example, see article by Aboitiz *et al.*, 2003, and commentaries on it). However, many issues related to this question still remain uncertain and controversial. Nevertheless, the combination of developmental, paleontological, and adult anatomical plus functional data, analyzed using a cladistic approach, has proven to be very useful for evolutionary studies; this combined approch has helped to clarify some aspects of cortical evolution and has offered some light on what direction to follow in this research (for example, Northcutt and Kaas, 1995; Striedter, 1997, 2005; Medina and Reiner, 2000; Puelles, 2001; Butler and Molnár, 2002; Aboitiz *et al.*, 2003). Here I will review evidence based on this approach that suggests that: (1) the pallium of birds and reptiles contains a sector that is homologous as a field to the mammalian neocortex (i.e., they evolved from the same primordium present in stem amniotes); (2) this pallial sector contains a primary visual and a primary somatosensory area that might be homologous to V1 and S1, respectively, of mammalian neocortex; and (3) the frontal part of this pallial sector contains a somatomotor control area in birds (apparently overlapped with the somatosensory field) and mammals (M1), but these areas likely evolved independently and are, therefore, nonhomologous (see Evolution of the Nervous System in Reptiles, Visual Cortex of Turtles, The Origin of Neocortex: Lessons from Comparative Embryology, Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications, The Evolution of Motor Cortex and Motor Systems).

2.07.2 Finding the Homologue of Neocortex in the Pallium of Nonmammals

The neocortex is a six-layered structure located in the dorsolateral part of the telencephalon in mammals, above the ventricle, and it covers the central and basal region that is occupied by the basal ganglia and other basal telencephalic cell groups (Figure 1a). The neocortex is also located above the rhinal fissure, which separates it from the piriform cortex and olfactory tract. In contrast to the neocortex, the basal ganglia and other basal telencephalic cell groups show a nuclear (nonlaminar) organization. The difference between laminar versus nuclear organization together with the relative position of the cell masses with respect to the ventricle was once considered a criterion to identify cortical (pallial) and basal (subpallial) regions in the telencephalon of nonmammals, and based on it the telencephalon of birds and reptiles was thought to be made of a very large basal ganglia and a very tiny cortical region (reviewed in Medina and Reiner, 1995; Striedter, 1997; Reiner *et al.*, 1998; Jarvis *et al.*, 2005). This is now known to be wrong, and there is a large amount of evidence showing that the telencephalon of birds and reptiles contains a large pallial region, a major part of which shows a nuclear organization and is located below the lateral ventricle (Figures 1b–1d) (Karten and Hodos, 1970; Reiner, 1991, 1993; Butler, 1994b; Striedter, 1997, 2005; Smith-Fernández *et al.*, 1998; Medina and Reiner, 2000; Puelles *et al.*, 2000; Jarvis *et al.*, 2005). The evidence showing this includes developmental and adult anatomical and functional data, and it has mainly been obtained after the development of modern techniques that allowed detection of gene products (such as enzymes, proteins and, more recently, mRNAs), tracing of axonal pathways, fate mapping, and functional studies of the brain.

The cortical region of mammals is subdivided into medial, dorsal, and lateroventral units during development and in the adult (Figures 1a and 2c), and the neocortex derives from the dorsal subdivision (Holmgren, 1925; Striedter, 1997). The cortical region (pallium) of birds and reptiles is also subdivided into medial, dorsal, and lateroventral units (Figure 1) (Reiner, 1991, 1993; Butler, 1994b; Striedter, 1997; Puelles *et al.*, 2000). The problem comes when trying to compare one-to-one these subdivisions of the avian/reptilian pallium with those of mammals. Some authors employing only adult anatomical and functional data (including connectivity patterns) believe that the dorsal subdivision of the avian/reptilian pallium is homologous to the dorsomedial part of the neocortex, whereas a large part of the lateroventral pallium of birds/reptiles (called dorsal ventricular ridge or DVR; in particular, its ventral part in birds) is homologous to the dorsolateral part of the neocortex or to specific cell groups of it (Karten, 1969, 1997; Reiner, 1993; Butler, 1994b). However, homologous structures must originate from the same embryonic primordium (Striedter, 1997; Puelles and Medina, 2002; see Field Homologies). In this sense, developmental studies indicate that only the dorsal subdivision of the avian/reptilian pallium can be compared to the neocortex, but not the DVR (in particular, its ventral part, which includes the nidopallium in birds) (Striedter, 1997; Puelles *et al.*, 2000). Rather, both developmental and some adult connectivity data suggest that the avian/reptilian DVR is homologous to the claustrum and pallial

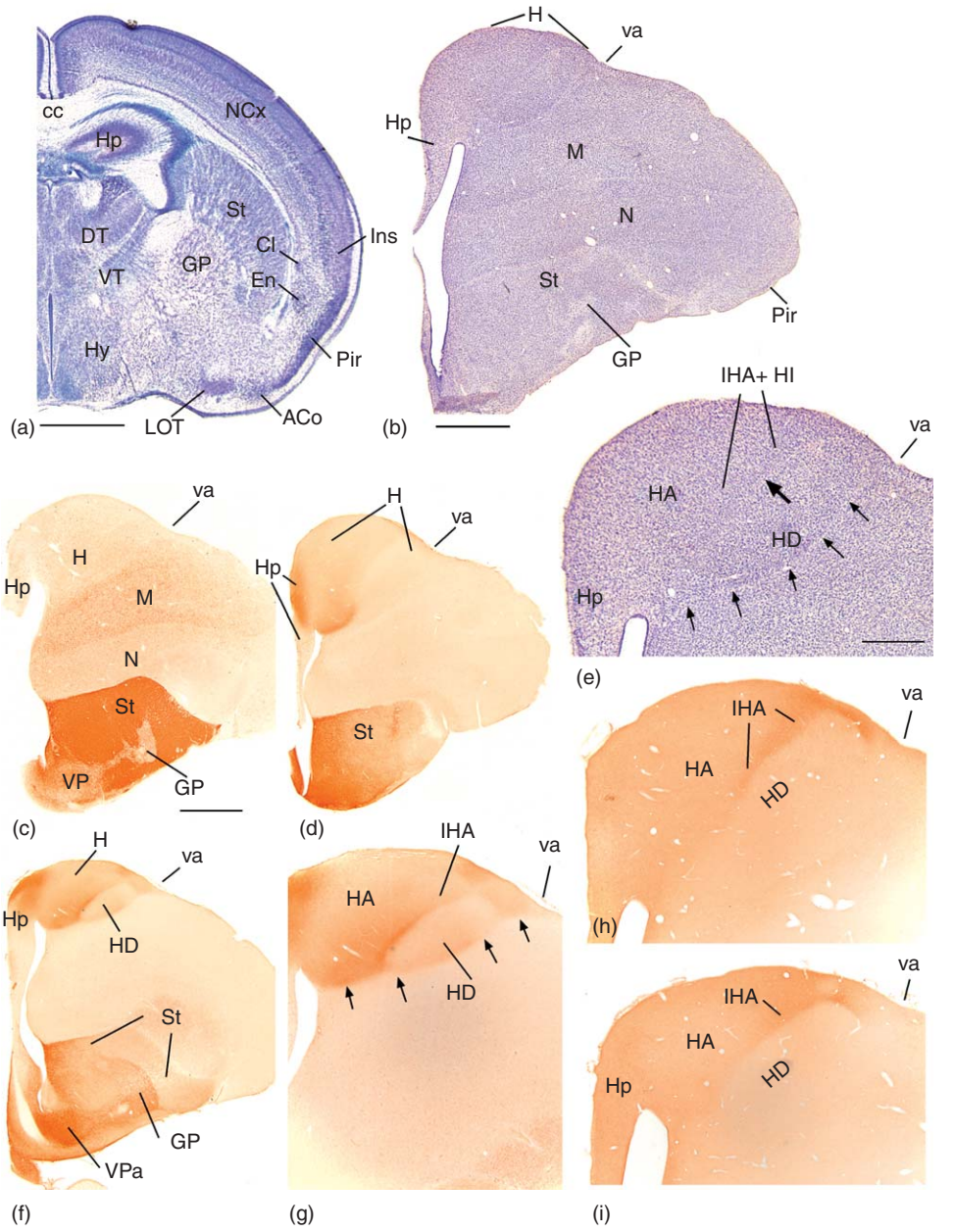

Figure 1 Photomicrographs of frontal sections through the telencephalon of a postnatal mouse (a) or adult pigeon (b–i), showing the general cytoarchitecture, as observed in Nissl staining (a), (b), (e), and some subdivisions based on immunostaining for tyrosine hydroxylase (c), substance P (d), (f), (g), of choline acetyltransferase (h), (i). Note the typical lamination in the cerebral cortex of mouse (a), that differs from the nuclear-like organization in the basal ganglia (striatum and pallium). In pigeon (b), as in other birds and reptiles, most of the telencephalon is not laminated. Nevertheless, neurochemical data help to locate the main intratelencephalic boundary in birds and reptiles, separating subpallium and pallium. The subpallium is relatively rich in tyrosine hydroxylase (c) and substance P (d), (f), and includes the basal ganglia (striatum and pallidum). The avian pallium includes four major subdivisions, including the hippocampal formation (medially), the hyperpallium (dorsally), the mesopallium (laterodorsally), and the nidopallium (lateroventrally). At caudal levels, the avian lateroventral pallium includes the arcopallium and part of the amygdala. The avian hyperpallium appears to be the only derivative of the dorsal pallium and is therefore comparable (homologous as a field) to the mammalian neocortex (see Figure 2). The avian hyperpallium (H) has four mediolateral subdivisions, called apical (HA), interstitial nucleus of apical (IHA), intercalated (HI), and densocellular (HD) hyperpallium (e–i). These subdivisions are not comparable to neocortical layers, although they show some functional features that resemble them. The lateral extension of the hyperpallium coincides with a cell-free lamina called superior frontal lamina (arrows in (e) and (g)), and generally relates to a superficial groove called vallecula (va), although this is not true at rostral levels. See text for more details. ACo, anterior cortical amygdalar area; cc, corpus callosum; Cl, claustrum; DT, dorsal thalamus; En, endopiriform nucleus; GP, globus pallidus; H, hyperpallium; HA, apical hyperpallium; HD, densocellular hyperpallium; HI, intercalated hyperpallium; Hp, hippocampal formation; Hy, hypothalamus; IHA, interstitial nucleus of the apical hyperpallium; Ins, insular cortex; LOT, nucleus of the lateral olfactory tract; M, mesopallium; N, nidopallium; NCx, neocortex; Pir, piriform cortex; St, striatum; va, vallecula; VPa, ventral pallidum; VT, ventral thalamus. Scale bars: 1 cm (a, b); 0.5 cm (c, d, f; scale in c); 1 cm (e, h, i; scale in e).

amygdala (Bruce and Neary, 1995a, 1995b, 1995c; Striedter, 1997; Guirado *et al.*, 2000; Puelles *et al.*, 2000; Puelles, 2001; Dávila *et al.*, 2002; Martínez-García *et al.*, 2002). Below I review the evidence that supports that the dorsal part of the avian/reptilian cortical region is homologous as a field to the mammalian neocortex, and that both evolved from a similar pallial subdivision present in the telencephalon of stem amniotes.

2.07.2.1 Developmental Evidence: Histogenetic Origin and Transcription Factors

During development, the telencephalon of vertebrates becomes parcellated into radial histogenetic divisions and subdivisions that are comparable across species (Striedter, 1997; Puelles and Medina, 2002). Each division/subdivision shows a unique molecular profile and produces specific cell groups, most of which stay within the radial domain, except for some selective cell populations that undergo tangential migration across boundaries (Striedter and Beydler, 1997; Striedter *et al.*, 1998; Puelles *et al.*, 2000; Cobos *et al.*, 2001; Marín and Rubenstein, 2001, 2002; Puelles and Medina, 2002). This conclusion is strongly supported by data on developmental regulatory genes (encoding transcription factors or signaling proteins that regulate the expression of other genes), which are expressed in specific and generally comparable spatiotemporal patterns in the telencephalon of different vertebrates during development (Smith-Fernández *et al.*, 1998; Puelles *et al.*, 2000; Brox *et al.*, 2003, 2004; Medina *et al.*, 2005), and play key roles in the regional specification and formation of telencephalic divisions and subdivisions (Marín and Rubenstein, 2002).

2.07.2.1.1 Pallial subdivisions in mammals and neocortical origin Classical and modern developmental studies, including data on developmental regulatory genes, indicate that the mammalian neocortex derives from the pallium, one of the major divisions of the telencephalon (Figure 2c) (Holmgren, 1925; Källén, 1951b; Puelles *et al.*, 2000). During development, the pallium shows specific expression of numerous transcription factor-expressing genes, including *Pax6*, *Emx1/2*, *Tbr1/2,* and several *LIM-homeobox* (*Lhx*) genes (Simeone *et al.*, 1992; Stoykova and Gruss, 1994; Bulfone *et al.*, 1995, 1999; Rétaux *et al.*, 1999; Puelles *et al.*, 2000; Bulchand *et al.*, 2001, 2003; Medina *et al.*, 2004), which play key roles in pallial specification and parcellation, cell proliferation, and/or cell differentiation (Stoykova *et al.*, 1996, 2000; Zhao *et al.*, 1999; Bulchand *et al.*, 2001; Hevner *et al.*, 2001, 2002; Yun *et al.*, 2001; Bishop *et al.*, 2002, 2003; Muzio *et al.*, 2002; Campbell, 2003). For example, *Pax6, Emx1,* and *Emx2* are involved in pallial specification and parcellation (Bishop *et al.*, 2002, 2003; Muzio *et al.*, 2002). *Emx1* and *Emx2* are also involved in pallial growth (cell proliferation) (Bishop *et al.*, 2003). On the other hand, *Tbr1* appears to be involved in the differentiation of glutamatergic neurons, which are typical in the pallium (Hevner *et al.*, 2001).

What part of the pallium gives rise to the neocortex? Classical developmental studies and studies on the expression and function of developmental regulatory genes indicate that the pallium of mammals contains three main radial subdivisions (Figure 2c): (1) a medial pallium, giving rise to the hippocampal formation; (2) a dorsal pallium, giving rise to the neocortex; and (3) a lateroventral pallium, giving rise to the piriform cortex, claustrum, and pallial amygdala (the lateroventral pallium is sometimes referred to as the piriform lobe, and has the olfactory tract at the surface) (Holmgren, 1925; Striedter, 1997; Puelles *et al.*, 2000; Puelles, 2001). The lateroventral pallium is also subdivided into dorsal and ventral parts (called lateral and ventral pallia, respectively, by Puelles *et al.*, 2000), which show distinct expression of the several developmental regulatory genes, including *Emx1* and *Dbx1*, and give rise to different parts of the claustrum and pallial amygdala (Figure 2c) (Puelles *et al.*, 2000; Yun *et al.*, 2001; Medina *et al.*, 2004). During early development, the lateral pallium expresses strongly *Emx1* but not *Dbx1*, whereas the ventral pallium expresses *Dbx1* in the ventricular zone but only shows *Emx1* expression in the subpial surface (Puelles *et al.*, 2000; Yun *et al.*, 2001; Medina *et al.*, 2004). However, a recent fate-mapping study indicates the existence of numerous *Emx1*-expressing cells in both lateral pallial and ventral pallial parts of the amygdala around and after birth (Gorski *et al.*, 2002), suggesting that there may be a high degree of cellular mixing between these subdivisions (Figure 2c).

The pallial subdivisions are apparently formed by the early action of: (1) signaling proteins (such as Wnt proteins) that diffuse from organizer centers such as the cortical hem (Figure 2c), which, by way of receptors, apparently control the expression of downstream genes (including genes encoding transcription factors) in adjacent pallial areas in a concentration-dependent way (Ragsdale and Grove, 2001); (2) transcription factors that are expressed in opposing gradients in the pallium during early development, involved in regional specification, parcellation, cell proliferation, and/or cell differentiation of the pallium (for example, *Pax6, Emx2, Lhx2*; Donoghue and Rakic, 1999;

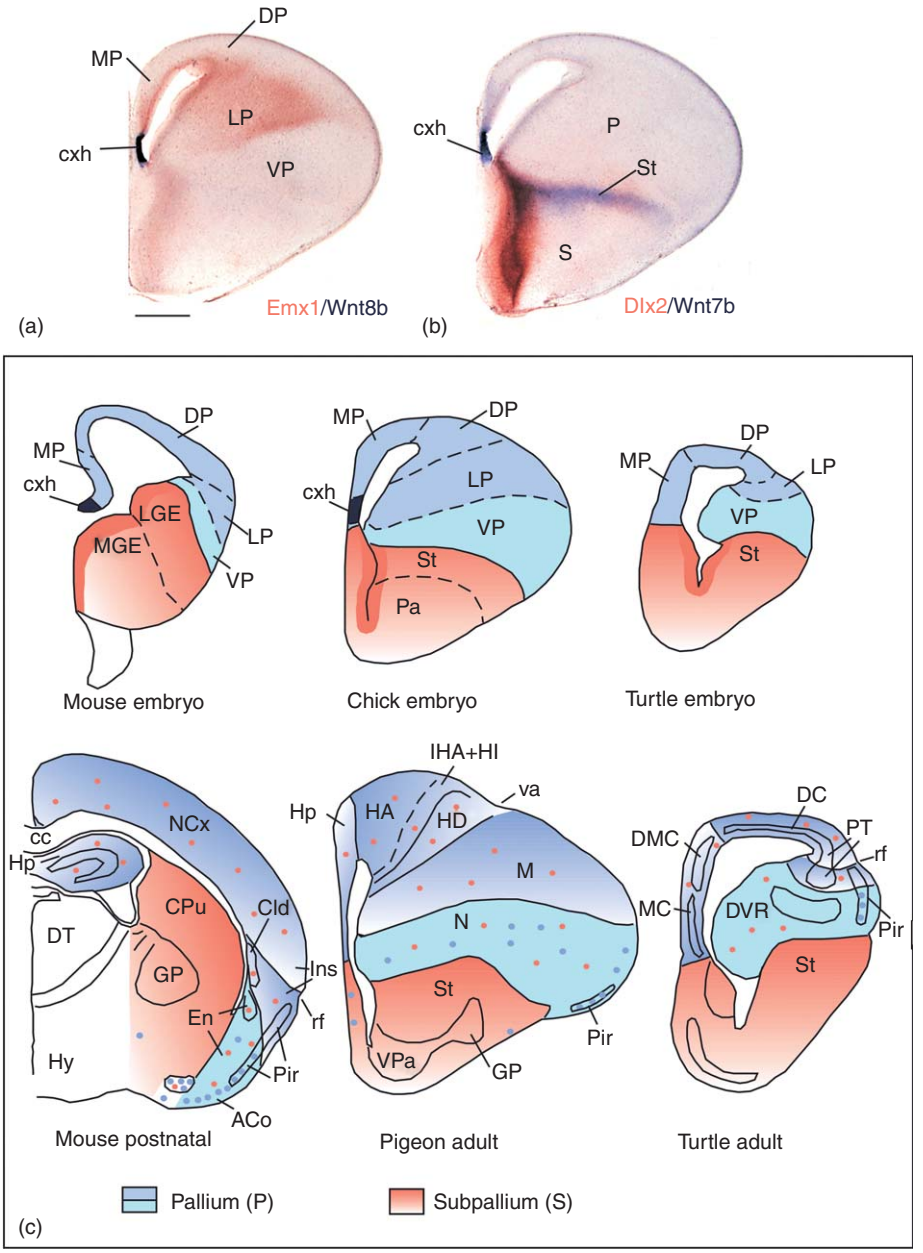

Figure 2 a and b, Photomicrographs of frontal sections through the telencephalon of chick embryos (10 days of incubation), showing expression of the chick genes *Emx1* (red in a), *Wnt8b* (blue in a), *Dlx2* (red in b), or *Wnt7b* (blue in b). The genes *Dlx2* and *Wnt7b* are expressed in the subpallium, either in the ventricular/subventricular zone of the whole subpallium (*Dlx2*) or the ventricular zone and mantle of the striatum (*Wnt7b*). Expression of *Emx1* helps to distinguish a ventral pallial (VP) subdivision, poor in *Emx1* expression and poor in subpallial marker genes. Note the expression of *Wnt* genes in the avian cortical hem, a putative secondary organizer comparable to the cortical hem of mammals. c, Schematics of the telencephalon of a mammal (mouse), a bird (chick or pigeon), and a reptile (turtle), as seen in frontal sections during development or in the adult. The pallial and the major subpallial subdivisions are represented in the different species, based on known expression patterns of developmental regulatory genes observed during development. Four major pallial subdivisions appear to exist in all groups, although the lateral and ventral subdivisions appear to have a large degree of cellular mixing in the adult (which occurs at the level of the ventral pallium). The dorsal pallium gives rise to the neocortex (NCx) in mammals, to the hyperpallium (H) in birds, and to the dorsal cortex (DC) in reptiles. The pallial thickening (PT) is often considered a lateral part of the dorsal cortex. However, available data suggest that only part of it may be a dorsal pallial derivative, and more studies are needed to know where the exact boundary between the dorsal and lateral pallium is, in reptiles. ACo, anterior cortical amygdalar area; cc, corpus callosum; Cld, dorsolateral claustrum; CPu, caudoputamen (dorsal striatum); cxh, cortical hem; DC, reptilian dorsal cortex; DMC, reptilian dorsomedial cortex; DP, dorsal pallium; DT, dorsal thalamus; DVR, dorsal ventricular ridge; En, endopiriform nucleus; GP, globus pallidus; H, hyperpallium; HA, apical hyperpallium; HD, densocellular hyperpallium; HI, intercalated hyperpallium; Hp, hippocampal formation; Hy, hypothalamus; IHA, interstitial nucleus of the apical hyperpallium; Ins, insular cortex; LGE, lateral ganglionic eminence; LP, lateral pallium; M, mesopallium; MC, reptilian medial cortex; MGE, medial ganglionic eminence; MP, medial pallium; N, nidopallium; NCx, neocortex; P, pallium; Pa, pallidum; Pir, piriform cortex; PT, pallial thickening; rf, rhinal fissure; S, subpallium; St, striatum; va, vallecula; VP, ventral pallium; VPa, ventral pallidum. Scale bar: 400μm.

Bulchand *et al.*, 2001; Bishop *et al.*, 2002); (3) cofactors (activators or repressors) and other regulatory proteins that regulate directly or indirectly the expression of specific transcription factors, that show sharp expression boundaries between subdivisions (for example, the LIM-only protein Lmo3, with a sharp expression boundary between dorsal and lateroventral pallial subdivisions; Bulchand *et al.*, 2003; Vyas *et al.*, 2003). The main pallial subdivisions are differentially affected by mutations targeting some of the above-mentioned developmental regulatory genes, supporting that they represent distinct histogenetic compartments. For example, a mutation in the LIM-homeobox gene *Lhx2* produces a severe malformation of the hippocampal formation and neocortex, but the piriform lobe appears unaffected (Bulchand *et al.*, 2001; Vyas *et al.*, 2003).

2.07.2.1.2 Pallial subdivisions in nonmammals: the dorsal pallium in birds and reptiles Since developmental regulatory genes generally show highly conserved sequences and expression patterns, they have become very useful tools for identifying comparable brain regions in different vertebrate species and for studies of brain evolution (Puelles *et al.*, 2000; Medina *et al.*, 2005). Classical and modern developmental studies, including radial glial analysis, fate-mapping studies, and expression of developmental regulatory genes, indicate that the telencephalic pallium in reptiles and birds contains three main radial divisions (Figure 2c): (1) a medial pallium, which gives rise to the medial/dorsomedial cortices in reptiles and to the hippocampal formation (hippocampus and parahippocampal area) in birds; (2) a dorsal pallium, which gives rise to the dorsal cortex in reptiles and the hyperpallium or Wulst in birds; and (3) a lateroventral pallium, which gives rise to the lateral or piriform cortex, to a large nuclear structure called the DVR and to some pallial amygdalar nuclei in reptiles and birds (Holmgren, 1925; Källén, 1951a, 1953, 1962; Striedter, 1997; Striedter and Beydler, 1997; Striedter *et al.*, 1998; Puelles *et al.*, 2000; Cobos *et al.*, 2001; Martínez-García *et al.*, 2002). As in mammals, the pallium of reptiles and birds shows specific expression of *Pax6*, *Tbr1/2,* and *Emx1* during development (Smith-Fernández *et al.*, 1998; Bulfone *et al.*, 1999; Puelles *et al.*, 2000; Garda *et al.*, 2002). Similarly to mammals, in birds there is an organizer center at the medial edge of the pallium expressing *Wnt*-family genes (the avian cortical hem; Figures 2a–2c), which may control the formation of the medial pallial subdivision (Garda *et al.*, 2002). This indicates that the specification and parcellation of the avian/reptilian pallium are controlled by many of the same regulatory genes and mechanisms that control pallial development in mammals.

Further, as in mammals, the lateroventral pallium of birds and reptiles is subdivided into a lateral pallium, showing broad and strong expression of *Emx1*, and a ventral pallium, which expresses *Emx1* only in a thin band of the subpial mantle (Figures 2a–2c) (Smith-Fernández *et al.*, 1998; Puelles *et al.*, 2000). These two pallial subdivisions of birds also differ by their distinct expression of *Dachsund* and several *Cadherin* genes during development (Redies *et al.*, 2001; Szele *et al.*, 2002). However, as in mammals, the derivatives of the lateral and ventral pallial subdivisions of birds apparently display a high degree of cellular mixing (Figure 2c), based on radial glial fiber disposition and fate-mapping analysis in chick embryos (Striedter and Beydler, 1997; Striedter *et al.*, 1998; Striedter and Keefer, 2000). The lateral pallium includes the so-called mesopallium and posterior amygdalar nucleus of birds, whereas in reptiles it appears to include a small dorsolateral part of the DVR plus the dorsolateral amygdalar nucleus (Smith-Fernández *et al.*, 1998; Guirado *et al.*, 2000; Puelles *et al.*, 2000; Martínez-García *et al.*, 2002). The ventral pallium includes the so-called nidopallium and arcopallium of birds, whereas in reptiles it appears to include most of the DVR plus the lateral and other amygdalar nuclei (Smith-Fernández *et al.*, 1998; Guirado *et al.*, 2000; Puelles *et al.*, 2000; Martínez-García *et al.*, 2002). The olfactory tract is located at the surface of the ventral pallium (or ventral DVR) in mammals, birds, and reptiles (Striedter, 1997; Guirado *et al.*, 2000; Puelles *et al.*, 2000; Puelles, 2001). Both the relative (topological) position and molecular profile of the pallial subdivisions (including expression of *Pax6, Tbr1,* and *Emx1*) suggest that the dorsal pallial subdivision of reptiles and birds, from which derive the reptilian dorsal cortex and avian hyperpallium, is comparable and possibly homologous as a field to the dorsal pallium of mammals, which gives rise to the neocortex (Striedter, 1997; Puelles *et al.*, 2000). As in mammals, in reptiles the rhinal fissure separates the dorsal cortex from the piriform cortex and olfactory tract (Figure 2c). These data also indicate that the ventral pallial part of the reptilian/avian DVR (which has the olfactory tract and piriform cortex at the surface, and is poor in *Emx1* expression) is not comparable and cannot be homologized to the neocortex, since they derive from different embryonic primordia (Striedter, 1997; Striedter and Beydler, 1997; Smith-Fernández *et al.*, 1998; Striedter *et al.*, 1998; Puelles *et al.*, 2000).

However, one important issue that developmental studies have not yet resolved is where to locate the exact boundary between the dorsal and lateral pallial subdivisions, since both subdivisions express many of the same developmental regulatory genes (for example, *Emx1*) and the morphological landmarks are not clear in birds and many reptiles. In other words, what is the exact lateral extension of the dorsal pallium in birds and reptiles? In mammals, some developmental regulatory genes are expressed differently in the lateral and dorsal pallium (for example, the LIM-only genes *Lmo2* and *Lmo3*), but, unfortunately, data on the orthologue genes are lacking in nonmammalian vertebrates (Medina *et al.*, 2005). I will return to this issue below.

2.07.2.2 Adult Anatomical Evidence: Morphological Landmarks, Molecular Markers, and Connections

2.07.2.2.1 Morphological landmarks and molecular markers: problematic delimitation of the dorsal pallium in birds and reptiles As noted above, the dorsal cortex of reptiles and the hyperpallium of birds appear to derive from the same pallial embryonic subdivision as the neocortex. In adult animals, these structures show cellular and molecular features typical of pallium. For example, they contain a majority of excitatory (glutamatergic) neurons (the principal or projection neurons) and only a relatively small subpopulation of inhibitory (GABAergic) interneurons (Ottersen and Storm-Mathisen, 1984; Reiner, 1993; Veenman and Reiner, 1994, 1996; Swanson and Petrovich, 1998; Fowler *et al.*, 1999; Medina and Reiner, 2000; Broman *et al.*, 2004). In mammals, birds, and reptiles, the principal pallial neurons have excitatory projections to the striatum and brainstem (Ottersen and Storm-Mathisen, 1984; Veenman and Reiner, 1996; Kenigfest *et al.*, 1998; Fowler *et al.*, 1999; Broman *et al.*, 2004). Some of the strongest evidence showing that the principal pallial neurons are glutamatergic has been provided recently by the localization of vesicular glutamate transporters VGLUT1 and VGLUT2, although data on these transporters exist only in mammals (Fujiyama *et al.*, 2001; Herzog *et al.*, 2001; Broman *et al.*, 2004; Fremeau *et al.*, 2004), but are lacking in birds and reptiles. The GABAergic interneurons of the mammalian neocortex and avian hyperpallium, as those of the rest of the mammalian and avian pallium, originate in the subpallium and migrate tangentially to the pallium during development (Figure 2c) (Anderson *et al.*, 1997, 2001; Pleasure *et al.*, 2000; Cobos *et al.*, 2001; Marín and Rubenstein, 2001; Nery *et al.*, 2002; Legaz *et al.*, 2005). This situation appears to be typical in all tetrapods, since it is also described in amphibians (Brox *et al.*, 2003).

In addition to these and other molecular and cellular features typical of the whole pallium, there are no comparative data on molecular markers that clearly distinguish the neocortex/dorsal pallium from other pallial subdivisions in adult animals. In mammals, the neocortex can be distinguished from the adjacent pallial subdivisions because of its typical six-layered structure and the presence of the rhinal fissure on its lateral egde. However, in birds and reptiles there are no clear morphological landmarks for distinguishing the lateral boundary of the dorsal pallium. The absence of dorsal pallial molecular markers has become an additional obstacule for delimiting the lateral extension of the dorsal pallium in adult birds and reptiles. As noted above, more comparative studies on the expression of developmental regulatory genes are also needed to resolve this issue. In reptiles, the dorsal cortex appears to include a rostrolateral extension called pallial thickening (reviewed in Reiner, 1993; Medina and Reiner, 2000). However, the identification of the pallial thickening varies between authors and reptilian species, and it appears that a ventral part of it is located deep to the piriform cortex and, thus, may be part of the lateral pallium (Figure 2c). Analysis of radial glial fiber disposition in that part of the reptilian pallium (Monzón-Mayor *et al.*, 1990) suggests that only the dorsal-most part of the pallial thickening may belong to the dorsal pallium (Figure 2c). This dorsal part of the pallial thickening appears located above the rhinal fissure (visible in only some reptiles), which is consistent with its dorsal pallial nature. As in reptiles, so also in birds, there is some confusion on where to locate the lateral boundary of the hyperpallium or Wulst. According to numerous studies, the hyperpallium includes the so-called apical, interstitial nucleus of apical, intercalated, and densocellular hyperpallium (HA, IHA, HI, and HD, respectively), and its lateral (or lateroventral) boundary coincides with both the superior frontal lamina and a superficial groove called vallecula (Figures 1b–1i and 2c) (Karten *et al.*, 1973; Shimizu and Karten, 1990; reviewed in Medina and Reiner, 2000). However, although this is generally true, the HD exceeds laterally the vallecula at rostral levels (Shimizu and Karten, 1990), and the superior frontal lamina appears to bend laterally when approaching the vallecula (Suárez *et al.*, 2006; see Figures 1e, 1g, and 2c). Further, the HD is sometimes misidentified and either confused

with the dorsal part of the mesopallium (a part of the DVR that belongs to the lateral pallium) or vice versa. This is partly due to the fact that the HD (or part of it) shares with the mesopallium expression of some molecular markers, such as some glutamate receptor subunits (Wada *et al.*, 2004). This has raised the question of whether the HD should or should not be considered part of the hyperpallium. However, the HD also differs from the mesopallium in many other molecular features, such as expression of calcium-binding proteins (Suárez *et al.*, 2006), expression of delta and mu opiate receptors (Reiner *et al.*, 1989), and expression of GluR1 glutamate receptor subunit, neurotensin receptors, or the neuropeptide substance P (Reiner *et al.*, 2004) (Figures 1f and 1g). Further, recent evidence indicates the existence of an additional pallial division (the laminar pallial nucleus) clearly located at the boundary between HD and mesopallium, showing distinct expression of calcium-binding proteins throughout development and in adult chicks (Suárez *et al.*, 2006). Thus, the questions raised on the identity and nature of HD may be partially due to the use of different species. Whereas all four subdivisions of the hyperpallium are clearly distinguished in birds with a large hyperpallium (such as the owl), it appears that the more lateral hyperpallial subdivisions, HI or HD, are difficult to distinguish in either pigeons/chicks or songbirds, respectively.

2.07.2.2.2 Connections One of the most typical features of the mammalian neocortex is that it contains unimodal sensory and motor areas that receive their input directly from specific nuclei of the dorsal thalamus (Northcutt and Kaas, 1995) (Figure 3). These primary functional areas of mammals show a detailed point-to-point representation of the body and/or world (Krubitzer, 1995). The presence of these primary, unimodal sensory and motor areas makes the neocortex unique and different from adjacent pallial divisions (such as the hippocampal formation, which typically receives multimodal thalamic input, and the piriform cortex, which typically receives olfactory input from the bulb), and this has been used for identifying the dorsal pallium or specific dorsal pallial functional areas in other vertebrates. Nevertheless, the use of connections (or any other single data) alone for identification of homologies is highly risky since they may have changed during the course of evolution (Striedter, 2005). For this reason, when searching for homologies and for evolutionary interpretations, data on connections need to be used in combination with other data, including embryological origin and/or topological position.

The neocortex contains: (1) a primary visual area (V1), receiving input from the retinorecipient dorsal lateral geniculate nucleus; (2) a primary somatosensory area (S1), receiving input from specific nuclei of the ventrobasal (or ventral posterior) thalamic complex (including the ventral posterolateral (VPL) and ventral posteromedial (VPM) nuclei in rodents), which in turn receive somatosensory information from the head and body via the trigeminal sensory and dorsal column nuclei; and (3) a primary motor area (M1), receiving input from specific nuclei of the ventrobasal thalamic complex (including the ventral anterior (VA) and ventral lateral (VL) nuclei in rodents) that receive motor information from the basal ganglia and deep cerebellar nuclei (Figure 3) (Krubitzer, 1995; Groenewegen and Witter, 2004; Sefton *et al.*, 2004; Tracey, 2004; Guy *et al.*, 2005). These primary functional areas are present in most groups of mammals and have a similar relative position within the neocortex, with M1 and S1 being always rostral to V1 (Krubitzer, 1995; Medina and Reiner, 2000; Kaas, 2004). This suggests that these areas (at least V1 and S1, as well as some other sensory areas) were likely present in the origin of the mammalian radiation (Krubitzer, 1995; Slutsky *et al.*, 2000). Further, it appears that the neocortex of early mammals had multiple somatosensory representations of the body, each one corresponding to a distinct area (Krubitzer *et al.*, 1995, 1997; Catania *et al.*, 2000a, 2000b; Kaas, 2004). However, current available data suggest that early mammals did not possess a separate motor cortical area (M1), and this possibly appeared with the origin of placental mammals (Kaas, 2004). In mammals with a small neocortex, the visual and somatomotor areas show a close spatial contiguity (for example, in monotremes or in the hedgehog). In mammals with a large neocortex, such as rodents, carnivores, and primates, V1 becomes secondarily displaced to the most caudal part of the neocortex (occipital lobe) by the development of novel cortical areas involved in higher-order or multimodal information processing. In these mammals, S1 is located in the parietal lobe (in the postcentral gyrus of the primate parietal lobe), whereas M1 is located in the frontal lobe (in the precentral gyrus of the primate frontal lobe). In rodents and primates, the initial parcellation of these functional areas during development is related (among other things) to their expression of specific ephrin ligands and receptors, some of which can be used as early markers of S1 (ephrinA5) or V1 (Ephrin receptor EphA6) (Donoghue and Rakic, 1999; Yun *et al.*, 2003).

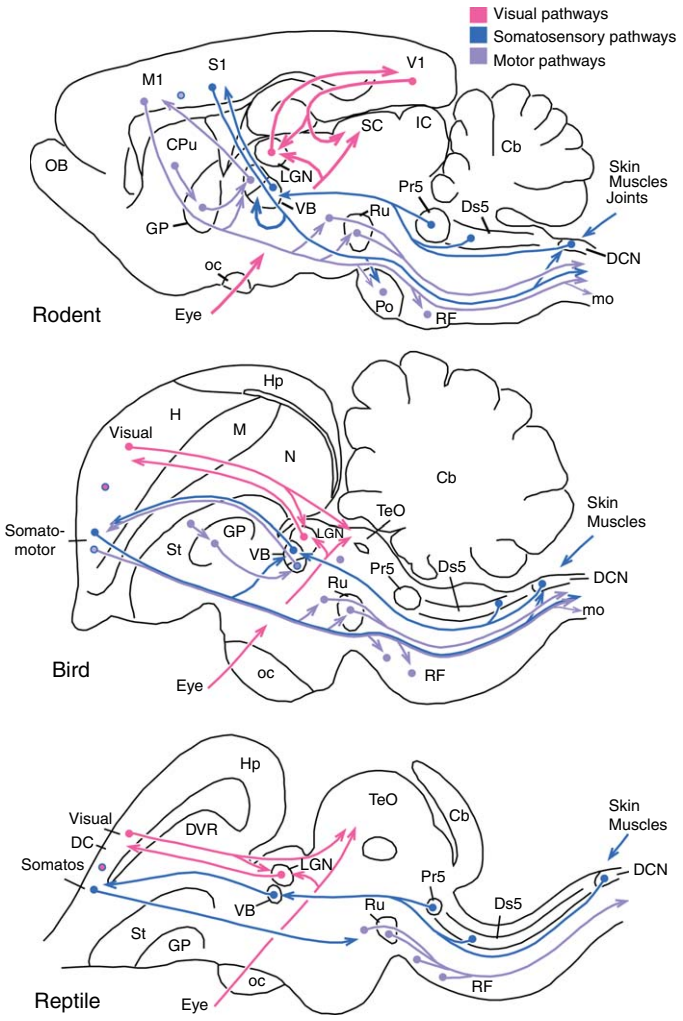

Figure 3 Schematics of lateral views of the brain of a rodent, a bird, and a reptile, showing the major connectivity patterns of the visual, somatosensory, and motor pathways. In mammals, birds, and reptiles, partially comparable visual and somatosensory pathways to the dorsal pallium are present. Visual information reaches a dorsal pallial primary visual area (V1 in mammals) by way of the lateral geniculate nucleus (LGN), located in the lemnothalamus. In turn, the visual cortical (dorsal pallial) area projects back to this thalamic nucleus and to the optic tectum. Somatosensory information reaches a dorsal pallial primary somatosensory area (S1 in mammals) by way of the ventrobasal complex of the lemnothalamus (VB). In birds, the body is mainly represented in the dorsal pallium, whereas in mammals and at least some reptiles, there is representation of both body and head. In birds and mammals, the ventrobasal complex has a motor subdivision that receives basal ganglia and cerebellar input, and projects to the dorsal pallium. In birds, this motor input ends in the somatosensory field (thus becoming a true somatomotor area). In placental mammals, such as rodents, the motor input ends mostly in a separate primary motor area (M1), although a small somatosensory–motor overlap occurs at the interface between M1 and S1. See text for details. In mammals, both S1 and M1 project back to the ventrobasal thalamic complex and to the brainstem and spinal cord by way of the pyramidal tract. S1 projections primarily end on precerebellar centers (such as the pontine nuclei) and somatosensory-relay centers (such as the dorsal column nuclei and the dorsal horn of the spinal cord). M1 projections primarily reach premotor precerebellar and reticulospinal centers (such as prerubral and rubral neurons), and also provide some input to motoneuron pools, such as those of the ventral horn of the spinal cord. In birds, the somatomotor dorsal pallial area shows descending projections resembling both S1 and M1 (especially in some avian species). In reptiles, the putative ventrobasal complex does not include any motor subdivision. The somatosensory area of the reptilian dorsal pallium only shows projections to diencephalic and midbrain tegmentum, reaching premotor precerebellar and reticulospinal cell groups, and suggesting that it may control or modulate motor behavior. However, this area lacks most of the connections typical of a true somatomotor area, suggesting that this likely evolved independently in birds and mammals. Cb, cerebellum; CPU, caudoputamen (dorsal striatum); DC, reptilian dorsal cortex; DCN, dorsal column nuclei; Ds5, descending sensory trigeminal nucleus; DVR, dorsal ventricular ridge; GLN, dorsal lateral geniculate nucleus; GP, globus pallidus; H, hyperpallium; Hp, hippocampal formation; IC, inferior colliculus; M, mesopallium; M1, primary motor area of neocortex; mo, motoneuron pools; N, nidopallium; OB, olfactory bulb; oc, optic chiasm; Po, pontine nuclei; Pr5, principal sensory trigeminal nucleus; RF, reticular formation; Ru, nucleus ruber; S1, primary somatosensory area of neocortex; SC, superior colliculus; St, striatum; TeO, optic tectum; V1, primary visual area of neocortex; VB, ventrobasal thalamic complex.

Are these areas present in the dorsal pallium of reptiles and/or birds? Data on ephrin ligands and receptors in the dorsal pallium of birds and reptiles are lacking but, in any case, the search for functional areas in the dorsal pallium of birds and reptiles requires the analysis of the thalamopallial projections in these vertebrate groups and/or electrophysiological recordings in the pallium.

Of interest, the adult hyperpallium of birds and dorsal cortex of reptiles show some patterns of connections with the dorsal thalamus similar to those of the neocortex (Figure 3). In particular, the patterns of connections suggest the existence of a primary visual area and a primary somatosensory area in the reptilian dorsal cortex and avian hyperpallium that are comparable and might be homologous to the primary visual (V1) and primary somatosensory (S1) areas of the mammalian neocortex (reviewed in Medina and Reiner, 2000; see also Wild and Williams, 2000). The somatosensory area found in the avian hyperpallium shows some connections, suggesting that it may represent a true somatomotor area, able to play a role in modulation of somatosensory input as well as in motor control, resembling aspects of mammalian S1+M1 (perhaps like the somatomotor area present in the origin of mammals). However, available data suggest that the motor control features of this avian hyperpallial area evolved independently from those found in M1.

The conclusion of homology of the visual and somatosensory cortical areas is partially based on assumption of homology of the thalamic nuclei of reptiles, birds, and mammals relaying the visual or somatosensory information to the dorsal pallium. But, for this to be true, these nuclei not only need to share similar connections, but also need to originate from the same embryonic primordium (or be located in the same histogenetic field). This will be analyzed in the following section.

2.07.3 Thalamopallial Projections and Sensory and Motor Areas in the Dorsal Pallium of Mammals, Birds, and Reptiles

2.07.3.1 Divisions of the Thalamus: Specific Relation of the Lemnothalamus with the Dorsal Pallium

To know whether the thalamic nuclei projecting to V1, S1, or M1 of mammalian neocortex are located in the same histogenetic unit as the avian and reptilian thalamic nuclei projecting to the hyperpallium/dorsal cortex, it is important to analyze the development and adult organization of the thalamus. In this sense, Butler (1994a) proposed the existence of two dorsal thalamic divisions, the lemnothalamus and the collothalamus, which receive sensory input through different systems and have different connections with the pallium (see The Dual Elaboration Hypothesis of the Evolution of the Dorsal Thalamus). The lemnothalamus includes nuclei receiving sensory input primarily from lemniscal systems and projecting to the medial and/or dorsal pallium (Butler, 1994a, 1994b). The collothalamus includes nuclei receiving a major collicular input and projecting to the lateroventral pallium (Butler, 1994a, 1994b). A similar but more complex subdivision of the thalamus was later proposed by other authors based on differential expression of cadherins or calcium-binding proteins by thalamic subdivisions during development or in the adult (Dávila *et al.*, 2000; Redies *et al.*, 2000). According to these authors, the dorsal thalamus is subdivided into three main histogenetic divisions, called dorsal, intermediate, and ventral tiers, each one showing a specific immunostaining profile and connections with a particular pallial subdivision (Dávila *et al.*, 2000; Redies *et al.*, 2000; Puelles, 2001). The dorsal tier corresponds roughly to the lemnothalamus, whereas the intermediate and ventral tiers roughly correspond to the collothalamus of Ann Butler (Butler, 1994a, 1994b; Dávila *et al.*, 2000; Redies *et al.*, 2000). Thus, the thalamic nuclei projecting to V1, S1, and M1 in mammals are all located in the dorsal tier or lemnothalamus (Puelles, 2001; Butler, 1994a). What about the visual and somatosensory/somatomotor thalamic nuclei projecting to the avian hyperpallium and reptilian dorsal cortex?

2.07.3.2 A Primary Visual Area in the Dorsal Pallium of Birds and Reptiles and Its Comparison to V1 of Mammals

In mammals, V1 (area 17) receives unimodal visual input from the dorsal lateral geniculate nucleus and projects back to this nucleus and to the superior colliculus (Figure 3) (Krubitzer, 1995; Sefton *et al.*, 2004). A comparable retinorecipient dorsal lateral geniculate nucleus is present in the lemnothalamus of birds and reptiles that projects to the avian hyperpallium and reptilian dorsal cortex (Figure 3) (Karten *et al.*, 1973; Hall *et al.*, 1977; Miceli and Repérant, 1982; Miceli *et al.*, 1990; Mulligan and Ulinski, 1990; Butler, 1994a, 1994b; Kenigfest *et al.*, 1997; Medina and Reiner, 2000; Zhu *et al.*, 2005). In lizards, this nucleus is sometimes called intercalatus (Bruce and Butler, 1984a) and apparently corresponds to the deeper part (cell plate) of the dorsal lateral geniculate nucleus of other authors (Kenigfest *et al.*, 1997; Dávila *et al.*, 2000). In lizards, the geniculate thalamic input only reaches a lateral extension of the dorsal cortex, called pallial

thickening (Bruce and Butler, 1984a; Kenigfest *et al.*, 1997). In birds, the geniculate thalamic input mainly reaches a hyperpallial subdivision called interstitial nucleus of the apical hyperpallium or IHA (Karten *et al.*, 1973; Watanabe *et al.*, 1983). As in mammals, the dorsal pallial area of birds and at least some reptiles (such as turtles) that receives visual input from the geniculate nucleus projects back to this thalamic nucleus and the optic tectum (Figure 3) (Karten *et al.*, 1973; Hall *et al.*, 1977; Miceli and Repérant, 1983, 1985; Reiner and Karten, 1983; Ulinski, 1986; Mulligan and Ulinski, 1990; Butler, 1994a, 1994b; Kenigfest *et al.*, 1998). Therefore, their similar position, histogenetic origin, and connections suggest that the visual lemnothalamic nuclei and related pallial areas of the neocortex, hyperpallium, and dorsal cortex of mammals, birds, and reptiles are homologous, and evolved from similar areas present in their common ancestor.

2.07.3.3 A Primary Somatosensory Area in the Dorsal Pallium of Birds and Reptiles and Its Comparison to S1 of Mammals

In the mammalian neocortex, S1 receives somatosensory input from the ventrobasal or ventral posterior thalamic complex (in particular, from VPL, receiving body information via the dorsal column nuclei, and from VPM, receiving head information via the principal sensory trigeminal nucleus; restricted parts of VPL/VPM also receive pain and temperature information directly from the spinal cord through the dorsal horn and spinal trigeminal nucleus) (Figure 3). In turn, S1 shows descending projections back to this thalamic complex and to the brainstem and spinal cord (reaching primarily precerebellar and/or somatosensory relay centers) (Weisberg and Rustioni, 1977; McAllister and Wells, 1981; Torigoe *et al.*, 1986; Krubitzer, 1995; Desbois *et al.*, 1999; Manger *et al.*, 2001; Martínez-Lorenzana *et al.*, 2001; Killackey and Sherman, 2003; Craig, 2004; Friedberg *et al.*, 2004; Gauriau and Bernard, 2004; Leergaard *et al.*, 2004; Oda *et al.*, 2004; Tracey, 2004; Waite, 2004; Guy *et al.*, 2005). Similarly to S1, the frontal part of the avian hyperpallium (Wulst) receives somatosensory input from the dorsointermediate ventral anterior thalamic nucleus (DIVA), which is a target of both the dorsal column nuclei (Wild, 1987, 1989, 1997; Funke, 1989a, 1989b; Korzeniewska and Güntürkün, 1990) and the spinal cord (Schneider and Necker, 1989) (Figure 3). The avian DIVA develops in the dorsal tier/lemnothalamus of the dorsal thalamus (Redies *et al.*, 2000) and, thus, appears comparable in position, histogenetic origin, and connections to the mammalian ventrobasal thalamic complex (mainly to VPL). In addition to receiving somatosensory input from DIVA, the frontal part of the Wulst (hyperpallium) projects back to this thalamic nucleus, to the brainstem, and, in some species of birds, to the cervical spinal cord (Figure 3) (Wild, 1992; Wild and Williams, 2000). As with S1, the frontal hyperpallial descending projections predominantly reach precerebellar areas and somatosensory relay areas, such as the thalamic DIVA, the dorsal column nuclei, and the vicinity of medial lamina V in the cervical spinal dorsal horn, which suggests that the frontal hyperpallium may be primarily concerned with the control/modulation of somatosensory input (Wild and Williams, 2000). This suggests that the avian frontal hyperpallium contains a primary somatosensory area that appears comparable to S1 of mammals (Wild, 1992; Medina and Reiner, 2000; Wild and Williams, 2000). However, unlike S1, a sensory trigeminal representation (with head information) has not been found in the hyperpallium (Wild *et al.*, 1985). To know whether these primary somatosensory areas of the avian hyperpallium and mammalian neocortex are homologous we need to analyze if a similar pallial area is present in the dorsal pallium of reptiles.

The frontal part of the reptilian dorsal cortex was previously thought to contain a somatosensory area based on input from a spinorecipient thalamic nucleus (Ebbesson, 1967, 1969, 1978; Hall and Ebner, 1970). Modern tract-tracing data in lizards indicate that the rostral dorsal cortex receives distinct ipsilateral input specifically from a ventral part of the dorsolateral thalamic nucleus (Guirado and Dávila, 2002), which receives somatosensory input from the dorsal column and trigeminal sensory nuclei as well as from the spinal cord (Figure 3) (Hoogland, 1982; Desfilis *et al.*, 1998, 2002). Of interest, the dorsolateral thalamic nucleus of lizards is located in the dorsal tier/lemnothalamus of the dorsal thalamus, and its ventral part DLV, which differs from the rest of the nucleus by its connections and calbindin immunostaining profile – shows a location that resembles that of avian DIVA and ventrobasal complex of mammals (Dávila *et al.*, 2000). The projection from the dorsal column and sensory trigeminal (both descending and principal) nuclei and from the spinal cord to the dorsolateral thalamic nucleus appears to reach specifically its ventrolateral part or DLV (plus the area adjacent to it, called intermediodorsal nucleus; Ebbesson, 1967, 1969, 1978; Hoogland, 1982). Thus, DLV of lizards appears to be a distinct subnucleus of the lemnothalamus that may be primarily involved in

somatosensory information processing. Using modern tract-tracing techniques, similar thalamocortical projections have also been described in crocodiles (Pritz and Stritzel, 1987; these authors also found a specific dorsolateral cell population projecting only ipsilaterally to the dorsal cortex) and in adult and developing turtles (Hall *et al.*, 1977; Cordery and Molnár, 1999). In turtles, the thalamocortical nucleus appears located in a perirotundal position, medially adjacent to the dorsal lateral geniculate nucleus and ventral to the dorsolateral thalamic nucleus (a position that resembles the DLV of lizards), and this perirotundal area is the site of termination of spinal and dorsal column nuclei (but not retinal) projections (Künzle and Schnyder, 1983; Siemen and Künzle, 1994). However, other authors studying turtles have not found any thalamic relay center projecting to the dorsal cortex other than the geniculate nucleus (Zhu *et al.*, 2005). This may be due to the more caudal location of the injections in the dorsal cortex (note that the somatosensory area is located at its rostral pole) or to the employment of *in vitro* tract-tracing techniques in the study done by Zhu *et al.* (2005). Based on the evidence presented above, two different thalamic relay centers conveying either visual or somatosensory information to the dorsal cortex are present in most reptilian groups, and were likely present in their common ancestor. Thus, the frontal part of the dorsal cortex of most reptiles contains a primary somatosensory area that is comparable and may be homologous to those present in the hyperpallium of birds and the neocortex of mammals. Consistent with this, the relative position of the primary somatosensory area in the dorsal pallium is similar in mammals, birds, and reptiles, being always located rostral (in a more frontal position) to the primary visual area.

2.07.3.4 Do Birds and/or Reptiles Possess a Somatomotor Dorsal Pallial Area Comparable to M1 of Mammals?

In the mammalian neocortex, M1 (area 4) receives motor input from a specific part of the lemnothalamic ventrobasal complex (VA/VL nuclei in rodents), and shows descending projections back to this thalamic complex, and to the brainstem and the spinal cord (Figure 3), where the projections reach precerebellar (including the red and pontine nuclei) and sensory-relay areas (including dorsal column nuclei and dorsal horn of the spinal cord), but also reach premotor reticulospinal cell groups (including the prerubral and rubral neurons) and, in some species (such as rodents and primates), motor neuron pools such as those of the ventral horn in the spinal cord (Weisberg and Rustioni, 1977; Humphrey *et al.*, 1984; Torigoe *et al.*, 1986; Liang *et al.*, 1991; Krubitzer, 1995; Song and Murakami, 1998; Kuchler *et al.*, 2002; Leergaard *et al.*, 2004). In general, the descending projections of M1 are similar to those of S1, and axons from both areas contribute to form the pyramidal tract. However, the descending projections of S1 and M1 are somewhat different. For example, in the brainstem, S1 projects significantly more heavily to the precerebellar pontine nuclei than M1 (Leergaard *et al.*, 2004), whereas M1 is the major source of corticorubral axons (Giuffrida *et al.*, 1991; Burman *et al.*, 2000) (Figure 3). Further, in the spinal cord, S1 axons primarily reach dorsal horn laminae, whereas M1 axons, but not S1 axons, also reach the motoneuron pools in the ventral horn (Figure 3) (Ralston and Ralston, 1985; Martín, 1996). Current available data suggest that early mammals lacked a separate motor cortical area (M1), and that a separate M1 likely evolved with the origin of placental mammals (Kaas, 2004). Thus, it appears that early mammals only had an S1 where somatosensory and motor attributes were overlapped, a situation which resembles that found in marsupials (Kaas, 2004).

Similarly to M1, the frontal part of the avian hyperpallium (Wulst) receives input from a putative motor thalamic nucleus, the ventrointermediate area (VIA), which receives input from the avian globus pallidus, substantia nigra pars reticulata, and deep cerebellar nuclei (Medina *et al.*, 1997) (Figure 3). The avian VIA resembles the motor part of the mammalian ventrobasal thalamic complex (VA/VL) in both its position (located in the lemnothalamus and adjacent to the somatosensory part of the avian ventrobasal complex or DIVA), and its connections (Medina *et al.*, 1997). Both DIVA and VIA project to the frontal part of the hyperpallium (Wild, 1987, 1989; Funke, 1989a, 1989b; Korzeniewska and Güntürkün, 1990; Medina *et al.*, 1997), where somatosensory and motor information may be completely overlapped (Medina and Reiner, 2000) (Figure 3). Of note, as S1 and M1 of mammals, the frontal hyperpallium of birds projects back to the thalamus (including DIVA), to the brainstem and, in some avian species, to the cervical spinal cord (Wild, 1989, 1992; Medina and Reiner, 2000; Wild and Williams, 2000) (Figure 3). In the brainstem and spinal cord, the frontal hyperpallial projections reach precerebellar (including pretectal and rubral nuclei), sensory-relay cell groups (including the dorsal column nuclei and the dorsal horn in the spinal cord), premotor reticulospinal neurons (such as rubrospinal neurons) and, in some birds, a few axons reach the ventral horn of the cervical spinal

cord, where motoneuron pools are located (Wild, 1992; Wild and Williams, 2000). Thus, the frontal hyperpallium contains a somatosensory/somatomotor area that appears at least partially comparable to the overlapped S1+M1 of marsupials, and possibly of early mammals. To know whether these areas are homologous we need to know if a similar sensorimotor field is present in the dorsal pallium of reptiles.

As noted above, in some reptiles (lizards), the frontal part of the dorsal cortex appears to receive somatosensory input from a specific subdivision of the dorsolateral thalamic nucleus, the DLV (Guirado and Dávila, 2002) (Figure 3). Further, this part of the reptilian dorsal cortex has descending projections to diencephalic and midbrain tegmetum (Hoogland and Vermeulen-vanderZee, 1989; Guirado and Dávila, 2002). In the prerubral tegmentum, these cortical projections reach at least the nucleus of the medial longitudinal fascicle, which is a well-known premotor precerebellar and reticulospinal cell group (Figure 3) (ten Donkelaar, 1976; Woodson and Künzle, 1982; Wolters *et al.*, 1986). This feature has been used to suggest that this part of the reptilian dorsal cortex may represent a rudimentary sensorimotor area, partially comparable to that in other amniotes (Medina and Reiner, 2000; Guirado and Dávila, 2002). However, this putative sensorimotor area of the reptilian dorsal cortex does not possess a distinct motor field comparable to M1 of mammals, since its thalamic input does not include a basal ganglia-recipient nor a cerebellar-recipient nucleus. No part of the dorsolateral thalamic nucleus and no part of the reptilian dorsal thalamus receives direct basal ganglia input (Reiner *et al.*, 1984, 1998; Medina and Smeets, 1991) nor input from the deep cerebellar nuclei (Künzle, 1985). Further, in some reptilian species (including the pond turtle) the descending projections of the dorsal cortex are rather modest and do not reach rubral and, perhaps, not even prerubral levels (Zhu *et al.*, 2005). Thus, at present it is unclear whether ancestral reptiles had a rudimentary somatomotor area in the dorsal cortex, and more data are needed in other reptilian species before any conclusion can be reached. If a rudimentary somatomotor area was present in the dorsal cortex of reptiles, this area lacked many of the connections that characterize the true somatomotor cortical area found in birds and mammals (including basal ganglia and cerebellar indirect input, or output to additional precerebellar and reticulospinal fields and to the spinal cord), meaning that these features likely evolved independently in the avian and mammalian radiations.

2.07.3.5 Other Functional Areas in the Pallium of Birds and Reptiles and Comparison to Mammals

In birds and reptiles, there are other sensory (visual, somatosensory, and auditory) areas in the pallium that are located in the DVR (Karten and Hodos, 1970; Dubbeldam *et al.*, 1981; Bruce and Butler, 1984b; Wild, 1987, 1994; Wild *et al.*, 1993, 1997; Guirado *et al.*, 2000; reviewed by Karten and Shimizu, 1989; Butler, 1994b; Reiner, 2000). These sensory areas are mainly located in the ventral pallial part of the DVR (called nidopallium in birds; Reiner *et al.*, 2004) and receive visual, somatosensory, or auditory input from specific nuclei of the collothalamus or directly from the brainstem (see above-cited references; this is described in detail in Evolution of the Nervous System in Reptiles, Visual Cortex of Turtles). In birds, some of these DVR areas appear to have a better (more detailed) sensory representation than those present in the hyperpallium, such as the nucleus basalis of the budgerigar, which shows a highly somatotopically organized representation of head and body (Wild and Farabaugh, 1996; Wild *et al.*, 1997). In addition, the caudal part of the DVR in birds (including the caudal nidopallium and the region called arcopallium) and reptiles contains associative and/or motor centers that project to the basal ganglia, hypothalamus, and/or, in birds, also to premotor brainstem centers (Zeier and Karten, 1971; Bruce and Neary, 1995a, 1995b, 1995c; Davies *et al.*, 1997; Dubbeldam *et al.*, 1997; Lanuza *et al.*, 1997, 1998; Kröner and Güntürkün, 1999; Bottjer *et al.*, 2000; Martínez-García *et al.*, 2002). Further, in songbirds and budgerigars, the caudal DVR (arcopallium) contains a specific motor area that projects directly to motor brainstem nuclei, the ambiguus, and/or hypoglossal motor nuclei, which control syringeal, respiratory, and tongue muscles (Nottebohm, 1991; Vicario, 1991a, 1991b; Wild, 1993; Brauth *et al.*, 1994; Striedter, 1994; Durand *et al.*, 1997; see details on this motor pallial area and its connections in The Evolution of Vocal Learning Systems in Birds). In songbirds and budgerigars, this motor area is well developed and plays a key role in vocalization (including vocal learning and vocal production), and apparently evolved independently in songbirds and budgerigars (Striedter, 1994). These sensory, associative, and motor areas of the DVR play very important roles in sensory processing, sensorimotor integration, and motor control, and in birds are also involved in cognitive tasks such as learning, memory, and spatial orientation, and they have been compared to specific areas or specific cell populations of the mammalian temporal,

frontal, and prefrontal neocortex (for example, Karten, 1969, 1997; Morgensen and Divac, 1993; Veenman *et al.*, 1995; Kröner and Güntürkün, 1999; see The Evolution of Vocal Learning Systems in Birds and Humans). Although this makes sense from a functional point of view, the different histogenetic origin of the DVR (lateroventral pallium) and neocortex (dorsal pallium) indicates that the similarities of such sauropsidian DVR and mammalian neocortical areas represent cases of analogy (homoplasy). Consistent with this, the thalamic nuclei that project to the DVR sensory areas are not comparable in location to those that project to the neocortex. Thus, the DVR receives sensory information via thalamic nuclei that are located in the intermediate and ventral tiers of the dorsal thalamus, whereas those that project to the neocortex are generally located in the dorsal tier or lemnothalamus (Dávila *et al.*, 2000, 2002; Puelles, 2001). Further, the avian motor area(s) of the caudal DVR projecting to the premotor and/or motor brainstem are not present in the caudal DVR of reptiles (some of them are not even present in all birds), which means that they evolved as novelties in some birds. Thus, it appears that, in contrast to mammals, the repertory of complex behaviors shown by birds and reptiles depends primarily (although not exclusively) on a large variety of cell groups that develop in the ventrolateral pallial histogenetic division, but the contribution of dorsal pallial areas to these behaviors is likely more modest (especially in reptiles).

2.07.4 Pallial Lamination in Birds and Mammals: Evidence for Independent Evolution

2.07.4.1 Different Development and Adult Organization of Neocortical Layers and Hyperpallial Subdivisions

In mammals, the neocortex shows a laminar structure of six layers, and each layer has a similar cytoarchitecture and general pattern of connections throughout all areas, including V1, S1, and M1 (Figure 4). Thus, the dorsal thalamic input mainly contacts cells in neocortical layer 4, a layer that is called granular layer because of its typical granule or stellate cells (Humphrey *et al.*, 1977; Kharazia and Weinberg, 1994). In this layer, thalamic axons contact granule cells as well as apical dendrites of pyramidal neurons located below, in layers 5/6 (Mountcastle, 1997). In addition, thalamocortical axons ending in layer 4 provide collaterals that terminate in layer 5 and/or 6, but other thalamocortical axons terminate in layer 1 (Figure 4) (Jones, 1975; Rausell *et al.*, 1992; Lu and Lin, 1993; Zhang and Deschenes, 1998; Groenewegen and Witter, 2004; Sefton *et al.*, 2004). Layers 2 and 3 are called supragranular layers, located superficially to layer 4, and typically contain small to medium-sized pyramidal neurons involved in corticocortical (associational) projections (Gilbert and Kelly, 1975; Jones and Wise, 1977; Swadlow and Weyand, 1981; Sefton *et al.*, 2004). Layers 5 and 6 are called infragranular layers, located deep to layer 4, and typically contain large pyramidal neurons that show descending projections to the striatum, thalamus, and brainstem (Figure 4). Layer 5 mainly projects to the striatum and brainstem (to the midbrain tectum in the case of V1 and to the brainstem tegmentum and spinal cord in the case of S1 and M1), whereas layer 6 typically projects to the thalamus (Gilbert and Kelly, 1975; Jones and Wise, 1977; Swadlow and Weyand, 1981; Sefton *et al.*, 2004; Tracey, 2004). The pyramidal neurons of the supra- and infragranular layers of the neocortex typically have a long apical dendrite that span the cortical layers above its cell body (Figure 4), which provides one of the anatomical bases for the columnar functional organization of the neocortex (Mountcastle, 1997; Lübke *et al.*, 2000).

In birds, the dorsal pallium (corresponding to the so-called hyperpallium) also shows cytoarchitectonic subdivisions, considered by some authors as the layers of the neocortex (for example, Karten *et al.*, 1973; Shimizu and Karten, 1990; reviewed by Medina and Reiner, 2000). From rostral (frontal) to caudal levels, each hyperpallial subdivision is characterized by a specific pattern of connections which partially resembles the connectivity organization of neocortical layers (Figure 4). For example, the thalamic input ends primarily in an intermediate hyperpallial subdivision called the IHA, in both the visual and somatomotor areas (Karten *et al.*, 1973; Watanabe *et al.*, 1983; Wild, 1987, 1997), resembling neocortical layer 4. Nevertheless, some thalamic axons appear to end in the hyperpallium outside IHA (Figure 4). Further, the descending projections to the striatum, thalamus, and brainstem mainly originate in the apical hyperpallium or HA (Reiner and Karten, 1983; Wild, 1992; Wild and Williams, 1999, 2000), resembling neocortical layers 5–6. Morever, the densocellular hyperpallium (HD) appears to be mainly involved in connections with other pallial and subpallial areas (Veenman *et al.*, 1995; Kröner and Güntürkün, 1999; Wild and Williams, 1999), thus partially resembling neocortical layers 2–3. In addition to the apparently similar laminar organization of both neocortex and hyperpallium, there is evidence suggesting that they also

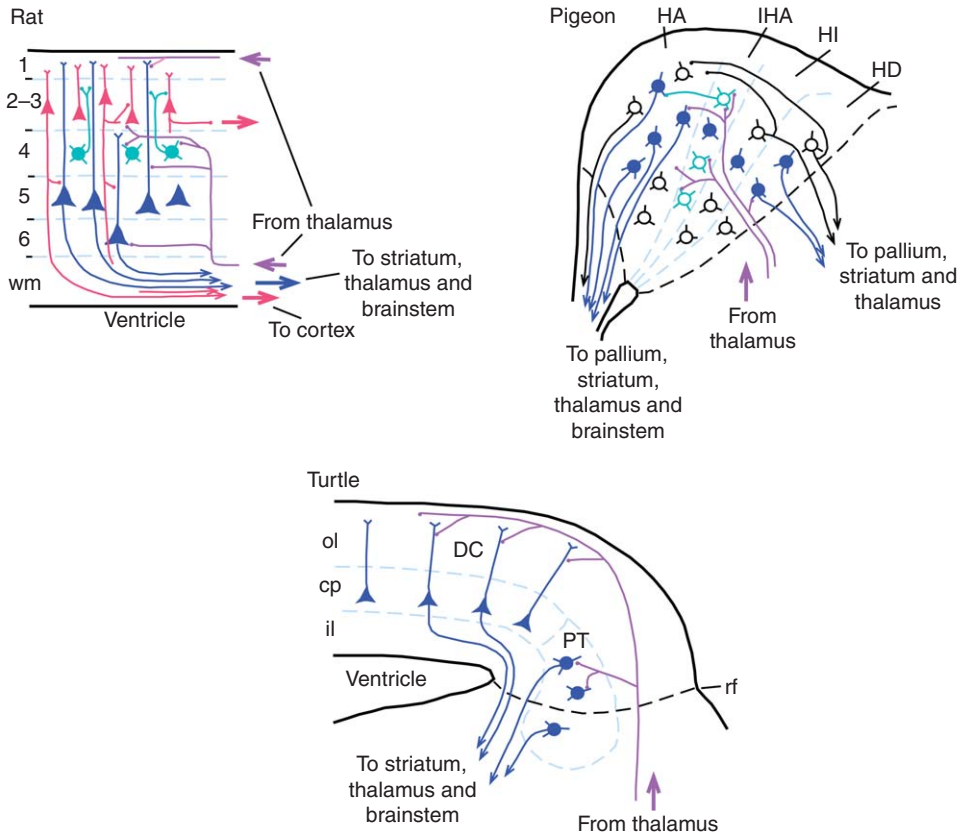

Figure 4 Schematics of the layers/subdivisions, cell types, and major connections of the mammalian neocortex, avian hyperpallium, and reptilian dorsal cortex. The mammalian neocortex shows a six-layered organization, and each layer shows specific cell types and connections. The thalamic input primarily reaches the intermediate layer 4 (also called granular layer, because of its typical granule or stellate cells). In this layer, thalamic axons contact stellate cells as well as apical dendrites of pyramidal neurons located in infragranular layers (layers 5 and 6). Some thalamic axons also reach layers 6 or 1. Infragranular layers contain large pyramidal neurons having apical dendrites that radially span the layers above, and give rise to descending projections to the striatum, thalamus, and brainstem. Supragranular layers contain small to medium pyramidal neurons involved in corticocortical (associational) connections. This anatomical and cellular organization, with radially oriented dendrites that span most layers, constitutes one of the basis of the functional columnar organization of neocortex. In contrast to this organization, the avian hyperpallium shows four mediolateral subdivisions that are formed and organized in a radically different way. These subdivisions contain multipolar or stellate-like neurons having star-like oriented dendrites that do not span adjacent subdivisions (i.e., they lack the translayer, radial dendritic organization typical of neocortex). In contrast to the neocortex, the connections between subdivisions occur by way of tangential projections, instead of the radial connections typical of neocortical columns. Nevertheless, the patterns of connections of hyperpallial subdivisions partially resemble those of neocortical layers. For example, thalamic input primarily ends in an intermediate subdivision (IHA), which is sandwiched between a subdivision (HA) giving rise to descending projections to the striatum, thalamus, and brainstem, and another subdivision (HD) giving rise to pallial projections. However, HA also projects to other pallial areas, whereas HD also projects to the striatum and thalamus, indicating that their similarity with specific neocortical layers in terms of connectivity is only partial. The reptilian dorsal cortex is very simple but resembles, in a very rudimentary way, both the laminar organization of neocortex and the mediolateral subdivisions of hyperpallium. Thus, the reptilian dorsal cortex contains a medial subdivision (dorsal cortex proper or DC) that resembles HA, and a lateral subdivision (pallial thickening or PT) that resembles HD. Further, the reptilian dorsal cortex shows a three-layered structure, with a main cell layer located between superficial and deep cell-sparse layers. The main cell layer contains pyramidal neurons having apical dendrites that span the superficial layer, where they are contacted by incoming thalamic axons. Further, these pyramidal neurons give rise to descending projections to the striatum, thalamus, and brainstem. This basic laminar and cellular organization partially resembles that of the neocortex, with pyramidal neurons located in deeper layers (5/6) giving rise to long descending projections, and thalamic axons contacting the apical dendrites of these cells. Cell types sharing these features and connections were likely present in the dorsal pallium of the common ancestor of mammals, birds, and reptiles (represented in dark blue in schematics). However, many of the cell types present in the mammalian neocortex and avian hyperpallium likely evolved independently in birds and mammals (such as the thalamorecipient stellate or stellate-like cells, or many of the neurons involved in corticocortical connections). cp, main cell layer; DC, reptilian dorsal cortex; HA, apical hyperpallium; HD, densocellular hyperpallium; HI, intercalated hyperpallium; IHA, interstitial nucleus of the apical hyperpallium; il, inner layer; ol, outer layer; PT, pallial thickening; wm, white matter.

share a similar functional columnar organization (Revzin, 1970). Both in the neocortex and the hyperpallium, the sensory input is topographically (retinotopically or somatotopically) organized (Pettigrew and Konishi, 1976; Wilson, 1980; Wild, 1987; Funke, 1989a; Manger *et al.*, 2002). In each single neocortical unit, the excitation reaches layer 4 by way of thalamocortical axons, and then spreads primarily in a columnar way first to supragranular and later to infragranular layers (Petersen and Sakmann, 2001), and this appears to be similar in the avian hyperpallium (Revzin, 1970).

However, the similarity of hyperpallial subdivisions and specific neocortical layers in terms of connectivity is only partial (Veenman *et al.*, 1995; Wild and Williams, 1999). More importantly, developmental and cellular analysis of the avian hyperpallium and mammalian neocortex indicates that the subdivisions of the avian hyperpallium are not true layers (as least, as defined from a developmental point of view, but see Striedter, 2005) and show important organizational differences with neocortical layers (reviewed in Medina and Reiner, 2000). For this reason, the subdivisions of avian hyperpallium have been called pseudolayers, meaning false layers (Medina and Reiner, 2000). This is based on the following facts. First, although the layers of the mammalian neocortex are aligned parallel to the ventricular surface and develop perpendicular to radial glial fibers, the hyperpallial subdivisions are generally organized parallel to radial glial fibers (Striedter and Beydler, 1997; Medina and Reiner, 2000). Since, during development, the majority of neurons migrate from ventricular to mantle positions following radial glial fibers (Rakic, 1972, 1995; Alvarez-Buylla *et al.*, 1988; Striedter and Beydler, 1997), the different disposition of neocortical layers and hyperpallial subdivisions with respect to the radial glial fibers likely reflects that they are formed in radically distinct ways (Medina and Reiner, 2000). Second, as a consequence of their apparently different development, whereas for any single neocortical area the majority of neurons of all layers are born in the same ventricular sector (except interneurons, which immigrate from the subpallium; Anderson *et al.*, 1997), in the avian hyperpallium the neurons of each subdivision (HA, IHA, and HD) are primarily born in different ventricular sectors (Medina and Reiner, 2000). Third, also as a consequence of their development, whereas many layers of the neocortex typically contain pyramidal neurons with an apical dendrite that span the layers above (where they can be contacted by axons of extrinsic origin ending in other layers, as well as by axon collaterals of local neurons of other layers; Mountcastle, 1997), the subdivisions of avian hyperpallium contain neurons showing multipolar or stellate-like morphology, with star-like oriented dendrites that generally do not cross subdivision boundaries (Figure 4) (Watanabe *et al.*, 1983; Tömbol, 1990; Medina and Reiner, 2000). This means that, whereas in the mammalian neocortex, the radial (translayer) disposition of dendrites allows functional integration of layers (and constitutes one of the anatomical basis of functional columns; Lübke *et al.*, 2000), the neuronal communication between subdivisions of the avian hyperpallium is apparently only possible by way of tangential, inter-area axonal connections (Figure 4) (Kröner and Güntürkün, 1999; Medina and Reiner, 2000; Wild and Williams, 2000).

2.07.4.2 Layers and Subdivisions of the Reptilian Dorsal Cortex. Possibilities and Uncertainties on Dorsal Pallial Evolution

How did the different pallial organizations found in neocortex and hyperpallium evolve and which was the primitive condition in stem amniotes? Extant reptiles have a very simple dorsal pallium, but this shows some features that partially resemble, in a very rudimentary way, both the medial–lateral subdivisions of avian hyperpallium and the lamination of mammalian neocortex (Figure 4). The reptilian dorsal pallium appears to have two parts that show different cytoarchitecture and connections: a medial part or dorsal cortex and a lateral part or pallial thickening (as noted above, only part of the pallial thickening may be part of the dorsal pallium) (Figure 4). In lizards, the thalamic input primarily reaches the pallial thickening, whereas the dorsal cortex gives rise to the descending projections to the striatum and brainstem (see references in previous section; reviewed in Medina and Reiner, 2000). In turtles, thalamic input reaches both the pallial thickening and the dorsal cortex (Mulligan and Ulinski, 1990), and extratelencephalic projections originate in the dorsal cortex (Hall *et al.*, 1977; Ulinski, 1986; Zhu *et al.*, 2005). In lizards and turtles, the pallial thickening shows important intratelencephalic connections (Medina and Reiner, 2000). Thus, the organization of the connections and the relative position of these two divisions in the reptilian dorsal pallium suggests a similarity of the reptilian dorsal cortex and avian HA and the reptilian pallial thickening and avian HD. As the dorsal cortex or pallial thickening, the avian HA and HD also appear to receive a minor direct input from the sensory thalamus (Karten *et al.*, 1973; Watanabe *et al.*, 1983; Wild, 1997; Wild and Williams, 2000). On the other hand, the reptilian dorsal cortex shows a simple three-layered structure, with a main, intermediate cell layer

containing pyramidal-like cells, flanked by a superficial and a deep cell sparse layer (Figure 4) (Reiner, 1993; Medina and Reiner, 2000; Colombe *et al.*, 2004). As neocortical layers, those of the reptilian dorsal cortex are disposed parallel to the ventricular surface and perpendicular to the radial glia. Further, the pyramidal cells of the main cell layer show apical dendrites that span the layer above, where they are contacted by thalamic afferent axons, and they give rise to long descending projections reaching the striatum and brainstem (Figure 4) (Mulligan and Ulinski, 1990; Colombe *et al.*, 2004). Thus, the reptilian dorsal cortex shares with the neocortex some aspects of its laminar and cellular organization. In both the neocortex and reptilian dorsal cortex, the thalamic axons contact the apical dendrites of deep pyramidal neurons, and in both these, deep pyramidal neurons are the source of long descending projections. Of interest, in the neocortex, some thalamic afferent axons travel tangentially in layer 1, where they contact apical dendrites of pyramidal neurons (Rausell *et al.*, 1992), resembling the trajectory of thalamic axons in the reptilian dorsal cortex. Further, analysis of chemically different neurons in the dorsal pallium of mammals, birds, and reptiles indicates that only the cell types present in neocortical layers 5–6 (which contain the deep pyramidal neurons giving rise to long descending projections) are found in birds and reptiles, suggesting that only layers 5–6 were present in the common ancestor (Reiner, 1991). Further, comparative developmental studies suggest that only the subpial layer 1 and the deepest neocortical layers may have been present in the common ancestor of extant reptiles, birds, and mammals (Marín-Padilla, 1998).

All these data together suggest that the pyramidal neurons found in the cell layer of the reptilian dorsal cortex may be homologous to the pyramidal cells of layers 5–6 of the mammalian neocortex, and possibly to some of the multipolar projection neurons of avian HA (at least including the neurons that, in addition to giving rise to long descending projections, receive thalamic input). In contrast, the thalamorecipient granule (or stellate) cells found in neocortical layer 4 have no counterpart in reptiles and are not homologous to the thalamorecipient stellate-like cells found in avian IHA (Figure 4). Stellate cells of neocortical layer 4 and stellate-like cells of IHA apparently evolved independently (and were produced as novelties) in the mammalian and avian radiations. On the other hand, the pyramidal neurons of neocortical layers 2–3 and part of the projection neurons of avian hyperpallium involved in corticocortical connections may also be newly evolved. Finally, it is unclear what part of the mammalian neocortex (if any) is comparable to avian HD and reptilian pallial thickening, both involved in intratelencephalic connections. As noted above, since the pallial thickening and apparently HD receive retinal input, they may be comparable to a lateral part of V1. In relation to this, V1 in rats contains medial and lateral subdivisions which differ in cyto-, myelo-, and chemoarchitecture (Palomero-Gallagher and Zilles, 2004). The medial subdivision represents a monocular subfield, whereas the lateral subdivision represents a binocular subfield. Nonplacental mammals, such as marsupials, also show similar medial–lateral V1 subdivisions in the neocortex, representing areas of either complex or simpler waveform processing (Sousa *et al.*, 1978). Thus, it is possible that such mediolateral subdivisions were present in the origin of mammals and, if so, the lateral V1 part may be comparable to HD/pallial thickening of birds and reptiles. Another possibility is that HD and/or the pallial thickening are not comparable to any part of V1, but rather to a more laterally located cortical or subcortical pallial area, such as the insular cortex (or part of it) or the claustrum (Striedter, 1997). The position of these structures at the lateral extreme of the neocortex, either abutting the lateral pallium or within it, resembles that of both HD and pallial thickening (Figure 2). In contrast to this possibility, the connectivity patterns of HD and pallial thickening are very different from those of the insular cortex or claustrum. For example, in contrast to HD and pallial thickening, neither the insular cortex nor the claustrum receive direct input from the dorsal lateral geniculate nucleus (Clascá *et al.*, 1997; Sefton *et al.*, 2004). More studies will be needed to resolve this issue. If the pallial thickening of reptiles were not homologous (as a field) to any part of V1, it would challenge the existence of a primary visual area in the dorsal pallium of the amniote common ancestor (since in lizards the geniculate projection only reaches the pallial thickening but not the dorsal cortex proper), opening new and important questions on neocortical evolution.

2.07.5 Functional Properties of the Visual and Somatosensory Areas of Neocortex and Sauropsidian Dorsal Pallium: Do Mammals, Birds, and Reptiles See and Feel the Same?

2.07.5.1 Visual Area: Retinotopy, Signal Types, Binocularity, and Perception

The mammalian V1 contains a detailed point-to-point retinal map, received through the retinogeniculocortical pathway, which subserves conscious

vision (Kahn *et al.*, 2000; Sefton *et al.*, 2004; Wässle, 2004). Neurons in V1 respond to orientation, direction, or color, and this information reaches the cortex through mostly segregated parallel pathways (Wässle, 2004). This information is then processed and combined (a process that involves higher-order areas), making possible animals' visual perception of the world. Visual signals are first detected by retinal photoreceptors, rods (involved in detection of low light levels), and cones (involved in detection of lights of different wavelengths; i.e., they are color-sensitive). The signals detected at the photoreceptor level are then processed and filtered through a complex retinal system involving several cell types (including horizontal, bipolar, amacrine, and ganglion cells), connected through specific circuitries (Lee, 2004; Wässle, 2004). At the end of this process, different types of retinal ganglion cells respond to orientation, direction, motion, or color, and this information is then transmitted to the brain through the retinofugal pathways (one of which is the retinogeniculate system). In primates, achromatic retinofugal signals mainly reach the visual cortex by way of the magnocellular layer of the geniculate nucleus, whereas chromatic retinofugal signals reach V1 mainly via either the koniocellular or the parvocellular geniculate cells, and each pathway mainly ends on a separate layer or sublayer in V1 (Chatterjee and Callaway, 2003; Lee, 2004).

In V1, orientation and direction signals represent a first step for analysis of form or movement, in which higher-order visual areas participate (Sincich and Horton, 2005; Saul *et al.*, 2005; Shmuel *et al.*, 2005; van Hooser *et al.*, 2005). Neurons responsive (or sensitive) to orientation or direction appear to be present in V1 of a large variety of mammals (including placental and marsupial species; Murphy and Berman, 1979; Parnavelas *et al.*, 1981; Crewther *et al.*, 1984; Orban *et al.*, 1986; Vidyasagar *et al.*, 1992; Ibbotson and Mark, 2003; Priebe and Ferster, 2005), and many mammals appear to have at least a second visual area (V2) involved in higher-order processing (Kaas, 2004; Sefton *et al.*, 2004), suggesting that some basic aspects of form and movement perception are common to all mammals. Nevertheless, in some mammals (such as marsupials) only a low percentage of V1 neurons respond to motion (Ibbotson and Mark, 2003), whereas other mammals possess multiple higher-order visual areas, one of which (V5/MT of primates) is specially involved in motion perception (Riecansky, 2004; Sincich *et al.*, 2005; Silvanto *et al.*, 2005). Thus, it appears that some mammals have a better visual perception of movement and form than others. Further, in mammals (such as primates, cats, and rats, as well as marsupials), some or many neurons of V1 are characterized by binocular convergence (depending on the degree of orbital convergence, which is maximal in primates), and are involved in perception of depth (stereoscopic vision) (Vidyasagar *et al.*, 1992; Barton, 2004; Grunewald and Skoumbourdis, 2004; Heesy, 2004; Menz and Freeman, 2004; Read, 2005). But again, some mammals show higher binocular convergence and have more visual cortical areas involved in its analysis, indicating that some species apparently have better depth perception than others. Nevertheless, in many mammals, several noncortical areas (including pretectum, superior colliculus, and other subcortical areas) are involved in motion processing (Ibbotson and Price, 2001; Price and Ibbotson, 2001; Sefton *et al.*, 2004). The superior colliculus appears to be involved in the spatial localization of biologically significant stimulus rather than its recognition (where it is rather than what it is) (Schneider, 1969), and can influence head/eye movements and guidance toward or away from a stimulus (reviewed by Sefton *et al.*, 2004). The visual cortex (with the participation of higher-order areas) appears to be involved in perception of both what the stimulus is (form and pattern discrimination) and where it is, among other aspects of visual perception.

Regarding color perception, the majority of mammals appear to have dichromatic vision, whereas – among placental mammals – only some primates have trichromatic vision. Among nonplacental mammals, it appears that some Australian marsupials may also have trichromatic vision. This depends on the pigment (opsin) variety found in retinal cone photoreceptors, and in the existence of color opponent systems. It appears that most mammals have two cone types: a majority of cones are sensitive to medium or long wavelengths (M/L-cones, sensitive to green or red), depending on the species; and a minority of cones are sensitive to short wavelengths (S-cones, sensitive to blue or ultraviolet (UV)), depending on the species (Peichl and Moutairou, 1998; Yokoyama and Radlwimmer, 1998, 2001; Shi and Yokoyama, 2003; Gouras and Ekesten, 2004). Among placental mammals, only some primates (including squirrel monkeys, New World monkeys, and humans) have a trichromatic color vision and their retina contains cones sensitive to green, red, or blue. Many marsupials also have M/L- and S-cones (Deeb *et al.*, 2003; Strachan *et al.*, 2004), and it seems that they were present in the retina of ancestral vertebrates well before the emergence of mammals (Shi and

Yokoyama, 2003). It has been suggested that the green-sensitive and red-sensitive cones present in mammals evolved from a single M/L cone present in the common ancestor well before the origin of mammals (Yokoyama and Radlwimmer, 1998). However, several species of Australian marsupials do have trichromatic retinas, with cones sensitive to short, medium, or long wavelengths (Arrese *et al.*, 2002, 2005), which appears to be due to retention from the ancestor (see below). It seems that the retina of ancestral placental mammals became dichromatic when these animals adopted nocturnality and some primates, subsequently, re-evolved trichromacy (Arrese *et al.*, 2002).

It seems that color perception involves a comparison of the relative activities of different cones by way of an opponent process, which starts in the retina and is conveyed to the visual cortex by parallel, anatomically segregated color-opponent systems (Dacey, 2000). In mammals having a dichromatic retina, only one opponent system exists, a blue–yellow system, in which signals from blue cones are opposed to signals from red or green cones. For trichromatic retinas (such as those of some primates), there are two color opponent systems, one for a red–green system, in which signals from red- and green-sensitive cones are opposed, and another one for a blue–yellow system, in which signals from blue cones are opposed to a combined signal from red and green cones (Dacey, 2000; Chatterjee and Callaway, 2003). Specific retinal ganglion cells exist for each color system. The blue–yellow information is conveyed to V1 through the koniocellular geniculate pathway, whereas the red–green information is conveyed by the parvocellular geniculate pathway (Lee, 2004). The information reaching V1 is later combined in higher-order visual areas. It is likely that ancestral placental mammals only had the blue–yellow system, and that the anatomical substrate for the red–green system evolved as a novelty in primates (Dacey, 2000; Lee, 2004).

In reptiles and birds, the retina contains cell types (including rod and cone photoreceptors, as well as horizontal, bipolar, amacrine, and ganglion cells) and circuitries in general similar to many of those present in mammals (Fernández *et al.*, 1994; Kittila and Granda, 1994; Ammermüller and Kolb, 1995; Haverkamp *et al.*, 1997, 1999; Luksch and Golz, 2003). Ganglion cells responsive to direction, motion, or color are found in the retina of both birds and reptiles, and cells responsive to orientation are also found in birds (Granda and Fulbrook, 1989; Guiloff and Kolb, 1994; Ammermüller *et al.*, 1995; Borg-Graham, 2001; Wilke *et al.*, 2001; Jones and Osorio, 2004). Retinal information is then conveyed to the dorsal pallium by way of a retinotopically organized retinogeniculodorsal pallial pathway (Bravo and Pettigrew, 1981; Miceli and Repérant, 1982; Ehrlich and Mark, 1984; Mulligan and Ulinski, 1990). This suggests that the dorsal pallium of birds and reptiles may be involved in some aspects of visual perception similar to those processed by V1 in mammals. Consistent with this, the visual hyperpallium of some birds (such as owls) contains neurons showing selectivity for orientation and movement direction (Pettigrew and Konishi, 1976), and has been shown to be involved in form discrimination, including some complex aspects such as subjective contour discrimination (Nieder and Wagner, 1999). In other birds (chicks or pigeons), the hyperpallium is involved in motion processing, far-field pattern discrimination, spatial discrimination acquisition, and in sun-compass associative learning (Gusel'nikov *et al.*, 1977; Leresche *et al.*, 1983; Britto *et al.*, 1990; Budzynski *et al.*, 2002; Watanabe, 2003; Budzynski and Bingman, 2004). Among birds, the complexity of visual processing by the hyperpallium appears to be higher in owls (which are frontal-eyed birds) than in other birds. In fact, the hyperpallium of owls shows a larger size, a more detailed retinotopic map, a much higher binocular convergence, and a more complex visual processing than that of lateral-eyed birds, such as pigeons (Pettigrew and Konishi, 1976; Nieder and Wagner, 1999, 2000, 2001; Liu and Pettigrew, 2003). Thus, the visual hyperpallium of owls is involved in depth perception and detection of visual illusions (subjective contours), exhibiting a functional complexity analogous to that of higher-order visual areas of highly visual mammals such as primates and cats (Nieder and Wagner, 1999, 2000, 2001; Liu and Pettigrew, 2003; van der Willigen *et al.*, 2003). In contrast, binocularity in pigeons is low (Martin and Young, 1983; McFadden and Wild, 1986; Holden and Low, 1989). Further, although the hyperpallium in pigeons is involved in motion perception and far-field discrimination, other brain areas, such as the optic tectum and the areas involved in the tectothalamo-DVR pathway, also play very important roles in motion processing or in other aspects of visual discrimination (Gusel'nikov *et al.*, 1977; Leresche *et al.*, 1983; Macko and Hodos, 1984; Britto *et al.*, 1990; Wang *et al.*, 1993; Laverghetta and Shimizu, 1999; Crowder *et al.*, 2004; Nguyen *et al.*, 2004). Among these areas, the thalamic nucleus rotundus and its DVR target play an important role in processing of ambient illumination, near-field discrimination, spatial-pattern vision, motion, and color (Wang *et al.*, 1993).

In reptiles, the visual dorsal cortex shows a coarse retinotopic map (Mulligan and Ulinski, 1990), and it appears involved in some aspects of visual processing, such as motion, discrimination acquisition, and spatial learning, but not in brightness discrimination (Reiner and Powers, 1983; Grisham and Powers, 1989, 1990; Prechtl, 1994; Prechtl *et al.*, 2000; Nenadic *et al.*, 2002). However, as in pigeons, other brain areas of reptiles, such as those involved in the tectothalamo-DVR pathway, play a more important role in brightness and pattern discrimination than the dorsal cortex (Morenkov and Pivovarov, 1975; Reiner and Powers, 1983).

Regarding color perception, the retina of birds and reptiles also supports color vision, but this appears to be more complex than in mammals. Thus, it appears that the retina of many diurnal birds and reptiles contains four types of cones, sentitive to red, green, blue, or UV or near-UV light (Ammermüller *et al.*, 1995; Bowmaker *et al.*, 1997; Kawamura *et al.*, 1999; Ventura *et al.*, 2001; Smith *et al.*, 2002). The cones of many diurnal birds and reptiles also contain colored oil droplets, which act as filters and apparently enhance color discrimination (Bowmaker *et al.*, 1997; Vorobyev, 2003). Parallel opponent retinal pathways have been shown in some species of reptiles, suggesting the existence of tetrachromatic color vision in these animals (Ammermüller *et al.*, 1995; Ventura *et al.*, 2001). In turtles, the opponent color systems described in the retina include a blue–yellow system, a red–green system, and a UV–blue system, among other possibilities (Ventura *et al.*, 2001). It is unclear whether all these systems are present in other reptiles or in birds. As noted above, the blue–yellow opponent system is apparently present in most mammals and may have been present in stem amniotes. However, the anatomical substrate of the red–green pathway of turtles is likely nonhomologous to that found in some primates. As noted above, birds and reptiles possess a retinotopically organized retinogeniculodorsal pallial pathway comparable (likely homologous) to the retinothalamo-V1 of mammals, suggesting that the avian and reptilian dorsal pallium may be involved in color vision processing. However, only a few aspects of color vision (if any) may be processed in the dorsal pallium of birds, and it appears that color vision in birds and possibly reptiles is mainly (if not only) processed by other brain areas and pathways, such as the tectothalamo (rotundal)-DVR pathway (Güntürkün, 1991; Chaves *et al.*, 1993; Wang *et al.*, 1993; Chaves and Hodos, 1997, 1998).

All of these data together indicate that, although the visual area of the reptilian dorsal cortex, avian hyperpallium, and mammalian V1 are involved in some similar basic aspects of visual perception, many complex functions shown by the visual hyperpallium of some birds and by V1 of highly visual mammals, such as depth perception (associated to binocularity) and subjective contour discrimination, among others, likely evolved independently. Consistent with this, the anatomical substrate for the binocularity is different in birds and mammals (Casini *et al.*, 1992; Medina and Reiner, 2000). Further, the role of V1 in color processing and the anatomical pathways related to it may have evolved only in mammals. Regarding motion, the dorsal pallial visual area of reptiles (at least turtles), birds, and mammals appears involved in its processing, and this may have characterized the dorsal pallial visual area of stem amniotes. All these data suggest that the retinogeniculodorsal pallial pathway found in birds and reptiles is mainly comparable to part of the magnocellular retinogeniculocortical pathway of mammals, but not to the parvocellular pathway (conveying mainly chromatic information of the red–green system) nor possibly the koniocellular pathway (conveying mainly chromatic information of the blue–yellow system).

In reptiles and many birds, the retinotectothalamo (rotundal)-DVR pathway is more developed than the retinogeniculodorsal pallial pathway, and appears to play an important role in some aspects of visual processing, such as motion, color, and pattern discrimination (perhaps important for knowing both what the stimulus is and where it is). This general pattern may have characterized stem amniotes. It appears that early mammals were nocturnal animals, which may explain why many extant mammals have dichromatic vision (instead of the tetrachromatic vision that characterizes many birds and reptiles). Perhaps this was accompanied by a regression in visual perception abilities and their anatomical substrate, and an improvement of other sensory systems, such as the somatosensory and the auditory systems. The evolution of new mammalian species living in diurnal niches was likely accompanied by the great development of the retinothalamodorsal pallial pathway, and by the development of more visual neocortical areas (Husband and Shimizu, 2001). An increase in size and complexity of the retinothalamodorsal pallial pathway also occurred in birds, but this was particularly important in some frontal-eyed birds (such as the owl). Did this involve the development of higher-order visual areas in the dorsal pallium of owls? As noted above, the visual hyperpallium of birds is involved in highly complex visual functions comparable to those carried out by higher-order

visual areas of the mammalian neocortex. However, physiological studies have not analyzed the existence of multiple visual areas in the hyperpallium of owls. A recent study has shown the existence of at least two somatosensory representations in the frontal hyperpallium of owls (Manger *et al.*, 2002), and it is likely that more than one visual representation exists in the large hyperpallium of owls.

Finally, regarding the question of whether mammals, birds, and reptiles see the same, it is clear that not all mammals have the same degree of depth, color, and/or form perception, and this is also true in birds. Regarding color vision, although many diurnal reptiles and birds appear to have tetrachromatic vision and most mammals have dichromatic vision, there are examples of color-blind or trichromatic animals within mammals. Further, a few mammals (such as mouse and rat) and many birds and reptiles detect UV light, whereas most mammals (including humans) do not. Thus, the question of whether mammals, birds, and reptiles see the same is nonsense since visual perception differs among mammals, among birds, and possibly among reptiles. Nevertheless, some basic aspects of visual perception appear to be similar between many amniotes. Of particular interest is the fact that some complex visual functions related to form and depth perception appear to be similar between frontal-eyed birds (such as owls) and some highly visual mammals such as cats and some primates. As noted above, the anatomical substrate for the complex visual processing by the dorsal pallium found in these animals likely evolved independently. Further, in birds and reptiles, many aspects of visual perception (including color perception) appear to be processed in the DVR (ventrolateral pallium), rather than the dorsal pallium.

2.07.5.2 Somatosensory Area: Somatotopy, Signal Types, Perception, and Multiple Maps

In mammals, S1 contains a somatotopically organized map of the whole body (contralateral side) (Tracey, 2004). The information received by S1 via the ventrobasal thalamic complex includes tactile (touch, pressure), vibration, and proprioceptive (postural) signals, as well as pain and temperature. The somatosensory information reaching the frontal hyperpallium in birds by way of DIVA is also somatotopically organized, and includes at least tactile (light touch and pressure signals) and vibration information, mostly from the contralateral body surface (Wild, 1987; Funke, 1989a). Based on the external cuneate (which receives proprioceptive information from extraocular and wing muscles; Wild, 1985; Hayman *et al.*, 1995) and spinal inputs to DIVA (Schneider and Necker, 1989; Wild, 1989), it is likely that the frontal hyperpallium also receives proprioceptive, pain, and temperature signals. However, in contrast to mammals, mainly the body (including the neck) appears to be represented in the frontal hyperpallium in several avian species (Wild, 1987, 1997), although some studies have also reported representation of the beak (Korzeniewska, 1987; discussed in Wild, 1989). In birds, it appears that the head somatosensory information is mostly represented in another pallial area, called nucleus basalis, located in the DVR (Berkhoudt *et al.*, 1981; Dubbeldam *et al.*, 1981; Wild *et al.*, 1997). The somatosensory information reaches this DVR nucleus by way of a direct, somatotopically organized projection from the principal sensory trigeminal nucleus (Dubbeldam *et al.*, 1981; Wild and Zeigler, 1996; Wild *et al.*, 2001). Further, in some birds, such as the budgerigar, the nucleus basalis of the DVR includes not only head but also body representation, and this appears to be more detailed than that in the hyperpallium (Wild *et al.*, 1997). Thus, it appears that birds possess two different systems for pallial somatosensory representation, which show different degrees of development depending on the species. As noted above, only the hyperpallial representation appears comparable to S1 of mammals. The frontal dorsal cortex of reptiles also appears to receive somatosensory information of the body (in lizards, turtles, and possibly crocodiles) and, at least in some lizards, also the head (Desfilis *et al.*, 1998). More studies are needed to know whether this pattern is common in other reptiles. It is unclear whether the somatosensory information reaching the frontal dorsal cortex in reptiles is or is not topographically organized. Although it seems likely that the primary somatosensory area observed in the dorsal pallium of extant birds, reptiles, and mammals evolved from a homologous area present in their common ancestor (stem amniotes), the scarcity of data in reptiles does not allow any suggestion on the specific features of this primitive area. In any case, this area was likely very small, and likely lacked many of the attributes (in terms of anatomical organization, connections, and functional complexity) found in S1 of mammals and in the frontal hyperpallium of birds. As noted above, the cytoarchitectural organization and intrinsic columnar circuitry shown by the neocortex and hyperpallium evolved independently. Since in birds there is a small overlap of the primary visual and somatosensory areas in the hyperpallium (Deng and Wang, 1992), it is possible that a partial

ovelap of sensory areas characterized the dorsal pallium of stem amniotes (Figure 3).

Of interest, the neocortex of extant mammals contains multiple somatosensory representations, many of which (including S1, a secondary somatosensory area or S2, and the parietal ventral area) appeared to be already present in the origin of mammals (Krubitzer, 1995; Kaas, 2004). This provides an idea of the importance and high quality of somatosensory perception in these animals, and this great development may be related to the fact that ancestral mammals were nocturnal animals, primarily relying on senses other than vision. Further, somatosensory representation is even more complex in some mammals, such as primates, in which S1 contains four subdivisions (areas 3a, 3b, 1, and 2), each one showing a complete body representation (Tracey, 2004). In other mammals, including rodents, S1 has a single body representation, possibly comparable to area 3b of primates (Northcutt and Kaas, 1995). In birds having a large hyperpallium (such as the owl), two separate somatosensory representations of the claw have been observed, each showing a detailed somatotopic organization (Manger *et al.*, 2002). Thus, it appears that at least some birds have a more complex somatosensory representation in the hyperpallium, which may mean that they have a more elaborated analysis of this information and a more sophisticated somatosensory perception. Since the somatosensory area of the dorsal cortex of reptiles is apparently very small, it seems unlikely that multiple somatosensory representations were present in the dorsal pallium of stem amniotes. This means that the additional somatosensory hyperpallial area found in owls likely evolved independently and cannot be compared to any of the multiple S1 areas found in primates, to S2, nor to other somatosensory areas of mammalian neocortex (Manger *et al.*, 2002). Another interesting aspect of somatosensory representation in the neocortex of mammals is its activity-dependent plasticity, which is important for behavior modification and adaptation as a result of sensory experience (Kaas, 1995; Tracey, 2004). It appears that plasticity also characterizes the somatosensory hyperpallial area of owls (Manger *et al.*, 2002).

Regarding the question of whether mammals, birds, and reptiles feel the same, based on the number of pallial representations and variety of somatosensory receptors found in mammals (Kaas, 2004; Tracey, 2004), it appears that in general mammals have a much better somatosensory perception than reptiles and most birds. But again, it appears that somatosensory perception differs among mammals, as well as among birds and maybe among reptiles. One of the reasons is that the number of somatosensory representations and higher-order areas varies between species (Kaas, 2004). Another reason may be the existence of differences in peripheral receptors (in terms of quality, quantity, and/or location). For example, the complex receptor type found in owl claws (Manger *et al.*, 2002) may not be present in the claws of other birds, or may be present at a low number/area ratio. Further, different parts of the body and head have a different representation (in terms of relative size) in the neocortex/dorsal pallium in different species, which depends on their specific behavior. For example, the S1 of humans has a very large (or relatively large) representation of digits (which is related to the great tactile discrimination and exploratory and manipulatory use of our fingers), whereas in S1 of mouse and rat, the digits are not so well represented but the area related to the whiskers (barrel field area) is relatively large (which relates to the great importance of vibrissae in exploratory behavior and texture discrimination in rodents; reviewed by Waite, 2004). This rule also appears to be true for somatosensory areas in pallial regions other than the dorsal pallium. An example of this is found in the nucleus basalis of the budgerigar, which shows a larger size and more extensive representation of areas such as the beak, highly used by these animals (Wild *et al.*, 1997). Similarly, the claw of barn owl, used for perching and grasping prey and containing an elaborated tactile sensory receptor, likely has a larger representation in the frontal hyperpallium of this animal (including two areas, as noted above; Manger *et al.*, 2002) than that of the pigeon or the canary.

2.07.6 Conclusions

The neocortex contains specific sensory, associative, and motor areas that allow mammals to obtain a detailed map of the world and to adapt their behavior to it. Available data suggest that at least two such areas, the primary visual area and the primary somatosensory area, are also present in the dorsal pallium of birds and reptiles, and likely evolved from similar areas found in stem amniotes. However, these dorsal pallial areas present in the common ancestor likely had a very simple cytoarchitecture (possibly including a rudimentary three-layered structure plus at least two mediolateral subdivisions), and possessed fewer cell types and connections than those found in the mammalian neocortex and avian hyperpallium. For example, the complex six-layered organization of neocortex and the four mediolateral subdivisions of hyperpallium evolved independently in mammals or

birds. Further, the columnar functional organization of neocortex and the columnar-like organization of hyperpallium also evolved independently. In addition, these primitive areas of stem amniotes were likely involved in few aspects of visual or somatosensory perception. The role of the visual area in complex aspects of form and pattern discrimination or in depth perception (associated to binocularity) likely evolved independently in mammals and some birds, and its role in color perception (and the anatomical substrate related to it) apparently evolved only in the mammalian radiation. Finally, available data suggest that the dorsal pallium of stem amniotes may have lacked a true somatomotor area, and this evolved independently in birds and mammals (see A History of Ideas in Evolutionary Neuroscience, Phylogenetic Character Reconstruction, Field Homologies, Evolution of the Nervous System in Reptiles, Visual Cortex of Turtles, The Evolution of Vertebrate Eyes, The Evolution of Ultraviolet Vision in Vertebrates, What Fossils Tell Us about the Evolution of the Neocortex, The Origin of Neocortex: Lessons from Comparative Embryology, Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications, The Evolution of Motor Cortex and Motor Systems, The Dual Elaboration Hypothesis of the Evolution of the Dorsal Thalamus).

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2.08 Visual Cortex of Turtles

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Glossary

anterior dorsal ventricular ridge	A component of the telencephalon in reptiles and birds that is probably homologous to the neocortex of mammals.
archosaurian reptiles	A group of ancient reptiles that gave rise to modern crocodiles and birds.
clade	An evolutionary lineage of organisms.
dorsal lateral geniculate nucleus	A component of the thalamus that receives direct information from the retina.
fast spiking pattern	A pattern of neuronal firing in which a neuron produces relatively thin action potentials with little slowing of the firing rate of the cell.
isoazimuth lamellae	A band of neurons in the visual cortex of turtles that receives inputs from a band of neurons in the dorsal lateral geniculate nucleus.
lateral forebrain bundle	A group of axons that carry fibers from the thalamus and brainstem to the telencephalon and vice versa; equivalent to the internal capsule of mammals.
principal component analysis	A statistical method that identifies components in a complicated pattern, such as a cortical wave.
regular spiking pattern	A pattern of neuronal firing in which a neuron produces relatively thick action potentials and shows marked reduction in the firing rate of the cell with time.
spikelet	A small action potential, possibly of dendritic origin.
striatum	A ventral component of the telencephalon; equivalent to the basal ganglia.
Wulst	A bump on the telencephalon of some birds that appears to be a visual cortex.

2.08.1 Visual Telencephalon in Reptiles

Early comparative neuroanatomists were uncertain about the existence of visual projections to the telencephalon of reptiles (see Evolution of the Nervous System in Reptiles, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?). However, work in the 1970s with modern tract-tracing methods demonstrated that two visual pathways to the dorsal thalamus are present in representatives of each of the orders of reptiles (Northcutt, 1981). A tectofugal pathway consists of direct retinal projections to the optic tectum and a subsequent projection to nucleus rotundus in the dorsal thalamus. Nucleus rotundus projects to a large telencephalic structure, the anterior dorsal ventricular ridge, in all the orders of reptiles (Ulinski, 1983). A thalamofugal pathway consists of direct projections to the dorsal lateral geniculate nucleus (DLGN) in all the reptiles that have been studied (Repérant *et al.*, 1992). However, projections from the DLGN complex to the telencephalon vary substantially in members of the four orders of living reptiles and appear to be best developed in turtles. Turtles belong to the order Chelonia, which comprises two suborders of living turtles (Pritchard, 1967). The side-necked, or pleurodiran, turtles are restricted to Australia and fold their necks into their shells instead of retracting their necks. The cortex of the pleurodiran turtle, *Podocnemis*, contains a large nuclear mass in its lateral cortex that could be a telencephalic visual structure (Riss *et al.*, 1969), but there have been no experimental studies of visual cortex in pleurodiran turtles. This review will, consequently, be restricted to work on the visual cortex in cryptodiran turtles. Most experimental work has been done on freshwater

turtles of the genera *Pseudemys* (= *Trachtemys*), *Chrysemys*, and *Emys* in the family Emydidae.

2.08.2 Visual Cortex in Turtles

2.08.2.1 Cytoarchitecture

The cerebral cortex in turtles is a sheet of tissue that extends across the cerebral hemispheres. The anatomy and physiology of the cortex have been reviewed by Ulinski (1990, 1999). The cortex consists of three distinct layers. The outer layer (1) contains a relatively small number of loosely packed cells. The intermediate layer (2) contains many densely packed cells. The inner layer (3) contains a few loosely packed cells. The cortex can be divided into four cytoarchitectonic areas (Figure 1) based on variations in the histology of the three layers (Colombe and Ulinski, 1999). The medial cortex is situated at the medial edge of the cortex adjacent to the septum. The dorsomedial cortex is situated lateral to medial cortex. The medial and dorsomedial areas together appear to correspond to the hippocampal formation of mammals in terms of their connections with other parts of the brain. The lateral cortex is situated on the lateral surface of the hemisphere, receives direct projections from the main olfactory bulb, and clearly corresponds to the pyriform cortex of mammals. The dorsal cortex is situated between the dorsomedial and lateral areas of the cortex. Dorsal cortex is clearly divided into two subareas: the lateral part of dorsal cortex (D_L) and the medial part of dorsal cortex (D_M). Layers 1 and 3 are relatively thick in D_L and layer 2 forms a distinctive scroll-like configuration. D_L is called the pallial thickening in the classical literature. Layer 3 becomes narrow as it is traced from lateral to medial across the dorsal cortex so that layer 2 lies relatively close to the ependyma at the D_L/D_M border. Layer 3 becomes essentially nonexistent at the border of D_M with the dorsomedial area.

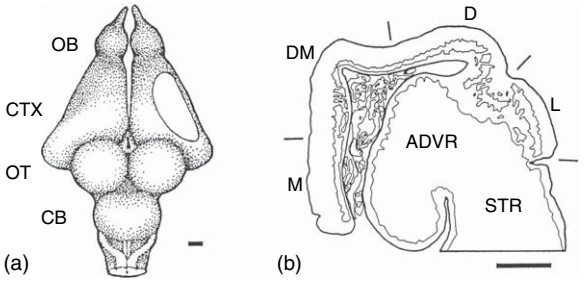

Figure 1 Visual cortex in a turtle. a, Dorsal view of the brain of a turtle, *Pseudemys scripta*. The white oval area is the visual cortex. b, Section through the telencephalon of *Pseudemys* at the level of the visual cortex. The visual cortex corresponds to the cytoarchitectonic area, D. ADVR, anterior dorsal ventricular ridge; CB, cerebellum; CTX, cortex; D, dorsal area; DM, dorsomedial area; L, lateral area; M, medial area; OB, olfactory bulb; OT, optic tectum; STR, striatum. Scale bars: 1 mm.

2.08.2.2 Geniculocortical Projections

The principal input to the visual cortex is from the DLGN, which lies internal to the optic tract and receives direct bilateral retinal projections (Ulinski and Nautiyal, 1987). DLGN contains two layers (Figure 2) situated concentric with the optic tract (Rainey and Ulinski, 1986). The neuropile layer is situated internal to the optic tract and contains a small number of cells whose dendrites extend parallel to the optic tract. The cell plate layer is the most noticeable and contains densely packed cells with dendrites that extend obliquely through the neuropile layer toward the optic tract. The dendrites end in finger-like processes that participate in a network of dendrodendritic synapses with flattened synaptic

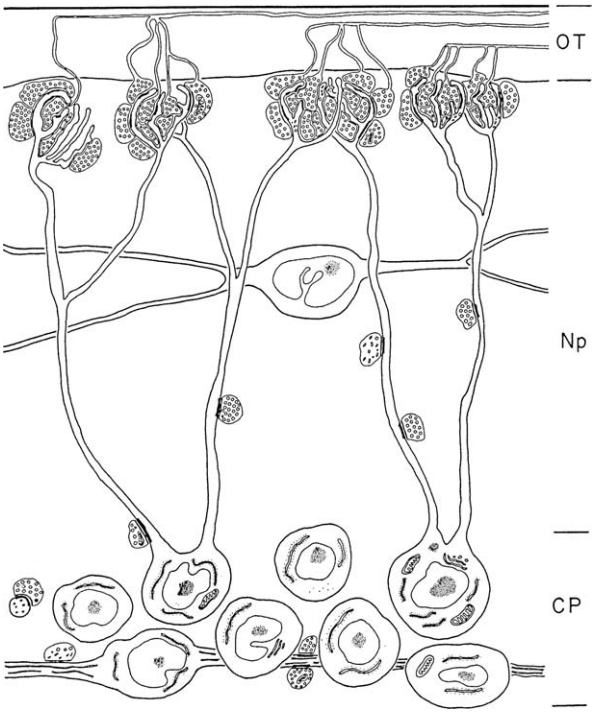

Figure 2 Organization of the OLGN. The basic organization of neurons in the dorsal lateral geniculate nucleus of turtles. The axons of retinal ganglion cells course over the outer surface of the geniculate complex in the optic tract (OT) and then turn into the neuropile (Np) layer of the geniculate complex. The majority of geniculate neurons have somata in the cell plate (CP) layer of the geniculate, and dendrites that extend into the neuropile layer. These dendrites end in finger-like processes that interact with each other through dendrodendritic synapses. The neuropile layer contains a few scattered cells with dendrites that extend parallel to the optic tract. Retinal ganglion cell terminals synapse on the dendrites of cell plate cell and on neuropile cells.

vesicles in their presynaptic elements (Ulinski, 1986a). It is reasonable to speculate that these synapses mediate inhibitory interactions between the cell plate cells.

Retinal ganglion cells show marked variations in their anatomy and physiology in turtles (e.g., Jensen and DeVoe, 1983; Granda and Sisson, 1992; Ammermuller and Kolb, 1995; Ammermuller *et al*., 1995; Dearworth and Granda, 2002). There is no agreement on the classification of turtle ganglion cells into groups in turtles. The simplest classification is that of Marchiafava and his colleagues, who recognize type A and type B cells, which differ in their soma sizes, the stratification of their dendritic trees in the inner plexiform layer of the retina, the diameters and conduction velocities of their axons, and their receptive field properties (Marchiafava and Weiler, 1980; Marchiafava *et al*., 1983). There is general agreement that a relatively large percentage of ganglion cells are directionally selective and prefer stimuli moving within specific ranges of speeds. Axons in the optic nerve can be placed in three conduction velocity groups and correspond to relatively thick-, medium-, and thin-diameter axons (Woodbury and Ulinski, 1986). The thick-diameter axons project to the basal optic nucleus, a component of the accessory optic system. Both the medium- and thin-caliber axons branch as they approach the brainstem and have collaterals that project to the optic tectum and the DLGN. The terminal arbors in the DLGN form two morphologically distinct groups (Sjöström and Ulinski, 1985). Relatively small arbors with many small boutons terminate in the outer half of the neuropile layer and are presynaptic to the dendrites of cell plate cells and to neuropile cells (Ulinski, 1986a). Large arbors with a few large boutons terminate in the inner half of the neuropile layer and are presynaptic to the dendritic shafts of the cell plate cells. The functional organization of the DLGN in turtles, thus, appears to be quite different from that of the DLGN complex in mammals. Projections from different groups of ganglion cells tend to be segregated into separate layers in the mammalian geniculate so that information from different physiological groups of ganglion cells is kept separate from the cortical level in mammals. By contrast, it appears that at least two types of ganglion cells (A and B) have convergent projections to the cell plate cells in turtles.

Retinal projections to the DLGN are bilateral, with the contralateral projection being the most extensive (Ulinski and Nautiyal, 1987). The nasotemporal axis of the retina is mapped along the rostrocaudal axis of DLGN and the dorsoventral axis of the retina is mapped along its ventrodorsal axis (Figure 3). A ventral rim of DLGN receives bilateral projections. The binocular region of turtle visual space includes a frontal field as well as a segment that extends above the turtle's head. Bilateral projections from the retinas to the DLGN extend from the caudal pole of the geniculate rostrally along its ventral rim.

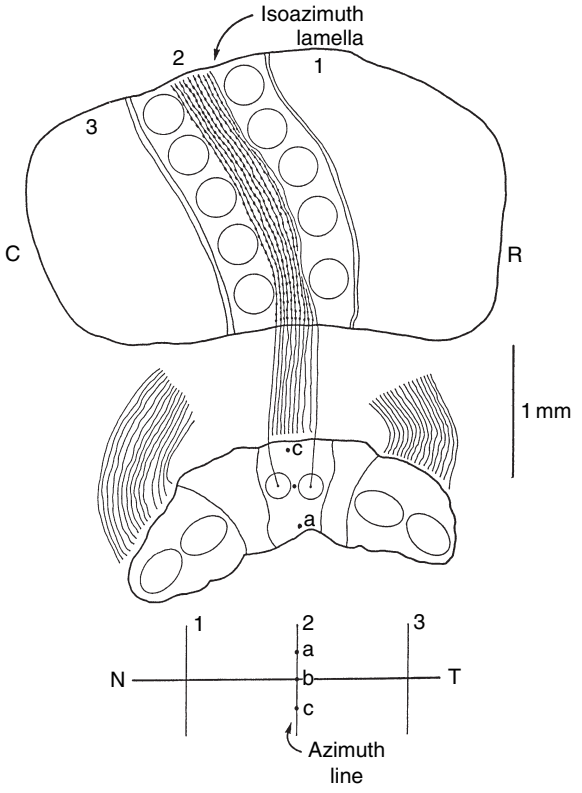

Figure 3 Organization of the geniculocortical projection. The nasal–temporal (N–T) axis of visual space and three azimuth lines (1, 2, and 3) are shown in the lower part of the figure. Three points of different elevation (a, b, and c) are shown on azimuth line 2. The dorsal lateral geniculate complex is shown in lateral view in the center of the figure. The N–T axis of visual space is mapped on to the rostral–caudal (R–C) axis of the geniculate. The superior–inferior axis of visual space is mapped on to the ventral–dorsal axis of the geniculate. Circles and ellipses on the geniculate represent the dendritic fields of individual geniculate cells. The visual cortex is shown in the upper part of the figure. Bundles of axons of geniculate neurons run across the visual cortex from lateral to medial, bearing synapses *en passant*. The circles in the visual cortex represent the dendritic fields of cortical neurons. The construction shows that a band of cortical neurons receives inputs from a band of geniculate neurons that contain information from the same azimuth of visual space. The cortical band is, thus, an isoazimuth lamella, and the diagram shows the approximate orientation of three isoazimuth lamellae that correspond to azimuth lines 1, 2, and 3.

Several studies have demonstrated that cell plate cells project to the dorsal cortex (Hall *et al*., 1977;

Belekhova *et al*., 1979; Desan, 1984, 1985; Zhu *et al*., 2005), but whether or not neuropile cells also project to visual cortex is controversial (Kenigfest *et al*., 1995). Geniculate axons leave the medial surface of DLGN and enter the telencephalon through the dorsal peduncle of the lateral forebrain bundle (Hall and Ebner, 1970; Heller and Ulinski, 1987). They turn dorsally as they approach the rostral recess of the lateral ventricle and enter the lateral edge of the dorsal cortex (Figure 3). Individual axons frequently course from lateral to medial across the dorsal cortex, bearing many varicosities *en passant* (Heller and Ulinski, 1987; Mulligan and Ulinski, 1990). The earliest studies of geniculocortical projections in turtles used anterograde degeneration techniques and interpreted degeneration in D_L as axons that passed through D_L without synapsing. However, more recent work has demonstrated that geniculate axons synapse on neurons in both D_L and D_M. Electron microscopic studies of degnerating synapses in D_M following large thalamic lesions show that thalamocortical synapses contain clear, round synaptic vesicles and synapse upon dendritic spines, dendritic shafts, and somata of cortical neurons (Ebner and Colonnier, 1975, 1978; Smith *et al*., 1980). The synapses are glutaminergic, access the α-amino-3-hydroxy-5-methyl-D-aspartate (AMPA) subtype of glutamate receptor and mediate fast excitatory postsynaptic potentials (EPSPs) in cortical neurons (Blanton and Kriegstein, 1991a, 1991b, 1992; Larson-Prior *et al*., 1991).

An interesting feature of the geniculocortical projection is that the laminar position of the bundle of geniculate axons shifts as it courses from lateral to medial across the dorsal cortex (Heller and Ulinski, 1987). It passes through the outer half of layer 3, layer 2, and the inner half of layer 1 as it courses through D_L, but then shifts its position to run in the outer third of layer 1 in D_M. Individual axons have slightly curved trajectories as they run across the dorsal cortex if the cortex is viewed from its dorsal surface (Mulligan and Ulinski, 1990). Axons cross over each other as the geniculate axons enter the lateral edge of cortex so that axons from the rostral pole of DLGN project to the caudal edge of the cortex and axons from the caudal pole of DLGN project to the rostral edge of the cortex. Since the nasotemporal axis of the retina is mapped along the rostrocaudal axis of the geniculate, a consequence of the geometry of the geniculocortical axons is that bands of cortical neurons receive synapses from a fascicle of geniculate axons that arise from a particular cluster of geniculate neurons. This band of cortical neurons receives inputs from points of all possible elevations along a given azimuth line of visual space and is called an isoazimuth lamella.

2.08.2.3 Cellular Structure of Turtle Visual Cortex

A rough estimate is that dorsal cortex contains approximately 100 000 neurons. Pyramidal cells account for approximately 80–90% of these neurons and are by far the most numerous neurons in layer 2 (Figure 4). They have vertically elongate somata that bear apical and basal dendrites. There are distinct differences between the pyramidal cells in D_L and D_M (Desan, 1984, 1985; Colombe and Ulinski, 1999). Those in D_L have roughly symmetric apical and basal dendritic trees that extend into layers 1 and 3, respectively (Figure 4a). The basal dendritic trees are reduced in pyramidal cells in D_M and become very short at the D_M/dorsomedial cortex border (Figure 4b). Because of the systematic shift in position of geniculate axons as they cross the dorsal cortex, pyramidal cells in D_L receive geniculate inputs on the somata and the proximal parts of both dendritic trees, while those in D_M receive geniculate inputs on the distal segments of their apical dendrites. Standard cable theoretic considerations predict that geniculate inputs should be far less effective in generating action potentials in D_M pyramidal cells than in D_L pyramidal cells. However, D_M pyramidal cells are electrotonically more compact than are D_L pyramidal cells and there is no systematic difference in the efficacy of geniculate synapses in the two groups of pyramidal cells (Ulinski, 1999). Intracellular current injections elicit two types of action potential in pyramidal cells (Connors and Kriegstein, 1986). The most common are large overshooting action potentials that appear to be somatic in origin. Smaller action potentials,

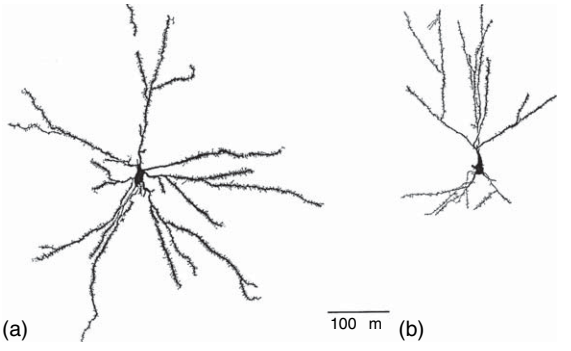

Figure 4 Morphology of pyramidal cells. a, Pyramidal cell from the lateral part of dorsal area, D_L. b, Pyramidal cell from the medial part of dorsal area, D_M. Both figures are drawings of pyramidal cells from Golgi preparations.

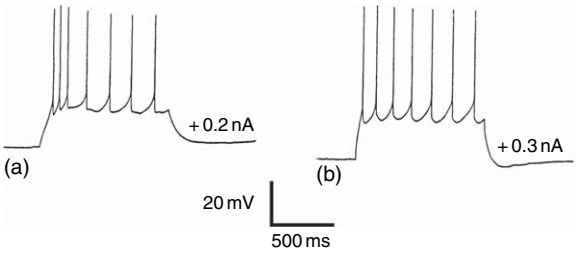

Figure 5 Firing patterns of cortical neurons. This figure shows the two most common firing patterns that are found in visual cortex neurons. a, A regular spiking cell, typical of pyramidal cells and subpial cells. b, A fast spiking cell, typical of stellate and horizontal cells. Each firing pattern was produced by injecting a square current pulse of + 0.2 or + 0.3 nA into a cell.

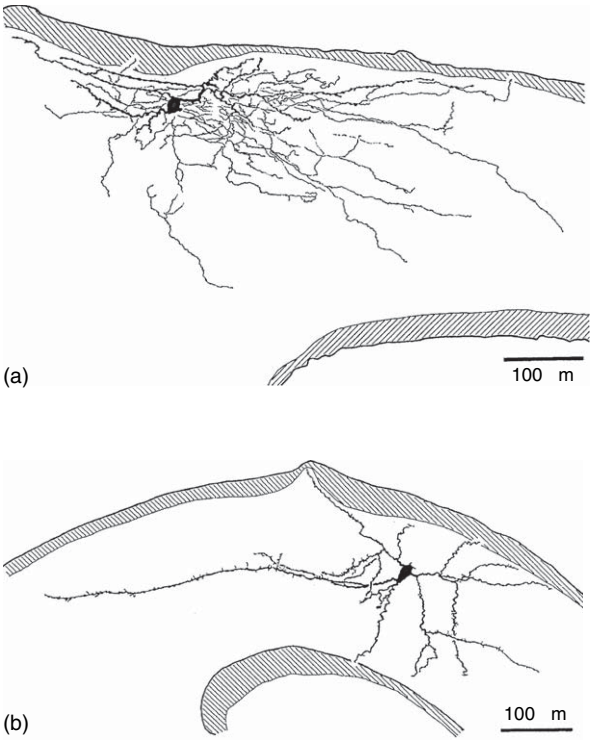

Figure 6 Inhibitory interneurons. a, A subpial cell from dorsal area. b, A stellate cell from dorsal area. Both drawings are of cells that were filled with Neurobiotin. Each is from a thick section. The pial and ventricular surfaces of the sections are shown with cross-hatching.

termed spikelets, are also seen frequently and may be dendritic spikes that propagate retrogradely into the somata of both D_L and D_M pyramidal cells (Millonas and Ulinski, 1997). Current injections can produce trains of action potentials that show a regular spiking pattern that is comparable to that seen in mammalian pyramidal cells (Figure 5a). The axons of pyramidal cells originate from their somata and extend into layer 3 where they bifurcate, one branch coursing medially into dorsomedial cortex and medial cortex and the other extending laterally into the lateral forebrain bundle (Connors and Kriegstein, 1986). These collaterals extend through the striatum and give secondary branches in the DLGN and optic tectum. In addition to these efferent projections, the primary branches of pyramidal cell axons give rise to a cone of collaterals that extends into layer 1. These collaterals form an ellipse-shaped field when viewed from the dorsal surface of the cortex, with the long axis of the ellipse extending along the isoazimuth lamellae (Cosans and Ulinski, 1990). The axonal field of individual pyramidal cells extends for approximately a third of the diameter of the dorsal cortex. Individual axons bear varicosities *en passant* and presumably synapse on other pyramidal cells as well as interneurons. Pyramidal cells are glutaminergic and their axons access both AMPA and *N*-methyl-D-aspartate (NMDA) receptors on other cortical neurons (Larson-Prior *et al.*, 1991). They mediate biphasic EPSPs that have both fast and slow components.

The remaining 20% of neurons in the dorsal cortex are nonpyramidal neurons that probably include both inhibitory and excitatory interneurons (Blanton *et al.*, 1987). Three groups of inhibitory interneurons are the best studied. The first of these are subpial cells (Figure 6a), which are completely embedded in the fascicle of geniculate afferents and consequently have somata positioned in the outer half of layer 1 (Colombe *et al.*, 2004). Their dendrites bear many distinct dendritic swellings or beads that appear to reduce the sensitivity of subpial cells to geniculate inputs. Like pyramidal cells, subpial cells show a regular spiking firing patterns following intrasomatic current injections. The axons of subpial cells arborize in the outer half of layer 1 and are presumably presynaptic to the apical dendrites of pyramidal cells and probably also synapse on other subpial cells. Subpial cells are GABAergic and appear to access both $GABA_A$ and $GABA_B$ receptors on their postsynaptic targets. The second major group of inhibitory neurons in D is stellate cells (Figure 6b), which have somata located in the inner half of layer 1 (Colombe *et al.*, 2004). They have dendrites that extend obliquely through layers 1 and 2 into layer 3. The dendritic arbors vary in size and individual dendrites are smooth or bear a small number of dendritic spines or appendages. They show relatively little adaptation in their firing rates following intrasomatic current injections and typically resemble the fast spiking firing patterns seen in many mammalian inhibitory interneurons (Figure 5b). Their axons ramify throughout all three layers of the dorsal cortex.

The third major group of inhibitory interneurons is horizontal cells, which have somata in layer 3 and dendrites that extend horizontally in layer 3 (Connors and Kriegstein, 1986). Like stellate cells, horizontal cells have a fast spiking firing pattern. Their axons appear to ramify in layers 3 and 2. Some horizontal cells are retrogradely labeled following injections of horseradish peroxidase into the thalamus, suggesting that they participate with pyramidal cells in forming the corticogeniculate projection system (Ulinski, 1986b). The anatomy and laminar positions of these three groups of neurons suggest that they have specific relationships to geniculate and intracortical projections. Subpial cells are literally embedded in geniculate afferents, suggesting that they play a dominant role in feedforward inhibition of pyramidal cells. Horizontal cells are embedded in the axons of pyramidal cells, suggesting they play a dominant role in intracortical feedback inhibition. Stellate cells are positioned to receive a mixture of geniculate and pyramidal cells inputs, raising the possibility that they are involved in both feedforward and feedback inhibition.

2.08.2.4 Responses of Visual Cortex Neurons to Visual Stimuli

The earliest physiological studies of turtle visual cortex used evoked potentials and flash stimuli to show that the cortical area now recognized as the dorsal cortex is responsive to visual stimuli (Orrego, 1961). Single-unit studies in alert turtles showed that neurons in this area have large, binocular receptive fields (Mazurskaya, 1974). The convergence of information from DLGN cells receiving inputs from all points along vertical meridians of visual space in the isoazimuth lamellae and the subsequent intracortical projections of pyramidal cells provide the anatomical substrate for the large receptive fields of neurons in the dorsal cortex. The cells respond robustly to small stimuli moving anywhere in binocular visual space or to two spots of light presented with temporal or spatial delays in an apparent motion paradigm (Mazurskaya, 1974). Since retinal ganglion cells in turtles frequently respond to stimuli moving with preferred directions or speeds in relatively small regions of visual space, they serve functionally as local motion detectors. Neurons in the dorsal cortex, thus, receive convergent inputs from many local motion detectors and serve as global motion detectors that can respond to large stimuli moving through visual space (Ulinski, 1999). Intracellular recordings from pyramidal and nonpyramidal neurons in the dorsal cortex can be efficiently carried out using an *in vitro* preparation of the turtle's eyes and brain (Kriegstsein, 1987). The cerebral hemisphere can be opened by a U-shaped incision that preserves the geniculate afferents that enter the cortex from its lateral end. The cortex is then unfolded, pinned flat in a recording chamber, and electrodes are inserted into the cortex from its ependymal surface. Intracellular recordings show that light flashes evoke combinations of EPSPs and inhibitory postsynaptic potentials (IPSPs) in neurons located in all three layers (Kriegstein, 1987; Mancilla *et al.*, 1998; Mancilla and Ulinski, 2001).

Recordings using multielectrode arrays in freely behaving turtles and in *in vitro* eye–brain preparations show that visual stimuli elicit waves of activity that propagate across the visual cortex. The spatiotemporal dynamics of the waves can be visualized using voltage-sensitive dyes (VSDs) and the eye–brain preparation (Prechtl, 1994, 1995; Senseman, 1996; Prechtl *et al.*, 1997, 2000; Senseman and Robbins, 1999). The waves appear to be triggered by the appearance of novel visual stimuli. VSD studies show that the waves always originate near the rostral pole of the dorsal cortex, regardless of the specific nature of the stimulus. Their propagation velocity depends on the nature of the stimulus, the light intensity, and the color of the light (Senseman, 1996). The waves are complex spatiotemporal events, but their properties can be analyzed quantitatively using a variation of a principal components method, the Karhunen–Loeve decomposition (Robbins and Senseman, 1998, 2004; Senseman and Robbins, 2002). This method can be used to represent individual waves in a low-dimensional phase space and to compare the dynamics of waves produced by different stimuli. Analysis of waves recorded in intact turtles, the eye–brain preparation, and a large-scale model of the dorsal cortex (Nenadic *et al.*, 2003) indicate that spots presented at different points in visual space or moving spots produce cortical waves with statistically different dynamics (Nenadic *et al.*, 2002; Du *et al.*, 2005). The dynamics of the wave can, consequently, be used to estimate the properties of visual stimuli, such as their positions in visual space or speed.

Analysis of waves using a large-scale model indicates that the waves are composed of three components (Wang *et al.*, 2005). The first is an initial depolarization that consists of a hill of depolarization that occurs near the rostral pole of the cortex and lasts approximately 200 ms after stimulus onset. In most cases, this depolarization triggers a primary propagating wave that moves from rostral to caudal across the cortex and reaches the caudal pole of the cortex after about 400 ms. In some cases,

a secondary propagating wave, or reflection, is generated at the caudal pole of the cortex as the primary wave begins declining. The secondary wave propagates from caudal to rostral across the cortex and typically subsides after about 800 ms. A long-lasting hyperpolarization can persist for more than 1.5 ms after stimulus presentation. Simulations of cortical waves in which the strengths of specific cortical synapses are systematically varied indicate that the initial depolarization is produced by the excitation of pyramidal cells in D_L by geniculate afferents and controlled by feedforward inhibition that is mediated by subpial cells (Wang *et al*., 2005). Consistent with this, intracellular recordings indicate that light flashes produce fast IPSPs in pyramidal cells that can regulate the amplitudes of geniculocortical EPSPs and the firing rate of pyramidal cells (Mancilla *et al*., 1998; Mancilla and Ulinski, 2001). Formation of the primary propagating wave is the result of recurrent excitation in the cortex that is mediated by pyramidal cell collaterals. Wave speed is regulated by the balance of excitation and by subpial cell-mediated inhibition. The duration of the primary and secondary waves is controlled by feedback inhibition mediated by horizontal cells.

2.08.3 Evolution of Visual Cortex

It is now well established that birds evolved from a group of archosaurian reptiles and that the four orders of living reptiles and birds are sister groups. Retinal projections to the optic tectum and to a dorsal thalamic nucleus are present in all of the species of reptiles and birds that have been studied, although the nomenclature of the dorsal thalamic retinorecipient nucleus varies from species to species (Ulinski and Margoliash, 1990). Tectal projections to nucleus rotundus and subsequent rotundal projections to the anterior dorsal ventricular ridge are also present in all reptiles and birds (Ulinski, 1983). This suggests that a retinorecipient structure in the dorsal thalamus and the tectofugal pathway are primitive, or symplesiomorphic, characters that were present in the stem reptiles and have been retained in all of the clades that evolved from these animals. The situation with regard to the thalamofugal pathway is less clear because telencephalic projections from the retinorecipient thalamic nucleus are quite variable and have not been carefully studied in many species. There are thalamic projections to a visual structure, the Wulst, that is situated on the anterior dorsal surface of the telencephalon in birds. The receptive field properties of neurons in the visual Wulst have been best studied in owls and shown to have many of the features associated with the receptive fields of neurons in the primary visual cortex of cats and monkeys such as a retinotopic map of visual space, orientation selectivity, and ocular dominance (Pettigrew and Konishi, 1976; Pettigrew, 1979). As reviewed above, the receptive field properties of neurons in turtle visual cortex are very different. There is no evidence for a retinotopic organization, all neurons have wide field receptive fields, and there is no indication of orientation selectivity. Thus, although the basic anatomy of a pathway from the retina, to a retinorecipient nucleus in the dorsal thalamus, and then to a dorsal telencephalic structure is a constant feature of reptiles, birds, and mammals, the functional organization of these telencephalic visual areas varies dramatically between species.

A reasonable working hypothesis is that visual cortex in turtles is involved in analyzing the movements of objects in visual space (Ulinski, 1999). Retinal ganglion cells in turtles tend to be directionally selective and can serve to analyze small objects or edges moving through restricted regions of visual space. However, naturally occurring visual stimuli usually have several edges with different orientations so that no individual retinal ganglion cell can adequately encode the motion of such a stimulus. Since neurons in DLGN and the dorsal cortex receive convergent inputs from many ganglion cells, they can integrate motion signals across both space and time and serve as global motion detectors. A similar set of transformations occurs in the motion pathways of primates, although it appears to take a longer sequence of transformations to reach essentially the same result. Retinal ganglion cells are typically not motion selective in the retinas of primates, but motion- and direction-selective neurons are found in the primary visual cortex and medial temporal areas of primates. A progressive increase in receptive field size occurs in successive structures in this pathway and neurons in extrastriate areas deep in the motion pathway have receptive fields that encompass all of visual space.

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2.09 The Evolution of Vocal Learning Systems in Birds

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Glossary

arcopallium Subdivision of the avian pallium located in the caudoventral telencephalon possessing descending projections to the brainstem.

mesopallium Large subdivision of the avian pallium dorsally adjacent to nidopallium.

nidopallium Large subdivision of the avian pallium dorsally adjacent to the striatum, receiving direct ascending sensory input of at least three modalities (auditory, visual, somatosensory).

pallium One of the two major subdivisions of the telencephalon, containing primarily glutamatergic projection neurons. In mammals, the vast majority of pallium consists of isocortex and hippocampus, but also includes the claustrum and parts of the amygdala. Evolutionary relationships between the pallial subdivisions of mammals and birds are still debated (see Evolution of the Amygdala in Vertebrates, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?).

parcellation Mechanism for evolving new structures in the central nervous system advocated by C. J. Herrick and S. O. E. Ebbesson. New structures are said to emerge as newly distinguished subdivisions within established structures. Once these new subdivisions are defined, they can restrict their connections to only a subset of the subregions that emerged from the original structure and acquire other derived characteristics.

2.09.1 Song: A Learned Behavior Used for Communication

Like many other vertebrates, birds in most orders of Aves make unlearned vocalizations that are used to communicate a variety of signals, e.g., begging or alarm calls (Kroodsma *et al.*, 1982). But three avian orders contain species capable of producing far more complex vocalizations that are learned through copying from other individuals. Such vocal learning is a rare trait shared only with cetaceans and humans (see Zeigler and Marler, 2004; and The Evolution of Vocal Learning Systems in Birds and Humans). The best-studied avian vocal learners are oscines (songbirds), constituting the great majority of species in order Passeriformes. Songbirds use their learned vocalizations, or songs, in courtship and territory defense, and perhaps for other poorly understood purposes. The capacity for learning, producing, and recognizing songs is imparted by a specialized set of telencephalic, diencephalic, and brainstem structures known collectively as the song system. The evolution of the oscine song system has long been a matter of interest to specialists, but this topic has recently attracted broader attention. Vocal learning in songbirds and humans shares a number of behavioral similarities (Doupe and Kuhl, 1999), and the recent realization that structures required for vocal learning in songbirds bear a close anatomical and physiological resemblance to the basal ganglia of mammals has raised the possibility that the similarities run deeper than mere behavioral analogies. Exactly how profound are the parallels between birdsong and human speech remains a matter of debate and active research, but it seems that there might at least be mechanistic similarities between song learning and the general processes of basal ganglia-dependent motor learning in mammals. Another factor drawing fresh attention to the evolution of the song system is the appreciation that surprisingly similar vocal control systems have appeared in three separate avian lineages. Conservation within Aves and across other vertebrate classes raises important questions about

how the song system evolved and how it is related to comparable systems in other taxa.

Behavioral studies in a variety of oscine species, carried out in field and laboratory settings, have uncovered a diverse array of song repertoires and learning abilities (Beecher and Brenowitz, 2005; Brenowitz and Beecher, 2005). Nonetheless, a consistent schematic pattern of song learning can be inferred (for review, see Williams, 2004). Songbirds learn their songs in two phases. During an initial sensory phase, a young bird memorizes the song(s) of a tutor bird. Later, the bird begins vocalizing in a sensorimotor phase where initially highly variable vocalizations become more refined over the course of several weeks into a highly stereotyped copy of the tutor song. Once the song is stereotyped, it is said to have crystallized. Normal hearing is critical for all phases of song learning, and is also essential for song maintenance in adult birds singing crystallized song. Although all oscine species studied to date conform to this general pattern, major variations occur in the degree to which both males and females sing, the number of songs learned, the degree of copying, and the degree of song stereotypy. It appears that a broad spectrum of behavioral features has evolved, which gives experimentalists a rich source of study material. Understanding both the conserved rules and the variations will be important in addressing how the neural structures underlying this complex behavior accomplish their tasks.

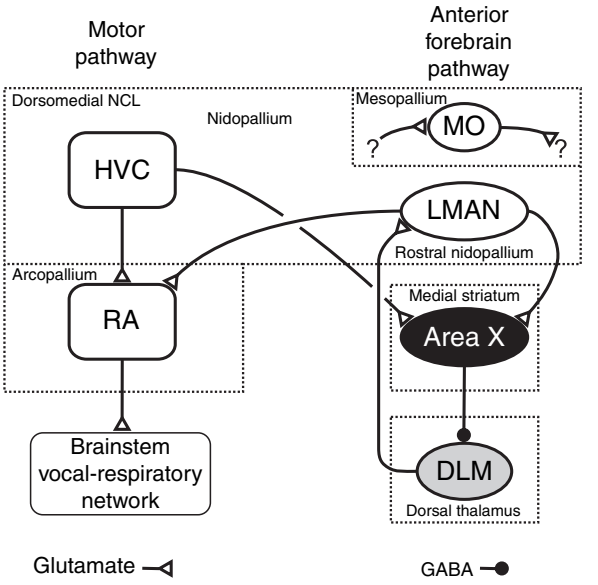

Figure 1 The oscine song system. This schematic shows the major components of the songbird vocal control system; abbreviations are defined in the text. For the sake of simplicity, some lesser known nuclei are omitted, including MMAN, DMP, NIf, and nucleus uvaeformis, a thalamic nucleus that projects to NIf and HVC. Within the forebrain, pallial (cortex-like) nuclei are white, subpallial (basal ganglia) nuclei are black, and thalamic nuclei are gray.

2.09.2 The Song System of Oscine Birds

The oscine song system consists of several discrete, interconnected brain nuclei that are devoted to song-related functions and are apparently absent in nonoscines (Farries, 2004). These nuclei and their connections were first reported in canaries (*Serinus canaria*), but have been most extensively studied in zebra finches (*Taeniopygia guttata*). The song system can be divided loosely into two major pathways (Figure 1). One pathway is a motor pathway that is essential for song production. This pathway is located in the arcopallium and caudal nidopallium, subdivisions of the avian telencephalon that appear functionally comparable to (but are not necessarily homologous to) mammalian isocortex. The motor pathway includes nucleus HVC (used as the proper name, not an abbreviation; see Reiner *et al.*, 2004b for nomenclature) of the caudal nidopallium, which receives strong auditory input from the interfacial nucleus of the nidopallium (NIf) and provides premotor output to the robust nucleus of the arcopallium (RA). RA, in turn, projects to several brainstem centers important for vocal control, including motor neurons in the tracheosyringeal portion of the hypoglossal nucleus (nXIIts) that control the syrinx, the avian vocal organ (Wild, 2004). RA also projects to a variety of respiratory premotor regions and to the dorsomedial nucleus of the intercollicular complex, which is involved in producing nonlearned vocalizations in all bird species studied to date.

HVC, in addition to sending projections to the motor pathway, also contains a separate population of neurons that project strongly to area X, a large nucleus located in the medial striatum. This connection initiates a circuit known as the anterior forebrain pathway (AFP). Area X projects to the medial part of the dorsolateral nucleus of the anterior thalamus (DLM), which then innervates the lateral magnocellular nucleus of the anterior nidopallium (LMAN). LMAN completes the AFP by projecting to RA, and collateral axons of the same neurons project back to area X. The AFP is not essential for song production *per se*, but is required for song learning and adult vocal plasticity. Lesions of LMAN or area X in juveniles dramatically disrupt learning, but lesions in adults do not alter normal song (Bottjer, 1984; Sohrabji *et al.*, 1990; Scharff and Nottebohm, 1991). In adults, however, the AFP does appear to play some role in active song maintenance, as lesions of LMAN prevent changes due to denervating the syrinx or deafening the

animal (Williams and Mehta, 1999; Brainard and Doupe, 2001). Since neurons in area X and LMAN exhibit auditory responses that are selective for the bird's own song (Doupe, 1997), one hypothesized role for the AFP is to compute some signal related to the degree of match between the bird's own song and his tutor's song and relay it to nucleus RA in the motor pathway, where it might influence behavioral plasticity. Three other nuclei in the oscine anterior forebrain should be considered a part of the song system, but whose functions are less well known. The medial magnocellular nucleus of the anterior nidopallium (MMAN) receives input from the posterior nucleus of the dorsomedial thalamus (DMP) and projects to HVC (Nottebohm *et al.*, 1982; Foster *et al.*, 1997). Finally, a nucleus just dorsal to LMAN, the oval nucleus of the mesopallium (MO), may also have song-related functions; it shows increased expression of the immediate early gene ZENK following bouts of singing (Jarvis *et al.*, 1998). However, the functions and anatomical connections of oscine MO are as yet unknown.

2.09.3 A Basal Ganglia Circuit Required for Song Learning

The AFP is of particular interest from an evolutionary point of view because it includes a specialized part of the oscine basal ganglia, area X. Since the basal ganglia have been implicated in some forms of learning in mammals (Graybiel, 1995), this raises the possibility that song learning in birds and motor learning in mammals employ related mechanisms. A brief glance at the AFP discloses some significant differences that undermine this idea. Although area X is part of the avian striatum, it projects directly to the thalamus (Bottjer *et al.*, 1989), while mammalian striatum communicates with the thalamus indirectly via the globus pallidus and substantia nigra, output stations of the basal ganglia (Parent and Hazrati, 1995). Furthermore, the projection neurons of area X are a sparsely distributed set of aspiny neurons (Bottjer *et al.*, 1989; Luo and Perkel, 1999), whereas the projection neurons of mammalian striatum are spiny neurons that comprise up to 95% of all striatal neurons (Gerfen, 1992). Nevertheless, a more thorough examination of area X uncovers a surprisingly long list of similarities. The vast majority of neurons in area X are spiny neurons that closely resemble their mammalian counterparts morphologically, histochemically, and physiologically (Farries and Perkel, 2002; Carrillo and Doupe, 2004; Reiner *et al.*, 2004a), even if they do not project out of the nucleus. All of the known interneuron types discovered in mammalian striatum are also present in area X (Farries and Perkel, 2002). As for the projection neurons, at least some (perhaps all) physiologically resemble neurons in the mammalian globus pallidus (Farries and Perkel, 2002; Farries *et al.*, 2005), suggesting that area X combines elements of the input (striatum) and output (globus pallidus) stations of the basal ganglia. Recent evidence suggests that area X may even contain functional equivalents of both major pathways through the mammalian basal ganglia (Farries *et al.*, 2005), the direct (having a net excitatory effect on the thalamus) and indirect (providing net inhibition to the thalamus). This curious pattern of conservation (or convergence?) and divergence raises new questions about the evolution of the basal ganglia, but also indicates that some highly specialized song-related circuitry has been built from common components that in their essentials remain unchanged after 300 million years of evolution.

Recently, parallels between birdsong and human speech received fresh interest when a possible molecular link between them surfaced. A familial speech and language disorder involving neuropathology of the cortex and basal ganglia results from mutations in the transcription factor FoxP2. A mutation in this gene also distinguishes humans, who are capable of vocal mimicry, from other primates, which are not. An intriguing hypothesis was that mutations in FoxP2 also distinguished bird taxa capable of vocal learning from those incapable, perhaps even enabling vocal learning in primates and birds. Two groups recently cloned FoxP2 from songbirds (Haesler *et al.*, 2004; Teramitsu *et al.*, 2004). Sequence analysis indicated that songbirds do not have the human-specific mutation. However, an intriguing fortuitous finding was that songbird FoxP1, a related member of the same gene family, is strongly expressed in several song nuclei. Since FoxP1 and FoxP2 can dimerize and influence transcription, it is possible that these proteins play some specific role in the development or function of the song system. On the other hand, given that these genes are relatively widely expressed, both within and outside the nervous system, it is also possible that they exert no specific function directly related to vocal learning, but rather a more general role in circuit development. Regionally and temporally specific manipulation of these genes will be needed to help determine any role these genes may play in vocal learning.

2.09.4 Vocal Control Circuits in Parrots and Hummingbirds

Oscines may be the most extensively studied vocal learners among birds, but they are not the only avian group that learns vocalizations. Parrots (order Psittaciformes) are famous for their vocal mimicry, with abilities ranging from the relatively modest (but still quite accomplished) budgerigar (Farabaugh and Dooling, 1996) to the African grey parrot capable of learning more than 70 English words (Pepperberg, 2002). Parrots also have an interconnected set of telencephalic vocal control centers bearing considerable resemblance to the oscine song system (Figure 2). Like songbirds, parrots have an arcopallial nucleus, the central nucleus of the anterior arcopallium (AAC), that directly innervates syringeal motor neurons and brainstem respiratory circuits (Paton *et al.*, 1981). This arcopallial nucleus receives input from a part of the nidopallium, the central nucleus of the lateral nidopallium (NLC), analogous to the oscine HVC (Paton *et al.*, 1981). Furthermore, parrots have a group of structures comparable to the songbird AFP, including a region of medial striatum containing sparsely distributed aspiny neurons that project directly to the dorsal thalamus (Striedter, 1994). The parrot AFP also incorporates an oval nucleus of the anterior MO which, unlike its oscine counterpart, has known connections to other vocal control nuclei (Striedter, 1994; Durand *et al.*, 1997). In addition to songbirds and parrots, at least some hummingbirds (family Trochilidae of order Apodiformes) appear capable of learning complex vocalizations (Baptista and Schuchmann, 1990). A study of the expression of the immediate early gene ZENK in hummingbirds after vocalization revealed a set of circumscribed telencephalic nuclei in locations similar to those of the vocal control nuclei of songbirds and parrots (Jarvis *et al.*, 2000). However, little is as yet known about the anatomical connections among these regions in hummingbirds.

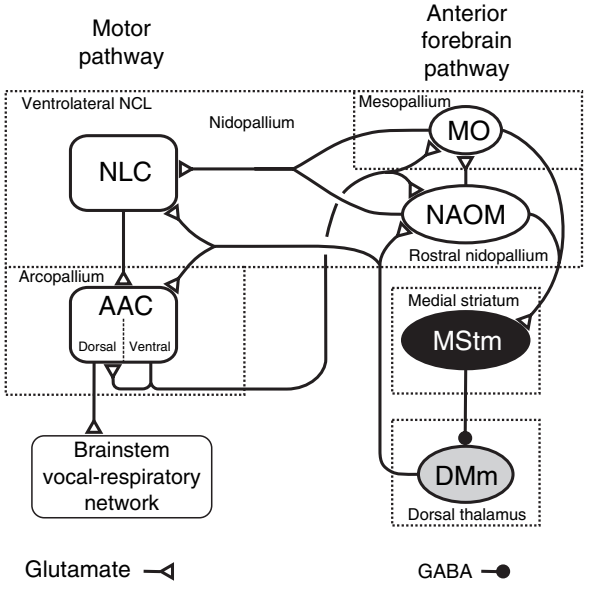

Figure 2 The psittacine vocal system. This schematic shows the known components of the parrot vocal control system. AAC is divided into distinct dorsal and ventral sectors; only the dorsal part has direct descending projections to the brainstem, while only the ventral part projects to NAOM and MO. Within the forebrain, pallial (cortex-like) nuclei are white, subpallial (basal ganglia) nuclei are black, and thalamic nuclei are gray. NAOM, medial part of the oval nucleus of the anterior nidopallium; MStm, magnocellular part of the medial striatum; DMm, magnocellular nucleus of the dorsomedial thalamus; other abbreviations are defined in the text.

2.09.5 Evolution of Avian Vocal Control Systems

The similarities between the vocal control systems of songbirds, parrots, and hummingbirds raise an obvious question: are these systems derived from an ancestral vocal control system that evolved just once somewhere in the lineage leading to these three taxa? At first glance, the similarities between these systems seem to demand an affirmative answer. Indeed, when components of the parrot vocal control system were first studied using modern tract-tracing techniques (Paton *et al.*, 1981), they were given names matching their oscine counterparts (i.e., NLC and AAC were originally known as HVC and RA, respectively), perhaps because the argument for homology seemed so compelling. However, songbirds, parrots, and hummingbirds are not closely related groups, and so the hypothesis of common ancestry for their vocal control systems implies that such systems were lost independently in the many other avian lineages descended from their last common ancestor – or that these other avian groups possess telencephalic vocal control systems in spite of the lack of evidence that they learn complex vocalizations. Furthermore, a closer look at the organization of the parrot vocal control system reveals several anatomical differences with the oscine song system (Striedter, 1994; Durand *et al.*, 1997), especially in the AFP (Figure 2). These two factors make the common ancestry hypothesis considerably less parsimonious than it first appears.

If we are to entertain the alternative hypothesis that the vocal control systems of songbirds, parrots, and hummingbirds evolved independently, we need to understand how these systems could nevertheless

be as similar as they are. To do that, we need to know something about the neural systems from which they might have evolved, found in birds that do not learn their vocalizations. Several anatomical studies conducted mainly in the pigeon (*Columba livia*) (Zeier and Karten, 1971; Kröner and Güntürkün, 1999) and domestic chick (*Gallus domesticus*) (Davies *et al.*, 1997; Metzger *et al.*, 1998), but also including the mallard (*Anas platyrhynchos*) (Dubbeldam *et al.*, 1997) and barn owl (*Tyto alba*) (Knudsen *et al.*, 1995), have elucidated a pathway in the caudal pallium that bears considerable resemblance to the motor pathway of the songbird (HVC and RA) and parrot (NLC and AAC) vocal control systems (hummingbirds appear to have comparable nuclei as well). This pathway (Figure 3) begins with a broad belt of caudolateral nidopallium (NCL) that projects topographically to a belt of intermediate arcopallium (AI). AI projects to many parts of the brainstem, including premotor neurons of the reticular formation, suggesting that at least parts of the NCL–AI system have motor functions (Wild *et al.*, 1985). However, AI does not directly innervate components of the brainstem vocal control circuitry in any avian species studied to date that does not learn complex vocalizations. NCL receives a variety of sensory inputs, including auditory input from field L1/3 confined mainly to the dorsomedial sector of NCL, and input from frontal trigeminal nidopallium (NFT) directed to the ventrolateral sector of NCL, potentially conveying both auditory and trigeminal somatosensory information (Metzger *et al.*, 1998; Kröner and Güntürkün, 1999). The NCL–AI system has received some attention in songbirds (Bottjer *et al.*, 2000), and the song system motor pathway appears embedded within, or at least lies immediately adjacent to, the dorsomedial segment of this system, i.e., the part receiving input from field L. The regions known in oscines as HVC shelf and RA cup are almost certainly homologous to this part of the NCL–AI system identified in pigeons and chicks (Vates *et al.*, 1996).

Given the location of HVC and RA relative to the oscine NCL–AI system, it is reasonable to suggest that this part of the song system emerged by parcellation of the pre-existing field L-recipient NCL–AI system (Farries, 2001). In this hypothesis, HVC and RA emerged as specialized subdomains of this system, acquiring some new connections (e.g., from RA to vocal–respiratory brainstem circuitry) and losing some old connections (e.g., from field L directly into HVC), but largely retaining the basic organization inherited from the NCL–AI system. It is less clear to what extent the AFP can also be regarded as a relatively simple alteration and specialization of a pre-existing system, but at least some of its connections have counterparts in the anterior telencephalon of pigeons and chicks (Kitt and Brauth, 1982; Wild, 1987; Veenman *et al.*, 1995; Montagnese *et al.*, 2003), including a projection from the dorsal thalamus to a region of anterior nidopallium (like the projection from DLM to LMAN) and a projection from that same nidopallial region to the medial striatum (like the projection from LMAN to area X). If the oscine song system inherited much of its organization from a larger system found in most or all birds, this could explain the similarities between the vocal control systems of songbirds, parrots, and hummingbirds – if all three

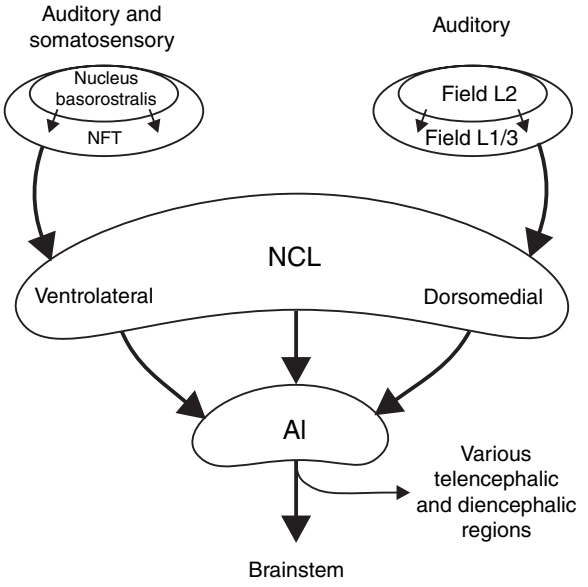

Figure 3 The NCL–AI pathway of avian caudal pallium. This schematic shows a pathway found in most or all birds that may be the evolutionary precursor to the vocal motor pathways of songbirds, parrots, and hummingbirds. NCL receives many sensory inputs, but only the ones involving auditory input are shown here. One major conduit for auditory information to the avian telencephalon passes through the thalamic nucleus ovoidalis, which projects to field L of the nidopallium, particularly to a subdivision called L2. Field L2 itself does not project widely in the telencephalon, but it does heavily innervate two other subdivisions of field L, L1, and L3, and they project to many areas, including the dorsomedial sector of NCL. A separate auditory pathway to the avian telencephalon arrives via an unusual direct projection from the intermediate nucleus of the lateral lemniscus in the brainstem to the nidopallial nucleus basorostralis, which also receives direct input from the principal trigeminal sensory nucleus. The nidopallium surrounding the nucleus basorostralis (NFT) relays this information to the ventrolateral sector of NCL. Songbird HVC is surrounded by dorsomedial NCL, whereas parrot NLC is surrounded by ventrolateral NCL. Auditory information also enters the NCL–AI pathway via direct projections from field L and NFT to AI. See Farries (2001) for a review of the primary literature describing this anatomy. Abbreviations are defined in the text.

systems are independent specializations of the same set of ancestral circuits (albeit without vocal functions), they would naturally share many characteristics inherited from those circuits.

This hypothesis predicts that the parrot and hummingbird vocal control systems are closely associated with or are embedded within the NCL–AI pathways of these taxa. At present, this is impossible to determine for hummingbirds due to a lack of anatomical data. In parrots, much less is known about the NCL–AI pathway than in songbirds, but regions of nidopallium have been identified that receive input from field L1/3 or NFT and that project to AI (Hall *et al*., 1993; Striedter, 1994). The parrot motor pathway nuclei are associated with this putative NCL–AI system, but not with the field L-recipient region as one might expect. Instead, the nidopallial tissue surrounding NLC receives input from NFT (and projects to arcopallial tissue adjacent to AAC) (Striedter, 1994). This part of the NCL–AI system probably also has access to auditory information, although via a different pathway, and thus may serve as an equally good base from which to evolve a system capable of vocal mimicry of an auditory model. The association of the parrot vocal control system with this putative NCL–AI system helps explain its similarities to the oscine song system, but at the same time its association with a different part of the NCL–AI system makes it extremely unlikely that these two vocal control systems could be homologous.

2.09.6 Conclusion

The vocal control systems of songbirds, parrots, and hummingbirds appear to be independently derived specializations of a common system found in most or all birds. This common system – the NCL–AI pathway and associated anterior forebrain circuitry – does not appear to play a significant role in vocal control outside of the three taxa that learn their vocalizations. Nevertheless, the avian vocal control systems appear to be relatively modest modifications of this basic plan. In spite of their striking specialization, avian vocal control systems are built from parts common to most birds, and to an extent from parts common to all amniotes. This is particularly true in the basal ganglia, where the oscine area X contains almost all of the basic components of the mammalian basal ganglia, with strikingly similar histochemistry, physiological properties, and functional organization. It is not unreasonable to hypothesize that the specialized basal ganglia domains of avian vocal learners share basic operating principles with the mammalian basal ganglia. In the specific case of human speech, one can imagine that convergent evolution makes the parallels even stronger because of the common requirements imposed by fundamentally similar tasks, although it is extremely unlikely that there is some deeper homology linking these instances of vocal learning. Study of the evolution of avian vocal control systems suggests that these specialized systems have much to tell us about general features of the amniote brain. Comparing vocal learning circuits in birds and humans also gives us at least two independent examples of evolution of a similar behavioral capacity from similar ancestral circuitry. Such comparisons could provide unique insights into whether these developments occurred through evolution by parcellation, and, if so, how this process yields new capabilities.

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2.10 The Evolution of Vocal Learning Systems in Birds and Humans

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Glossary

anterior vocal pathway Pathway in the anterior part of the forebrain that includes a pallial (cortical) region, striatal region, and thalamic region and that controls vocal learning and complex aspects of vocal production.

arcopallium Means arched pallium; a subdivision of the avian telencephalon with a border that is shaped like an arch along other pallial regions. It is a major output region of the telencephalon.

auditory learning The ability to make novel associations with sounds heard.

hyperpallium Means upper pallium; one of the dorsal-most subdivisions of the avian telencephalon. It has input, output, and intratelencephalic functions.

mesopallium Means middle pallium; a subdivision of the avian telencephalon that is located in between the nidopallium ventral to it and the hyperpallium dorsal to it. It is a major intratelencephalic region.

nidopallium Means nested pallium; a subdivision of the avian telencephalon that is shaped like a nest for the pallium regions dorsal to it and sits on top of the subpallium, or basal ganglia ventral to it. It has both input and intratelencephalic functions.

pallium Means mantle or covering. The pallium of vertebrates is the part of the embryonic brain that gives rise to all cortical regions in mammals and pallial named areas in birds.

posterior vocal pathway Pathway in the mid to posterior part of the forebrain, that includes pallial (cortical) regions, midbrain and medulla regions, and that controls production of learned vocalizations.

vocal learning The ability to modify acoustic and/or syntactic structure of sounds produced, including imitation and improvisation.

2.10.1 What is Vocal Learning

Vocal learning is the ability to modify acoustic and/or syntactic structure of sounds produced, including imitation and improvisation. It is distinct from auditory learning, which is the ability to make associations with sounds heard. Most, if not all, vertebrates are capable of auditory learning (see Organization and Correspondence of the Auditory Cortex of Humans and Nonhuman Primates, Shared Features of the Auditory System of Birds and Mammals), but few are capable of vocal learning. The latter has been found to date only in four distantly related groups of mammals (humans, bats, cetaceans, and recently elephants) and three distantly related groups of birds (parrots, hummingbirds, and songbirds) (Figure 1) (Nottebohm, 1972; Jarvis *et al.*, 2000; Poole *et al.*, 2005). Vocal learning is the behavioral substrate for spoken human language. An example helps in understanding the distinction between vocal learning and auditory learning. A dog can learn the meaning of the human words 'sit' (in English), 'sientese' (in Spanish), or 'osuwali' (in Japanese) or of a sentence ('come here boy'). Dogs are not born with this knowledge of human words or syntax. They acquire it through auditory learning. However, a dog cannot imitate the sounds 'sit', 'sientese', or 'osuwali'. A human, parrots, and some songbirds can. This is vocal learning, and though it depends upon auditory learning (Konishi, 1965), it is distinct from it.

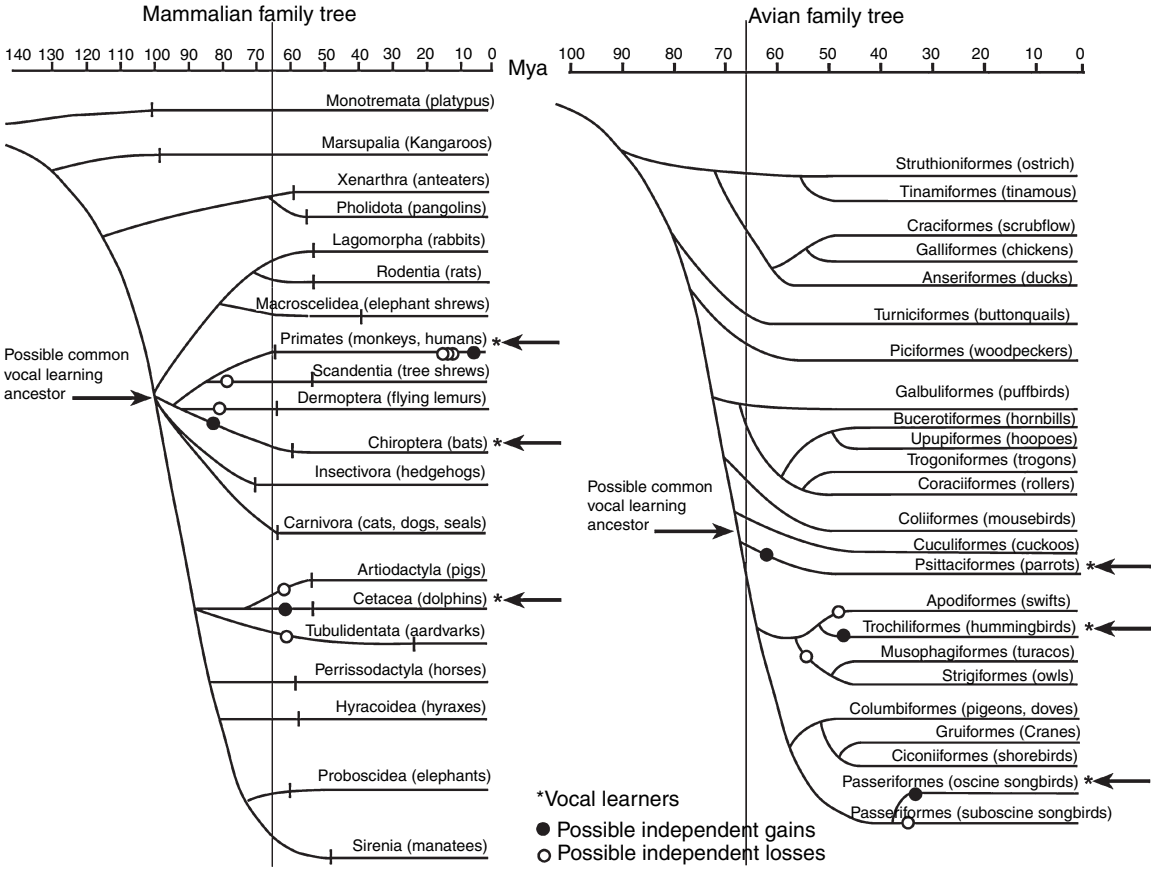

Figure 1 Family trees of living mammalian and avian orders. The mammalian tree is derived from the morphological analysis by Novacek (1992, 2001); horizontal lines indicate extant of geologic evidence from fossils. The avian tree was derived from DNA–DNA hybridization analysis by Sibley and Ahlquist (1990, p. 838). The Latin name of each order is given along with examples of common species. Passeriformes are divided into its two suborders, suboscine and oscine songbirds. The vertical line down the trees indicates the cretaceous–tertiary boundary; Mya = millions of years ago. Open and closed circles show the minimal ancestral nodes where vocal learning could have either evolved independently or been lost independently. Independent losses would have at least required one common vocal learning ancestor, located by the right facing arrows. Within primates, there would have to be least seven independent losses (tree shrews, prosimians, New and Old World monkeys, apes, and chimps) followed by the regain of vocal learning in humans (assuming that all nonhuman primates are vocal nonlearners). The trees are not meant to present the final dogma of mammalian and avian evolution, as there are many differences of opinion among scientists. Reproduced from Jarvis, E. D. 2004. Learned birdsong and the neurobiology of human language. *Ann. NY Acad. Sci.* 1016, 749–777, with permission.

Most vocal learners only imitate sounds of their own species, and not all vocal learning species have vocal abilities to the same degree. Humans are the most prolific vocal learners, as they learn to produce a seemingly infinite number of combinations of learned vocalizations. Not as prolific are some parrots, corvid songbirds, starlings, and mockingbirds, where they produce hundreds if not thousands of calls and/or learned warble/song combinations. Finally, less prolific are very stereotyped songbirds and hummingbirds, where they produce only one distinct song type with little variation (Catchpole and Slater, 1995; Farabaugh and Dooling, 1996; Ferreira *et al.*, 2006). Each of the vocal learning avian and mammalian groups has close vocal nonlearning relatives (Figure 1). Thus, it has been argued that vocal learning has evolved independently of a common ancestor in the three vocal learning bird groups (Nottebohm, 1972) and presumably in the four vocal learning mammalian groups (Jarvis, 2004). The question thus arises, is there something special about the brains of these animals that can imitate sounds.

2.10.2 Consensus Brain Systems of Vocal Learners

There is something special about the brain systems of vocal learners. Only vocal learners, songbirds, parrots, hummingbirds, and humans, have brain regions in their cerebrums (or telencephalon) that control vocal behavior (Jurgens, 2002; Jarvis *et al.*, 2000; see The Evolution of Vocal Learning Systems in Birds). Vocal control brain regions have not yet

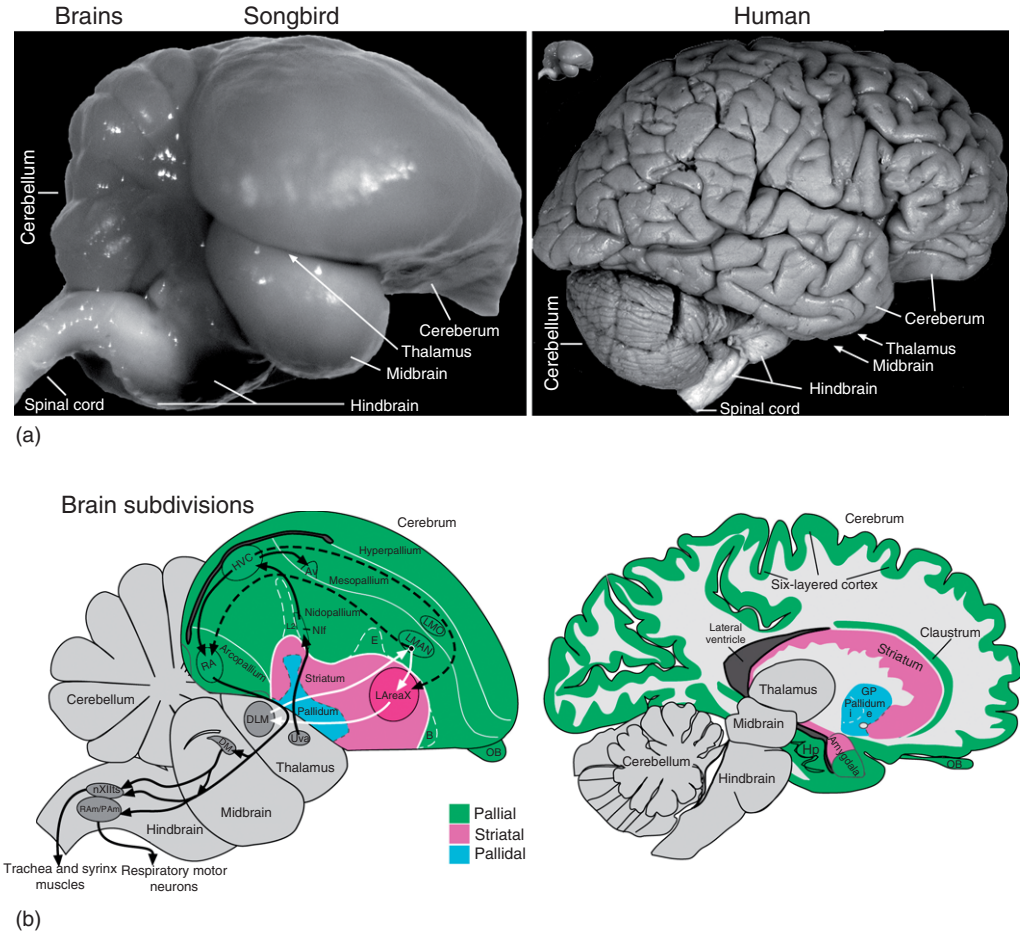

Figure 2 Avian and mammalian brain relationships. a, Side view of a songbird (zebra finch) and human brain to represent avian and mammalian species. In this view, the songbird cerebrum covers the thalamus and the human cerebrum covers the thalamus and midbrain. Inset (left) next to the human brain is the zebra finch brain to the same scale. b, Sagittal view of brain subdivisions according to the modern understanding of avian and mammalian brain relationships (Reiner *et al.*, 2004b; Jarvis *et al.*, 2005). Solid white lines are lamina (cell-sparse zones separating brain subdivisions). Large white areas in the human cerebrum are axon pathways called white matter. Dashed white lines separate primary sensory neuron populations from adjacent regions. Abbreviations in Table 1. Human brain image in (a), reproduced from, courtesy of John W. Sundsten, Digital Anatomist Project, Dept. of Biological Structure, University of Washington, with permission. b, Reprinted by permission from Macmillan Publishers Ltd: *Nat. Rev. Neurosci.* (Jarvis, E. D., Gunturkun, O., Bruce, L., *et al.* 2005. Avian brains and a new understanding of vertebrate brain evolution. *Nat. Rev. Neurosci.* 6, 151–159.), copyright (2005).

been investigated in cetaceans, bats, and elephants. Nonvocal learners, including nonhuman primates and chickens, only have midbrain and medulla regions that control innate vocalizations (Wild, 1997). Using this knowledge, it has been possible to generate a consensus vocal pathway, much like generating a consensus DNA sequence from comparing comparable genes of different species, by comparing vocal brain regions of different vocal learning and vocal nonlearning species. This comparison is facilitated by a recent revision to the nomenclature and understanding of avian brain organization relative to mammals and other vertebrates (Reiner *et al.*, 2004b; Jarvis *et al.*, 2005). Like mammals, birds have pallidal, striatal, and pallial subdivisions in their cerebrums. However, the pallial subdivision in mammals is layered in its cellular organization, whereas in birds it is nuclear (Figure 2). Even with this major difference, connectivity and other functional properties are similar. Below, the consensus brain regions, connectivity, and function studies of vocal learning species are described.

2.10.2.1 Brain Regions and Connectivity

Remarkably, all three vocal learning bird groups have seven comparable cerebral vocal brain nuclei: four posterior forebrain nuclei and three anterior forebrain nuclei (Figures 3a–3c; abbreviations in Table 1) (Jarvis *et al.*, 2000). These brain nuclei have been given different names in each bird group because of the possibility that each evolved their

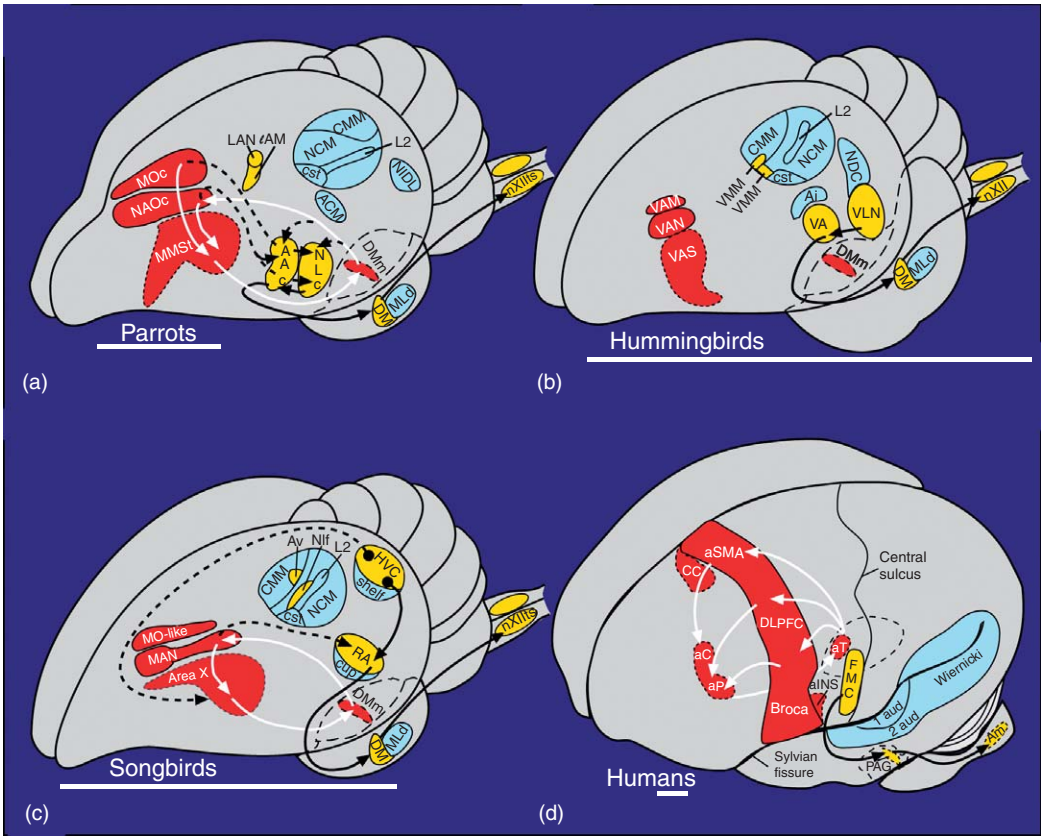

Figure 3 Proposed comparable vocal and auditory brain areas among vocal learning birds (a–c) and humans (d). Left hemispheres are shown, as this is the dominant side for language in humans and for song in some songbirds. Yellow regions and black arrows indicate proposed posterior vocal pathways; red regions and white arrows indicate proposed anterior vocal pathways; dashed lines indicate connections between the two vocal pathways; blue indicates auditory regions. For simplification, not all connections are shown. The globus pallidus in the human brain, also not shown, is presumably part of the anterior pathway as in nonvocal pathways of mammals. Basal ganglia, thalamic, and midbrain (for the human brain) regions are drawn with dashed-line boundaries to indicate that they are deeper in the brain relative to the anatomical structures above them. The anatomical boundaries drawn for the proposed human brain regions involved in vocal and auditory processing should be interpreted conservatively and for heuristic purposes only. Human brain lesions and brain imaging studies do not allow one to determine functional anatomical boundaries with high resolution. Scale bar: ~7 mm. Abbreviations are in Table 1. Modified from Jarvis, E. D. 2004. Learned birdsong and the neurobiology of human language. *Ann. NY Acad. Sci.* 1016, 749–777, with permission.

vocal nuclei independently of a common ancestor with such nuclei (Striedter, 1994; Jarvis *et al.*, 2000). In all three bird groups, the posterior nuclei form a posterior vocal pathway that projects from a nidopallial vocal nucleus (HVC, NLC, VLN) to the arcopallial vocal nucleus (RA, AAC dorsal part, VA), to the midbrain (DM) and medulla (nXIIts) vocal motor neurons (Figures 3a–3c, black arrows) (Striedter, 1994; Durand *et al.*, 1997; Vates *et al.*, 1997; Gahr, 2000); nXIIts projects to the muscles of the syrinx, the avian vocal organ. Vocal nonlearning birds have DM and nXIIts for production of innate vocalizations, but they appear not to have projections to these nuclei from the arcopallium (Wild *et al.*, 1997). The anterior nuclei (connectivity examined only in songbirds and parrots) are part of an anterior vocal pathway loop, where a pallial vocal nucleus (MAN, NAO) projects to the striatal vocal nucleus (area X, MMSt), the striatal vocal nucleus to a nucleus in DLM of the dorsal thalamus (DLM, DMM), and the dorsal thalamus back to the pallial vocal nucleus (MAN, NAO) (Figures 3a and 3c, white arrows) (Durand *et al.*, 1997; Vates *et al.*, 1997). The parrot pallial MO vocal nucleus also projects to the striatal vocal nucleus (MMSt) (Durand *et al.*, 1997). Connectivity of the songbird MO analogue has not yet been determined.

The major differences among vocal learning birds are in the connections between the posterior and anterior vocal pathways (Jarvis and Mello, 2000). In songbirds, the posterior pathway sends input to the anterior pathway via HVC to area X; the anterior pathway sends output to the posterior pathway via lateral MAN (LMAN) to RA and medial MAN (mMAN) to HVC (Figures 3c and 4a). In contrast,

Table 1 Some of the abbreviations used in this article

AAC	Central nucleus of the anterior arcopallium
AACd	Central nucleus of the anterior arcopallium, dorsal part
AACv	Central nucleus of the anterior arcopallium, ventral part
aCC	Anterior cingulate cortex
aCd	Anterior caudate
ACM	Caudal medial arcopallium
aDLPFC	Anterior dorsal lateral prefrontal cortex
Ai	Intermediate arcopallium
aINS	Anterior insula cortex
Am	Nucleus ambiguous
aP	Anterior putamen
area X	Area X of the striatum
aST	Anterior striatum
aT	Anterior thalamus
Av	Avalanch
CM	Caudal mesopallium
CSt	Caudal striatum
DLM	Medial nucleus of dorsolateral thalamus
DM	Dorsal medial nucleus of the midbrain
DMM	Magnocellular nucleus of the dorsomedial thalamus
FMC	Face motor cortex
HVC	A letter based name
L2	Field L2
MAN	Magnocellular nucleus of anterior nidopallium
MLd	Mesencephalic lateral dorsal nucleus
MMSt	Magnocellular nucleus of the anterior striatum
MOc	Oval nucleus of the mesopallium complex
NAOc	Oval nucleus of the anterior nidopallium complex
NCM	Caudal medial nidopallium
NDC	Caudal dorsal nidopallium
NIDL	Intermediate dorsal lateral nidopallium
NIf	Interfacial nucleus of the nidopallium
NLC	Central nucleus of the lateral nidopallium
nXIIts	Tracheosyringeal subdivision of the hypoglossal nucleus
Ov	Nucleus oviodalis
PAG	Periaqueductal grey
pre-SMA	Presupplementary motor area
RA	Robust nucleus of the arcopallium
St	Striatum
Uva	Nucleus uvaeformis
VA/VL	Ventral anterior/ventral lateral nuclei of the mammalian thalamus.
VAM	Vocal nucleus of the anterior mesopallium
VAN	Vocal nucleus of the anterior nidopallium
VAS	Vocal nucleus of the anterior striatum
VA	Vocal nucleus of the arcopallium
VLN	Vocal nucleus of the lateral nidopallium
VMM	Vocal nucleus of the medial mesopallium
VMN	Vocal nucleus of the medial nidopallium

in parrots, the posterior pathway sends input into the anterior pathway via ventral AAC (AACv, parallel of songbird RA) to NAO (parallel of songbird MAN) and MO; the anterior pathway sends output to the posterior pathway via NAO to NLC (parallel of songbird HVC) and AAC (Figures 3a and 4b) (Durand *et al.*, 1997).

In humans, imaging and lesion studies have revealed they have cortical and striatal regions that are active and necessary for learning and production of language (reviewed in Jarvis, 2004). These include what Jarvis has proposed is a lateral-to-medial strip of premotor cortex – from the anterior insula (aINS), the Brocas area, the anterior dorsal lateral prefrontal cortex (aDLPFC), the presupplementary motor area (pre-SMA), and the anterior cingulate (aCC) – below this level of cortex – an anterior region of the striatum and a posterior region of cortex – the face motor cortex (Figure 3d), as well as anterior parts of the thalamus. To date, these areas have not been found to be required for vocal behavior in vocal nonlearning mammals, including nonhuman primates. However, the anterior cingulate is required for voluntary control of when to vocalize, but not of the acoustic structure of vocalizations in vocal nonlearning mammals (Jurgens, 2002).

Ethical and practical issues prevent connectivity tract-tracing experiments in humans and thus the connectivity of vocal learning pathways is not known for any mammal. However, studies have been performed on the cerebrums of nonvocal learning mammals. Therefore, it is possible to make connectivity comparisons between vocal learning pathways in vocal learning birds with nonvocal pathways in vocal nonlearning mammals. In this regard, the avian posterior vocal pathways are similar in connectivity to mammalian motor corticospinal pathways (Figure 4). Specifically, the projecting neurons of songbird RA and parrot AACd are similar to pyramidal tract (PT) neurons of lower layer 5 of mammalian motor cortex (Matsumura and Kubota, 1979; Glickstein *et al.*, 1985; Karten and Shimizu, 1989; Keizer and Kuypers, 1989; Reiner *et al.*, 2003). The latter send long axonal projections out of the cerebrum through pyramidal tracts to synapse onto brainstem and spinal cord premotor or α-motor neurons that control muscle contraction and relaxation. The projection neurons of parrot NLC and the RA-projecting neurons of songbird HVC are similar to layer 2 and 3 neurons of mammalian cortex, which send intrapallial projections to layer 5 (Figure 4) (Aroniadou and Keller, 1993; Capaday *et al.*, 1998). Mammalian parallels to songbird NIf and Av are less clear.

The only connectivity determined among cerebral vocal brain areas of humans (Kuypers, 1958a) is the finding of face motor cortex projection to nucleus ambiguous (Am) of the medulla; Am projects to the muscles of the larynx, the main mammalian vocal organ (Zhang *et al.*, 1995; Jurgens, 1998) and is thus the mammalian parallel of avian nXIIts. This connectivity from face motor cortex in humans was

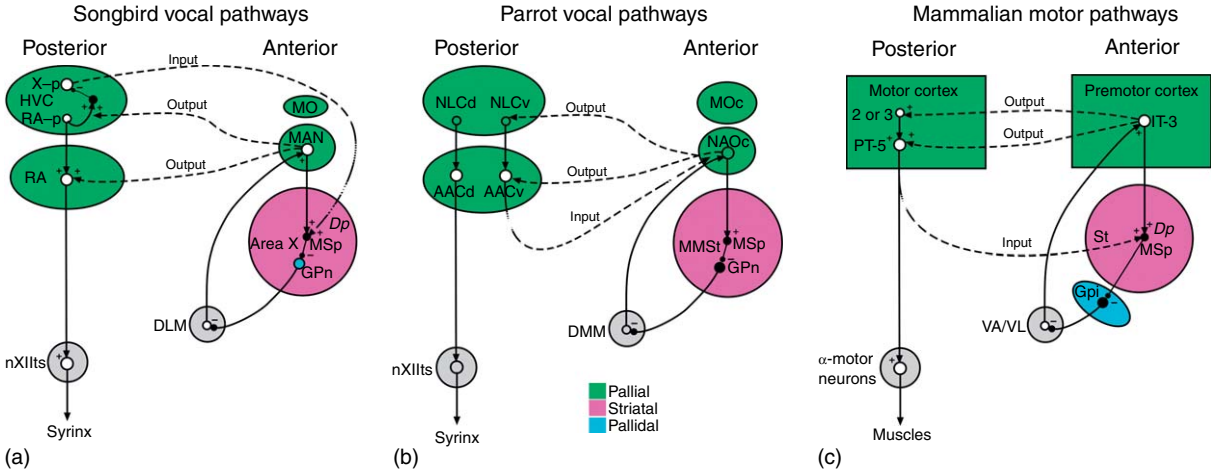

Figure 4 Comparative and simplified connectivity of posterior and anterior vocal motor pathways in (a) songbirds and (b) parrots and motor pathways in (c) mammals. Dashed lines: connections between anterior and posterior pathways; inputs and outputs are labeled relative to anterior pathways. Output from songbird MAN to HVC and RA are not from the same neurons; medial MAN (mMAN) neurons project to HVC, lateral MAN (LMAN) neurons project to RA. ○: excitatory neurons; •: inhibitory neurons; +: excitatory glutamate neurotransmitter release; −: inhibitory GABA release. MSp, medium spiny neuron; GPn, globus pallidus-like neuron in songbird area X and parrot MMSt. Only the direct pathway through the mammalian basal ganglia (St to GPi) is shown, as this is the one most similar to area X connectivity (MSp to GPn) (Reiner *et al.*, 2004a). X-p, X-projecting neuron of HVC; RA-p, RA-projecting neuron of HVC; PT-5, pyramidal tract neuron of motor cortex layer 5; IT-3, intratelencephalic projecting neuron of layer 3. Connections that need validation for this model to be correct are whether collaterals of the same neurons of mMAN project to mAreaX and to HVC, as opposed to different neurons, whether input from HVC into area X is onto the area X MSp neurons, whether the microcircuitry in parrot MMSt is the same as in songbirds, whether the collaterals of single IT-3 neurons of mammal cortex send branches to both layers 3 and 5 of motor cortex or just to one layer per IT-3 neuron. Abbreviations are in Table 1. Modified from Jarvis, E. D. 2004. Learned birdsong and the neurobiology of human language. *Ann. NY Acad. Sci.* 1016, 749–777, with permission.

determined using silver staining of degenerated axons in patients who had had vascular strokes to brain areas that included but were not limited to face motor cortex (Kuypers, 1958a). Kuypers (1958b) reproduced similar lesions in macaque monkeys and chimpanzees and found that their face motor cortex projects minimally, if at all, to Am, but it does project massively to the hypoglossal nucleus and to all other brainstem cranial motor nuclei as found in humans. The hypoglossal nucleus in mammals and the nontracheosyringeal part of nXII in birds controls muscles of the tongue (Wild, 1997). In this manner, the pallial nuclei of the songbird and parrot posterior vocal pathways combined are more similar to the human face motor cortex than to any other part of the human pallium.

The avian anterior vocal pathways are similar in connectivity to mammalian corticobasal ganglia-thalamic-cortical loops (Figure 4) (Bottjer and Johnson, 1997; Durand *et al.*, 1997; Jarvis *et al.*, 1998; Perkel and Farries, 2000). Specifically, the projection neurons of songbird MAN and parrot NAO (Vates and Nottebohm, 1995; Foster *et al.*, 1997; Durand *et al.*, 1997) are similar to intratelencephalic (IT) neurons of layer 3 and upper layer 5 of mammalian premotor cortex, which send two collateral projections, one to medium spiny neurons of the striatum ventral to it and the other to other cortical regions, including motor cortex (Figure 4) (Avendano *et al.*, 1992; Reiner *et al.*, 2003). Unlike mammals, the spiny neurons in both songbird area X, and presumably parrot MMSt, project to pallidal-like cells within area X and MMSt instead of to a separate structure consisting only of pallidal cells (Durand *et al.*, 1997; Perkel and Farries, 2000; Reiner *et al.*, 2004a). This striatal–pallidal cell intermingling may be a general trait of the anterior avian striatum (Farries *et al.*, 2005). The projection of the pallidal-like cells of songbird area X and parrot MMSt are similar to the motor pallidal projection neurons of the internal globus pallidus (GPi) of mammals, which project to the ventral lateral (VL) and ventral anterior (VA) nuclei of the dorsal thalamus (Figure 4) (Alexander *et al.*, 1986). Like songbird DLM and parrot DMM projections to LMAN and NAO, mammalian VL/VA projects back to layer 3 neurons of the same premotor areas, closing parallel loops (Jacobson and Trojanowski, 1975; Alexander *et al.*, 1986; Luo *et al.*, 2001).

Because connections between the posterior and anterior vocal pathways differ between songbirds and parrots, comparisons between them and mammals will also differ. In mammals, the PT-layer 5 neurons of motor cortex have axon collaterals, where one projects into the striatum and the other

projects to the medulla and spinal cord (Figure 4c) (Alexander and Crutcher, 1990; Reiner *et al.*, 2003). This is different from the songbird where a specific cell type of HVC, called X-projecting neuron, projects to the striatum separately from neurons of RA of the arcopallium that projects to the medulla. This is also different from the parrot, where AAC of the arcopallium has two anatomically separate neuron populations, AACd that projects to the medulla and AACv that projects to anterior pallial vocal nuclei NAO and MO (Durand *et al.*, 1997). Output of mammalian anterior pathways are proposed to be the collaterals of the IT–layer 3 and IT–upper layer 5 neurons that project to other cortical regions (Figure 4c) (Reiner *et al.*, 2003; Jarvis, 2004).

Taken together, the above analysis suggests that there are gross similarities between the connectivity of the consensus bird brain system for learned vocalizing and the nonvocal motor pathways (a posterior-like pathway) and cortical-basal-ganglia-thalamic-cortical loops (an anterior-like pathway) of mammals (Figures 4a–4c). Differences in connectivity between birds and mammals appear to be in the details, particularly with the pallidal cell types within the avian striatum and with connectivity between posterior and anterior pathways. Functions of these brain regions are now compared from lesion studies.

2.10.2.2 Brain Lesions

There are some gross similarities in behavioral deficits following lesions in specific brain areas of vocal learning birds (experimentally placed) and of humans (due to stroke or trauma). Lesions to songbird HVC and RA (Nottebohm *et al.*, 1976; Simpson and Vicario, 1990), on the left side in canaries, cause deficits similar to those found after damage to left human face motor cortex, this being muteness for learned vocalizations, i.e., for speech (Valenstein, 1975; Jurgens *et al.*, 1982; Jurgens, 2002). Innate sounds, such as crying and screaming, can still be produced. When the lesions are unilateral, both birds and patients often recover some vocal behavior, because the opposite hemisphere appears to take over some function; likewise, recovery is better when the canary is a juvenile or the patient a child (Nottebohm, 1977; Rey *et al.*, 1988; Hertz-Pannier *et al.*, 2002). Lesions to parrot NLC cause deficits in producing the correct acoustic structure of learned vocalizations, particularly for learned speech (Lavenex, 2000). The symptoms are similar to that of dysarthria in humans after recovery from damage to the face motor cortex. Lesions to the face motor cortex in chimpanzees and other nonhuman primates do not affect their ability to produce vocalizations (Kuypers, 1958b; Jurgens *et al.*, 1982; Kirzinger and Jurgens, 1982). Lesions to avian nXIIts and DM and mammalian Am and PAG result in muteness in both vocal learners and nonlearners (Brown, 1965; Seller, 1981; Nottebohm *et al.*, 1976; Jurgens, 1994, 1998; Esposito *et al.*, 1999). One difference is that lesions to songbird NIf or parrot LAN of the posterior pathway do not prevent production of learned vocalizations or cause dysarthric-like vocalizations, but lead to production of more varied syntax or impaired vocal imitation (Hosino and Okanoya, 2000; Plummer and Striedter, 2002).

Lesions to songbird MAN (Nottebohm *et al.*, 1990; Scharff and Nottebohm, 1991; Foster and Bottjer, 2001) cause deficits that are most similar to those found after damage to anterior parts of the human premotor cortex, this being disruption of imitation and/or inducing sequencing problems. In birds and humans, such lesions do not prevent the ability to produce learned song or speech. In humans, these deficits are called verbal aphasias and verbal amusias (Benson and Ardila, 1996). Damage to the left side often leads to verbal aphasias, whereas damage to the right can lead to verbal amusias (Berman, 1981). The deficits in humans, however, are more complex. Specifically, lesions to songbird LMAN (Bottjer *et al.*, 1984; Scharff and Nottebohm, 1991; Kao *et al.*, 2005) and lesions to the human insula and the Brocas area (Mohr, 1976; Dronkers, 1996; Benson and Ardila, 1996) lead to poor imitation with sparing or even inducing more stereotyped song or speech. However, in addition, lesions to the Brocas area and/or DLPFC (Benson and Ardila, 1996) lead to poor syntax production in construction of phonemes into words and words into sentences. Lesions to DLPFC also result in uncontrolled echolalia imitation, whereas lesions to pre-SMA and anterior cingulate result in spontaneous speech arrest, lack of spontaneous speech, and/or loss of emotional tone in speech, but with imitation preserved (Nielsen and Jacobs, 1951; Barris *et al.*, 1953; Rubens, 1975; Valenstein, 1975; Jonas, 1981). Lesions to songbird mMAN lead to a decreased ability in vocal learning and some disruption of syntax (Foster and Bottjer, 2001), as do lesions to the Brocas area.

Lesions to songbird area X and to the human anterior striatum does not prevent the ability to produce song or speech, but does result in disruption of vocal learning and disruption of some syntax in birds (Scharff and Nottebohm, 1991; Sohrabji *et al.*, 1990; Kobayashi *et al.*, 2001) or verbal aphasias and amusias in humans (Mohr, 1976; Bechtereva

et al., 1979; Leicester, 1980; Damasio *et al.*, 1982; Alexander *et al.*, 1987; Speedie *et al.*, 1993; Cummings, 1993; Lieberman, 2000). Specifically, songbirds do not crystallize onto correct syllable structure and syntax heard, and as adults they can stutter (Scharff and Nottebohm, 1991; Sohrabji *et al.*, 1990; Kobayashi *et al.*, 2001). Humans can have a combination of symptoms (Mohr, 1976) perhaps because, as in nonhuman mammals, large cortical areas send projections that converge onto relatively smaller striatal areas (Beiser *et al.*, 1997). Not many cases have been reported of lesions to the human globus pallidus leading to aphasias (Strub, 1989), but the fact that this can occur suggests some link with a striatal vocal area in humans.

Similar to a preliminary report on songbird DLM (Halsema and Bottjer, 1991), damage to anterior portions of the human thalamus (VA, VL, and A) leads to verbal aphasias (Graff-Radford *et al.*, 1985). In humans, thalamic lesions can lead to temporary muteness followed by aphasia deficits that are sometimes greater than after lesions to the anterior striatum or premotor cortical areas. This greater deficit may occur perhaps because there is further convergence of inputs from the striatum into the thalamus (Beiser *et al.*, 1997). However, the interpretation of thalamic lesions in humans is controversial (Benson and Ardila, 1996), perhaps because of small but important differences in lesion locations between patients among studies. The thalamus concentrates many functions into adjacent small nuclei, and thus, a relatively small variance in the location of a lesion may lead to a large difference in the brain function affected.

The lesions in birds and in humans can affect more than one modality. For example, lesions to LMAN or HVC in songbirds (Scharff *et al.*, 1998; Burt *et al.*, 2000) and to the Brocas area and anterior striatum in humans (Freedman *et al.*, 1984; Benson and Ardila, 1996) lead to decreased abilities in song/speech perception and discrimination. The perceptual deficits, however, are usually not as great as the motor deficits.

Taken together, the above evidence is consistent with the presence in humans of a posterior-like vocal motor pathway and an anterior-like vocal premotor pathway that are similar to the production and learning pathways of vocal learning birds. The relative locations of the brain regions in humans appear to be comparable to the relative location of the pathways in birds. The clearest difference between birds and humans appears to be the greater complexity of the deficits found after lesions in humans. Function of brain regions from activation studies is now compared.

2.10.2.3 Brain Activation

Brain activation includes changes in electrophysiological activity (recorded in both birds and in humans during surgery of patients), electrical stimulation (birds and humans), motor- and sensory-driven gene expression (birds and nonhuman mammals), and PET and magnetic resonance imaging of activated brain regions (in humans). In vocal learning birds, such studies have revealed that all seven comparable cerebral nuclei display vocalizing-driven expression of immediate early genes (Figure 5) (Jarvis and Nottebohm, 1997; Jarvis *et al.*, 1998, 2000 Jarvis and Mello, 2000); these are genes that are responsive to changes in neural activity. In deafened songbirds, these nuclei still display vocalizing-driven expression (Jarvis and Nottebohm, 1997), indicating that motor-driven gene activation is independent of hearing. Likewise, premotor neural firing has been found in HVC, RA, NIf, LAreaX, and LMAN when a bird sings (McCasland, 1987; Yu and Margoliash, 1996; Hessler and Doupe, 1999a; Hahnloser *et al.*, 2002). In deafened birds, similar singing-associated activity still occurs when a bird sings, at least for LMAN (Hessler and Doupe, 1999a). The firing in HVC and RA correlates with sequencing of syllables and syllable structure, respectively, whereas firing in LAreaX and LMAN is much more varied and in LMAN it correlates with song variability. In addition, neural firing and gene expression in LAreaX, LMAN, as well as RA differ depending upon the social context in which singing occurs (Jarvis *et al.*, 1998; Hessler and Doupe, 1999b); they are moderate when a bird sings directly facing another bird and high when a bird sings in an undirected manner. No difference has been observed between right (the dominant) and left HVC activity during singing in zebra finches, but in song sparrows activity in the left and right HVC is associated with production of specific sequences of song syllables (Nealen and Schmidt, 2002). Stimulation with electrical pulses to HVC during singing temporarily disrupts song output, i.e., song arrest (Vu *et al.*, 1998).

In humans, the brain area most comparable to songbird HVC and RA is one that is always activated (as measured with PET and fMRI) with all speech tasks: the face motor cortex (Figure 5) (Petersen *et al.*, 1988; Rosen *et al.*, 2000; Gracco *et al.*, 2005). Similar to other songbird vocal nuclei, other human vocal brain areas appear to be activated or not activated depending upon the context in which speech is produced. Production of verbs and complex sentences can be accompanied by activation in all or a subregion of the strip of cortex

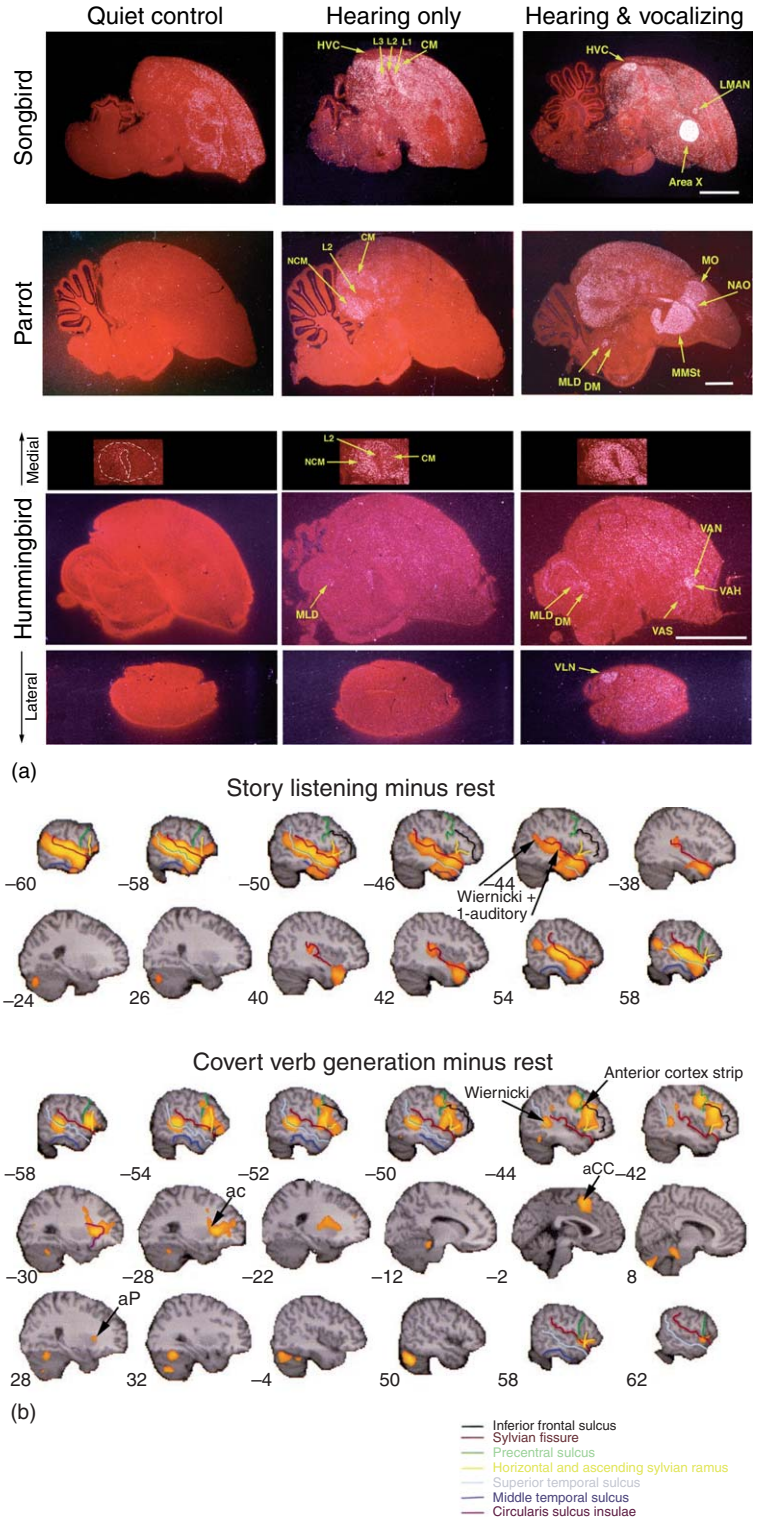

Figure 5 Hearing and vocalizing-driven brain activation patterns in vocal learning species. a, Example brain activation patterns in some brain regions of vocal learning birds (songbird-canary; parrot-budgerigar; hummingbird-sombre), as seen with hearing and vocalizing-driven *egr-1* gene expression (white). Shown are darkfield emulsion dipped sagittal sections reacted by *in situ* hybridizations to the *egr-1* gene; anterior is to the right, dorsal is toward the top. Red is cresyl violet staining. b, Example brain activation patterns in some brain regions of humans, as seen with hearing and vocalizing-driven PET signals minus rest. Shown are PET signals superimposed on sagittal slices, labeled with millimetric *x*-axis coordinates. The brighter the yellow signal the higher the activation. In all groups, different hearing and vocalizing tasks can differentially activate different brain areas. Not all activated brain areas are represented in these images. a, Figures based on Jarvis and Nottebohm (1997), Jarvis and Mello (2000), and Jarvis *et al.* (2000). b, Modified from Papathanassiou, D., Etard, O., Mellet, E., Zago, L., Mazoyer, B., and Tzourio-Mazoyer, N. 2000. A common language network for comprehension and production: A contribution to the definition of language epicenters with PET. *Neuroimage* 11, 347–357, Elsevier.

anterior to the face motor cortex: the anterior insula, Brocas area, DLPFC, pre-SMA, and anterior cingulate (Petersen *et al.*, 1988; Price *et al.*, 1996; Poeppel, 1996; Wise *et al.*, 1999; Crosson *et al.*, 1999; Papathanassiou *et al.*, 2000; Rosen *et al.*, 2000; Palmer *et al.*, 2001; Gracco *et al.*, 2005). Activation in the Brocas area, DLPFC, and pre-SMA is higher when speech tasks are more complex, including learning to vocalize new words or sentences, sequencing words into complex syntax, producing nonstereotyped sentences, and thinking about speaking (Hinke *et al.*, 1993; Poeppel, 1996; Bookheimer *et al.*, 2000; Buckner *et al.*, 1999). The left brain vocal areas show more activation than their right counterparts (Price *et al.*, 1996; Poeppel, 1996; Papathanassiou *et al.*, 2000; Rosen *et al.*, 2000). Like vocal nuclei in birds, premotor speech-related neural activity has been found in the Brocas area (Fried *et al.*, 1981). Further, low-threshold electrical stimulation to the face motor cortex, the Brocas area, or the anterior supplementary areas cause speech arrest or generation of phonemes or words (Jonas, 1981; Fried *et al.*, 1991; Ojemann, 1991, 2003).

In noncortical areas, speech production is accompanied by activation of the anterior striatum and the thalamus (Wallesch *et al.*, 1985; Klein *et al.*, 1994; Wildgruber *et al.*, 2001; Gracco *et al.*, 2005). Low-threshold electrical stimulation to ventral lateral and anterior thalamic nuclei, particularly in the left hemisphere, leads to a variety of speech responses, including word repetition, speech arrest, speech acceleration, spontaneous speech, anomia, and verbal aphasia (but also auditory aphasia) (Johnson and Ojemann, 2000). The globus pallidus can also show activation during speaking (Wise *et al.*, 1999). In nonhuman mammals and in birds, PAG and DM, Am, and nXII display premotor vocalizing neural firing (Larson, 1991; Larson *et al.*, 1994; Zhang *et al.*, 1995; Dusterhoft *et al.*, 2004) and/or vocalizing-driven gene expression (Jarvis *et al.*, 1998, 2000; Jarvis and Mello, 2000).

The cerebral vocal areas can also show neural firing during hearing, and this depends upon hearing task and species. In awake male zebra finches, firing is minimal in vocal nuclei (all the way down to nXIIts) when a bird hears playbacks of song, but greater when it is anesthetized or asleep and presented with playbacks of its own song (Williams and Nottebohm, 1985; Dave and Margoliash, 2000; Nealen and Schmidt, 2002; Cardin and Schmidt, 2003). In song sparrows, the reverse occurs: robust firing is observed in HVC when an awake bird hears playbacks of its own song, and this response is diminished when it is anesthetized (Nealen and Schmidt, 2002). In both species, the level or number of neurons firing in vocal nuclei during hearing is lower than that during singing. In humans, the face motor cortex, the Brocas area, and/or the DLPFC often show increased activation when a person hears speech or is asked to perform a task that requires thinking in silent speech (Hinke *et al.*, 1993; Price *et al.*, 1996; Poeppel, 1996; Wise *et al.*, 1999; Crosson *et al.*, 1999; Papathanassiou *et al.*, 2000; Rosen *et al.*, 2000; Palmer *et al.*, 2001). The magnitude of activation is usually lower during hearing than that seen during actual speaking. The anterior insula, the Brocas area, and DLPFC can also be activated by other factors, such as by engaging working memory (MacLeod *et al.*, 1998; Zhang *et al.*, 2003), which is a short-term memory that is formed before committing it to long-term storage. It is unclear, however, if this activation occurs in overlapping brain regions activated by speech or a speech perceptual task or whether the working memory tasks require language processing, or if there are separate but adjacent brain areas that are activated.

Taken together, the brain activation findings are consistent with the idea that songbird HVC and RA are more similar in their functional properties to face motor cortex than to any other human brain area and that songbird MAN, area X, and the anterior part of the dorsal thalamus are more similar in their properties to parts of the human premotor cortex, anterior striatum, and ventral lateral/anterior thalamus, respectively.

2.10.3 Consensus Auditory System

The above discussion focused solely on the motor component of vocal learning systems. This is because the motor component is what is specialized in vocal learners, whereas the auditory component is common among vocal learners and vocal nonlearners. Thus, the auditory component is only briefly discussed here; more detail is given in Jarvis (2004). Birds, reptiles, and mammals have relatively similar auditory pathways (Figure 6) (Webster *et al.*, 1992; Vates *et al.*, 1996; Carr and Code, 2000). The pathway begins with ear hair cells that synapse onto sensory neurons, which project to cochlea and lemniscal nuclei of the brainstem, which in turn project to midbrain (avian MLd, reptile torus, mammalian inferior colliculus) and thalamic (avian Ov, reptile reunions, mammalian medial geniculate) auditory nuclei. The thalamic nuclei in turn project to primary auditory cell populations in the pallium (avian L2, reptile caudal pallium, mammalian layer 4 of primary auditory cortex). Avian L2

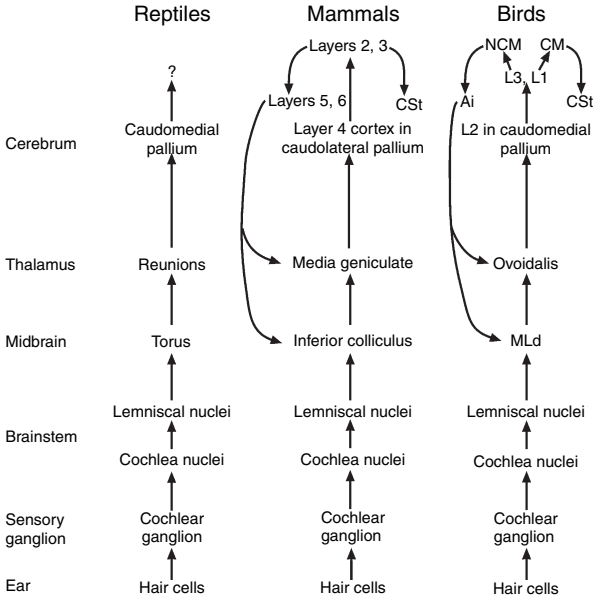

Figure 6 Comparative and simplified connectivity among auditory pathways in reptiles, mammals, and birds, placed in order from left to right of the most recently evolved. The connectivity from CM to CSt in birds needs verification by retrograde tracing. Abbreviations are in Table 1. Reproduced from Jarvis, E. D. 2004. Learned birdsong and the neurobiology of human language. *Ann. NY Acad. Sci.* 1016, 749–777, with permission.

then projects to other pallial cell (L1, L3, NCM, CM) and striatal (CSt) populations that form a complex network. Mammalian layer 4 cells then project to other layers of primary auditory cortex and to secondary auditory regions. Cerebral pathway connectivity is not known for reptiles. In terms of connectivity, avian L1 and L3 neurons are similar to mammalian layers 2 and 3 of primary auditory cortex, the latter of which receive input, like L2, from layer 4 (Karten, 1991; Wild *et al.*, 1993). Avian NCM and CM are also similar to layers 2 and 3 in that they form reciprocal intrapallial connections with each other and receive some input from L2.

The source of auditory input into the vocal pathways of vocal learning birds is unclear. Proposed routes include the HVC shelf into HVC, the RA cup into RA, Ov or CM into NIf, and from NIf dendrites in L2, in songbirds (Wild, 1994; Fortune and Margoliash, 1995; Vates *et al.*, 1996; Mello *et al.*, 1998). However, the location of the vocal nuclei relative to the auditory regions differs among vocal learning groups. In songbirds, the posterior vocal nuclei are embedded in the auditory regions; in hummingbirds, they are situated more laterally, but still adjacent to the auditory regions; in parrots, they are situated far laterally and physically separate from the auditory regions (Figures 3a–3c).

In humans, the primary auditory cortex information is passed to secondary auditory areas, which includes the Wernickes area (Figure 3d). When damaged, this area leads to auditory aphasias, sometimes call fluent aphasia. A patient can speak words relatively well, but produces nonsense highly verbal speech. One reason for this is that the vocal pathways may no longer receive feedback from the auditory system. Information from the Wernickes area has been proposed to be passed to the Brocas area through arcuate fibers that traverse a caudal–rostral direction (Geschwind, 1979), but this has not been demonstrated experimentally. Bilateral damage to primary auditory cortex and Wernickes area also leads to full auditory agnosia, the inability to consciously recognize any sounds (speech, musical instruments, natural noises, etc.) (Benson and Ardila, 1996).

No one has tested whether lesions to avian secondary auditory areas result in fluent song aphasias. Yet lesions to songbird NCM and CM result in a significant decline in the ability to form auditory memories of songs heard (MacDougall-Shackleton *et al.*, 1998). It is difficult to ascertain how nonhuman animals, including birds, perceive sensory stimuli, and therefore it is difficult to make comparisons with humans in regard to perceptual auditory deficits.

2.10.4 Evolution of Vocal Learning Systems from a Common Motor Pathway

Given that auditory pathways in avian, mammalian, and reptilian species are similar, whether or not a given species is a vocal learner, this suggests

that the auditory pathway in vocal learning birds and in humans was inherited from their common stem-amniote ancestor, thought to have lived approximately 320 Mya (Evans, 2000). Having a cerebral auditory area would explain why nonhuman mammals, including a dog, exhibit auditory learning, including learning to understand the meaning of human speech, although with less facility than a human. For vocal learning pathways, because the connections of the anterior and posterior vocal pathways in vocal learning birds bear some resemblance to those of nonvocal pathways in both birds and mammals, pre-existing connectivity was presumably a genetic constraint for the evolution of vocal learning (Durand *et al.*, 1997; Farries, 2001; Lieberman, 2002; Jarvis, 2004). In this manner, a mutational event that caused descending projections of avian arcopallium neurons to synapse onto nXIIts or mammalian layer 5 neurons of the face motor cortex to synapse onto Am may be the only major change that is needed to initiate a vocal learning pathway. Thereafter, other vocal brain regions could develop out of adjacent motor brain regions with pre-existing connectivity. Such a mutational event would be expected to occur in genes that regulate synaptic connectivity of upper pallial motor neurons to lower α-motor neurons. This hypothesis requires that avian nonvocal motor learning systems have up to seven areas distributed into two pathways in at least six brain subdivisions (the mesopallium, nidopallium, arcopallium, striatum, pallidal-like cells in the striatum, and dorsal thalamus). It would also require that mammalian nonvocal motor learning systems have brain regions distributed in two pathways involving at least four brain subdivisions (the six layers of the cortex, the striatum, the pallidum, and the dorsal thalamus). Not apparent in this view is the question of whether there is a genetic constraint for auditory information entering vocal learning pathways.

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2.11 Forebrain Size and Social Intelligence in Birds

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Glossary

adaptive specialization — Adaptation to a particular selective context, e.g., food caching, brood parasitism.

cooperative breeding — Care of juveniles that is done by individuals other than the parents. Often occurs in the form of helping at the nest, where fledged offspring from a previous clutch provide food or protection for their younger siblings, instead of dispersing and producing their own offspring.

cultural transmission — The effect of social learning at the population level, leading to local traditions and diffusion of new behaviors.

general process skills — Cognitive differences between taxonomic groups that are thought to reflect broad, unspecialized abilities based on large and/or diffuse neural substrates.

innovation — A behavior seen for the first time in animal and that is not the result of a genetic change or a pathology. The novel behavior is an attempt to solve a problem (feeding, social) that the standard repertoire cannot resolve.

module — Information relevant to one specialized context or domain that is not available in other contexts.

motor imitation — The copying of a particular action.

phyletically independent contrasts — The most widely used procedure for removing the effects of common ancestry on taxonomic similarities or differences between traits. The technique measures differences between related taxonomic groups in the values of biological traits, rather than actual trait values on extant taxa. Relatedness between groups is usually measured through mitochondrial or nuclear DNA data.

social facilitation — Performance of a behavior by a demonstrator which stimulates the expression of that behavior in an observer.

social learning — A generic term describing the effects that information coming from other animals has on learned behavior. In experiments, originators of the information are called demonstrators or tutors, while recipients are called observers. The term 'social learning' includes more specific ones such as social facilitation, stimulus enhancement, motor imitation, or cultural transmission.

social or Machiavellian intelligence — Cognitive complexity selected in the context of social interactions. Usually contrasted with ecological intelligence, selected in the context of interactions with the nonsocial, physical environment.

specialized cognitive skills — Cognitive abilities that are specific to a restricted selective context (e.g., spatial memory, learned song) and whose neural substrate is often a restricted brain area (e.g., hippocampus, nucleus HVC in Oscines). The terms 'adaptive specialization' or 'module' embody similar ideas. The term can be opposed to general process skills.

stimulus enhancement — Interaction of a demonstrator with a feature in the environment that directs the attention of an observer to that feature, thereby facilitating its learning.

2.11.1 Introduction

Grouping patterns vary among animal species and populations. For example, weaverbirds in Africa move in flocks that can include several thousand individuals. In North America, chickadees form small, stable winter groups with a dominance

hierarchy based on individual recognition. In Barbados, Zenaida doves defend year-round territories and feed most often alone. Social life is clearly less complicated when you are alone than when you have to deal with many individuals. It thus makes intuitive sense to predict that variation in grouping patterns might be associated with variation in social cognition and the neural centers that control it. The idea that social information processing demands might be a driving force for the evolution of cognition was first proposed by primatologists such as Jolly (1966), Humphrey (1976), and Byrne and Whiten (1988). These authors contrast social pressures on the evolution of intelligence with those derived from the nonsocial environment such as foraging and predator avoidance. Social pressures on intelligence are sometimes called Machiavellian (Byrne and Whiten, 1988), based on cases where animals appear to deceive and manipulate others (Byrne and Corp, 2004); nonsocial pressures are sometimes referred to as ecological (e.g. Schultz and Dunbar, 2006).

Most of the experimental tests and taxonomic correlations on social intelligence have been done on primates (Dunbar, 1998; Reader and Laland, 2002; Byrne and Corp, 2004; see Role of Spindle Cells in the Social Cognition of Apes and Humans), but similar work has recently been extended to birds. The theoretical framework is the same as the one used for primates, but the definition of groups is sometimes broader, including large flocks that do not necessarily show the close individual interactions typical of a monkey troop. This article reviews some of the key studies on the taxonomic and experimental approaches, then discusses theoretical issues on the evolution of social intelligence.

2.11.2 Analyses of Taxonomic Distributions

A common way of testing evolutionary predictions on animal cognition is to correlate the taxonomic distribution of a cognitive measure, the size of its neural substrate, and lifestyle variables. The prediction is that animals with particular ecological demands on information processing should have relatively larger neural centers that allow more of a particular cognitive skill. For example, birds that successfully colonize new habitats are larger-brained and more innovative in their locus of origin than birds that are unsuccessful (Sol *et al.*, 2005). Using this logic, one can predict that birds that breed cooperatively or live in larger flocks or more complex groups might benefit from larger neural centers that allow more social information processing. One might also predict that the cognitive requirements of social learning or social play should benefit from larger neural centers, estimated either as relative size of the whole brain (Ricklefs, 2004), the telencephalon (Burish *et al.*, 2004), or association areas such as the mesopallium and nidopallium (Bouchard, 2002; see Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?).

Taxonomic correlations on brains and social intelligence in birds have for the moment been done on social play (Diamond and Bond, 2003), complexity of the social system (Burish *et al.*, 2004; Ricklefs, 2004), social learning (Bouchard, 2002), cooperative breeding (Iwaniuk and Arnold, 2004; Ricklefs, 2004), and group size (Beauchamp and Fernandez-Juricic, 2004). Two of the analyses support the idea that social life is associated with large brain size in birds, while four do not. The study of Bouchard (2002) yields ambiguous results because of a statistical outlier. We will look at each of the analyses in more detail.

Bouchard (2002) examined avian social learning with the same method used by Reader and Laland (2002) for primates. She mapped the taxonomic distribution of all presumed cases of social learning in the field and correlated this distribution with relative size of the brain and of the mesopallium. Contrary to the data set collated by Reader and Laland (2002), field reports of avian social learning were relatively rare. Only 72 cases were found, compared to the 445 social learning observations collated for primates and the more than 2000 cases of innovation gathered by Lefebvre and colleagues on birds (Lefebvre and Bouchard, 2003). In birds, an innovation is operationally defined as the first report in the short notes section of an ornithology journal of a new or unusual food type or foraging technique, based on the presence in the paper of key words such as "never seen before," "not described in the handbook," "opportunistic," "we were surprised" (see table 1 in Lefebvre *et al.*, 1997, for examples). A gull that kills rabbits by dropping them from the air onto rocks (Young, 1987) is thus using an innovative form of its well-known shell- or crab-breaking technique.

Of the 72 reported cases of avian social learning, more than 70% belonged to the order Passeriformes, yielding an extreme outlier when the frequency is corrected for the usual biases of research effort or species number. The outlier cannot be discarded because it represents such a strong proportion of the data set and it makes the usual parametric multivariate statistics invalid. Rank

correlations of residual social learning rate against residual brain size or residual innovation rate yield nonsignificant values. This is consistent with the experimental data on the most complex form of social learning, motor imitation, which has been reported in large-brained species such as parrots (Dawson and Foss, 1965; Moore, 1992; Mottley and Heyes, 2003), small-brained species such as pigeons (Zentall *et al.*, 1996) and quail (Akins and Zentall, 1996, 1998), as well as species of intermediate brain size such as starlings (Fawcett *et al.*, 2002) and grackles (Lefebvre *et al.*, 1997).

The two studies on cooperative breeding (Ricklefs, 2004; Iwaniuk and Arnold, 2004) yield similar negative results. Ricklefs (2004) looked at all avian families and operationalized cooperative breeding as the proportion of species within each family for which helping behavior is reported in the literature. In birds, helping behavior is defined as a contribution to the care of nestlings by offspring from a previous clutch, at an age where they could theoretically be independent and move away from their parental area. Even though large-brained birds such as corvids and hornbills showed high proportions of helping, the overall relationship between the variables was nonsignificant. Iwaniuk and Arnold (2004) focused on 155 species of Corvida, using the extensive brain size database gathered by Iwaniuk (2003). They scored cooperative breeding on a four-point scale for percentage of nests including helpers; they also measured size of the cooperative breeding group. Most of their analyses yielded nonsignificant results, with the exception of one within-family comparison (honeyeaters) for presence versus absence of helpers and one phyletically uncorrected analysis of between family differences in percentage of nests with helpers.

Using extant species as well as phyletically independent contrasts, Beauchamp and Fernandez-Juricic (2004) looked at group size and forebrain size in three different data sets covering nearly 200 avian species. They operationalized group size as the mean and maximum size of avian flocks outside the breeding season, including individuals from more than one species when mixed species flocks occurred. They also used a dichotomous solitary versus gregarious measure to include cases where exact group numbers were not available. None of their analyses showed a relationship between flocking and relative brain size, although it is possible that an avian flock is more of a simple aggregation and less of a cohesive unit than is a mammalian group. A positive relationship has been reported between relative brain size and group size in several mammalian orders, including primates, ungulates, carnivores, and cetaceans (Worthy and Hickie, 1986; Sawaguchi and Kudo, 1990; Dunbar, 1992; Marino, 1996; Barton, 1999; Schultz and Dunbar, 2006; but see Connor *et al.*, 1998; van Schaik and Deaner, 2003).

Ricklefs (2004) defined sociality as presence or absence of a complex social structure. The large-brained parrots and corvids fall in the first category, while the smaller-brained ducks, cuckoos, doves, and quail fall in the second, but the overall trend among all birds is nonsignificant. Burish *et al.* (2004) classified 154 avian species as either solitary (one to three individuals), living in coveys (4–50 individuals with a dominance or lek structure), colonial (groups of hundreds and thousands), or transactional (presence of social cognition and individual communication). They found a significant relationship between social category and fraction of the whole brain represented by the telencephalon (see Role of Spindle Cells in the Social Cognition of Apes and Humans).

Finally, Diamond and Bond (2003) collated all reliable cases of social play in birds, which was found in 25 species from 5 families: parrots, corvids, hornbills, and babblers. Social play in birds is defined in much the same way as it would be in primates or dogs, where two individuals go through repeated, incomplete sequences of a behavior outside its context, using for example the biting, pushing, and grasping movements of fighting in a way that does not imply aggression (see examples in table 1 in Diamond and Bond, 2004). Diamond and Bond (2003) report a positive relationship between relative brain size and propensity to social play, as do the studies of Ortega and Bekoff (1987). Diamond and Bond (2004) show a further interesting difference between two closely related species of New Zealand parrots, the kea, *Nestor notabilis*, and the kaka, *N. meridionalis*. The kea is a highly exploratory generalist forager that goes through a prolonged social interaction period between juvenile groups and parents; the kaka has a much more specialized diet, is much less exploratory, and juveniles disperse early from their natal area. In accordance with these differences, social play is much more extensive in keas than it is in kakas.

In summary, the majority of taxonomic studies do not provide very strong support for the idea that social intelligence has co-evolved with brain size in birds.

2.11.3 Experimental Tests

An alternative to predictions on taxonomic distributions is to set up controlled experiments where a targeted species is given in captive conditions a test

that would imply intelligence if it were solved by a human. The advantage of this approach is that rigorous controls can be added to the study to eliminate simpler processes. Hypotheses about the cognitive rules animals follow can also be tested with the appropriate design. Solving an experimental problem does not prove humanlike cognition in an animal, but does provide empirical evidence consistent with operational predictions on a complex process. The best examples of this approach are the experiments of Templeton *et al.* (1999) on social learning, those of Bond *et al.* (2003) on the tracking of dyadic relationships, those of Bednekoff and Balda (1996) on memory for caches made by other birds, and those of Emery and Clayton (2001) on avoidance of cache pilfering. The common thread between these studies is that they are all conducted on corvids, one of the largest-brained avian taxa (Rehkämper *et al.*, 1991; Emery and Clayton, 2004).

Whether faced with a set of ordered color stimuli (Bond *et al.*, 2003) or actual conspecifics (Paz-y-Mino *et al.*, 2004), the highly social pinyon jay appears to track multiple dyadic relationships quickly and accurately, more so than the less-social western scrub jay (Bond *et al.*, 2003). A pinyon jay that observes pairs of unknown conspecifics aggressively interacting, then uses these observations of relative rank to attack or defer to these individuals when it meets them, is using a sophisticated form of social intelligence. Comparing the pinyon jay to the less-social Clark's nutcracker, Bednekoff and Balda (1996) have shown that the more social species is also better at remembering the location of food it sees a conspecific store. In contrast, a nutcracker is more efficient at remembering caches it has made itself. Templeton *et al.* (1999) also compared pinyon jays and nutcrackers on an individual learning and a social learning task. Pinyon jays learned faster from a tutor than they did on their own, while the two routes for learning did not differ in nutcrackers. Emery and Clayton (2001) studied recaching behavior of scrub jays and found that it was more frequent when the storer had been observed by a conspecific that could potentially steal the hidden food. Caching was also more often done in sites where the storer could not be seen by others, while recaching after observation was more frequent in individuals that were experienced pilferers themselves.

For the experimental studies on social intelligence to be complementary with the taxonomic correlations reviewed above, more tests need to be done on smaller-brained species to see if the abilities shown by corvids are unique to the larger-brained social birds. For the moment, there is no clear demonstration that relative brain size is a key determinant of the abilities demonstrated in social intelligence experiments with corvids.

2.11.4 Is Social Intelligence Different from Other Types of Intelligence?

The idea that intelligence in animals and humans is not a unitary ability, but a complex set of specialized operations supported by modular organization, has recently become popular in cognitive sciences. Proponents of this view argue that memory being limited, animals with different ecological requirements should trade off an increase in one type of cognition (including its neural substrate) by a decrease in others that are less important for that lifestyle (Sherry and Schacter, 1987; Shettleworth, 1998). The comparative experiments conducted by Kamil and co-workers on jays are based on this logic: differing demands in social lifestyle should have selected for differences in a restricted set of relevant cognitive abilities (Bond *et al.*, 2003). For example, Templeton *et al.* (1999) found that a social jay is better than a solitary jay at social learning, but not at individual learning. If all forms of learning were better in a species that differs only on the relevant ecological trait, in this case sociality, then the underlying cognitive process would have to be considered general, not modular (Lefebvre and Bolhuis, 2003).

Can social learning be considered a modular specialization? Apart from the results of Templeton *et al.* (1999), most of the evidence in birds (Lefebvre and Giraldeau, 1996) and primates (Reader and Lefebvre, 2001) suggests that social learning is the same thing as individual learning. Species that learn faster socially also tend to learn faster individually (Sasvari, 1985a, 1985b; reanalyzed by Lefebvre and Giraldeau, 1996). A consensus was reached on this point among social learning researchers at an international conference (Heyes and Galef, 1998) and workers in the area now speak of socially guided learning (Fragaszy and Perry, 2003) to express this similarity. The process that governs associations is currently seen as identical whether a stimulus that predicts an outcome comes from a conspecific or an inanimate feature of the environment.

Given this, are all other aspects of social intelligence also the same thing as nonsocial intelligence, even though the two presumed pressures on encephalization have traditionally been addressed separately? Researchers that have focused on the specific pressures that social life exerts on intelligence have long recognized that the ultimate

determinant of grouping patterns in animals is the spatial and temporal distribution of resources (see, for example, Kudo and Dunbar, 2001), but they have not explicitly thought of social and nonsocial cognition as correlated responses to similar ecological pressures. Resource defense theory can be useful here as a framework for making predictions. Brown (1964) was the first to propose that the distribution of resources in space and time determines whether animals should feed alone and defend access to food or whether they should live in relatively unaggressive groups. The more food is predictable in space and time and the more it occurs in low-density clumps, the more a single individual can profit by defending it. The more unpredictable food is and the more it occurs in densities that attract many individuals, the less profitable will be defense and, conversely, the more profitable will be group feeding. The actual size of the group should then be proportional to the average density of the food patches. There is a large body of theoretical and empirical work supporting these predictions (Warner, 1980; Davies and Houston, 1984; Grant, 1997; Goldberg *et al.*, 2001).

If food is unpredictable in space and time, this also implies that animals need to range over a wide area to find it and that using social information to find this food might be particularly critical. The wider the area and the more unpredictable the food, the higher the chance that animals will both encounter and need to take different food types. Similar patterns of resource distribution thus have parallel effects on both the solitary–group feeding continuum and the specialist–generalist continuum. All other things being equal, more predictable food should favor both more solitary feeding and specialization, while less predictable food should favor both more group feeding and more opportunistic generalism. There is experimental support for this idea. Rafacz and Templeton (2003) showed that starlings relied more on information provided by a conspecific tutor if they were foraging in an unpredictable environment than in a predictable one, while Gray (1981) showed that making food less predictable led rodents to select a more generalist diet. This implies that social and food-finding pressures on intelligence and brain size evolution might not be independent, but positively correlated.

2.11.5 Conclusions

Ecological theory suggests that social and nonsocial forms of intelligence might be correlated responses to similar patterns of resource distribution. In birds, there is consistent evidence that nonsocial forms of cognition (e.g., innovation, Timmermans *et al.*, 2000; tool use, Lefebvre *et al.*, 2002; bower building, Madden, 2001) are associated with the relative size of the whole brain and of the nidopallium–mesopallium complex. The evidence that brain evolution has been associated with the pressures of sociality in birds is not as clear. For the moment, the experimental evidence, gathered almost exclusively on corvids, suggests that complex forms of social cognition are present in large-brained birds. Experiments are needed on small-brained taxa, however, before an association can be made between avian encephalization and sociality. Taken together, the taxonomic distribution analyses that have tested this association directly provide poor evidence for it, with only two out of seven studies yielding a positive trend. More work is needed before social intelligence in birds is as well understood as it is in primates (Byrne and Whiten, 1988; Cheney and Seyfarth, 1990; Whiten and Byrne, 1997; Tomasello and Call, 1997), but recent reviews (Rehkämper and Zilles, 1991; Emery and Clayton, 2004; Lefebvre *et al.*, 2004) emphasizing the apparent convergence between the two taxa may help.

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2.12 The Hippocampal Formation in Food-Storing Birds

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Glossary

brain parcellation	Brain areas, or centers, may be delineated on the basis of cellular density, cellular morphology (e.g., cortical layers), neurochemical markers, or developmental origin.
cognitive map	An abstract representation of multiple factors (i.e., location, time or certain objects) contained in memory and dependent on the relationship between objects or stimuli.
food cache	A site where food storers keep pieces of collected food.
hippocampus	Part of the limbic system, a complex archicortical brain area consisting of several subdivisions. In mammals, it includes the Ammon's horn, dentate gyrus, and subiculum. The hippocampal formation is implicated in memory fomation.
hodological study	Connectivity studies of brain pathways, describing the afferent and efferent axonal projections of certain circumscribed areas.
medial pallium	The medial division of the cortical mantle (pallium) containing the hippocampal formation.
retrieval	Recovery of a once hidden food item from the food cache.
spatial navigation	An ability to explore and 'learn' space in search of food, nesting sites, or mates. Animals are then capable of identifying the exact location of an important site in relation to their environment.
storing (or hoarding)	Hiding selected food items in a cache.
synaptic plasticity	The neuromorphological/physiological basis for learning and memory; it includes the enlargement of synaptic surfaces, modification of synaptic proteins, or alterations in the firing pattern of the neurons.

2.12.1 Introduction

The avian hippocampal formation (HP) is considered to be homologous to the mammalian hippocampal complex on the basis of topography, connectivity, developmental origin, and its role in spatial memory. The HP of food-storing passerines and corvids exhibits an adaptive modification by showing certain enlargement relative to the rest of the telencephalon. The volumetric changes, also influenced by storing/retrieving experience, are paralleled by seasonal differences in neurogenesis and total cell number in storing passerines studied at different times of the year.

The underlying neuroanatomical organization is very different from that of mammals and equivalent morphological entities are not easily identified within the avian HP. Nevertheless, pathway tracing studies have revealed three distinct subdivisions (dorsolateral, dorsomedial, and ventral), which, on the basis of connectivity, may correspond to the subiculum, dentate gyrus, and cornu Ammonis of mammals, respectively. The transmitter and neuropeptide distribution of the avian HP differs, in terms of topography, substantially from that of the mammalian HP and offers an alternative subregional classification.

Certain neurochemical markers, detected by neurohistological methods or receptor autoradiography, including Calbindin, neuronal nitric oxide synthase, immediate early gene (IEG) products, or N-methyl-D-aspartate (NMDA)-receptor ligands show characteristic alterations in the storing HP when compared to that of related nonstoring passerines. Thus, the morphological framework, which is a neuronal basis of memory formation, has been modified to express an adaptive specialization to assist certain behavioral needs in parallel with the evolutionary changes. However, it has to be noted that other forebrain or brainstem nuclei connected to hippocampal subdivisions may express parallel structural changes in relation to storing behavior, as has been detected for the septal nuclei. Therefore, a hippocampopetal/fugal memory circuit has to be suggested in the processing of food-storing behavior.

2.12.1.1 Hippocampus – The Question of Homology

During the decade beginning in the mid-1990s, the avian HP has received considerable attention, due to certain birds' remarkable abilities in spatial navigation. Apart from their functional/behavioral parallels, the HP of avian and mammalian species share certain developmental, cytoarchitectonical, immunohistochemical, hodological, and electrophysiological characteristics, all suggesting homology (see Evolution of the Hippocampus, Sex and Species Differences in Hippocampal Volume).

The HP, or dorsomedial (DM) cortex, derives from the same medial pallial segment of the neural tube in mammals, birds, or reptiles as detected by genetical markers (Puelles *et al.*, 2000). Several authors (Montagnese *et al.*, 1996; Tömböl *et al.*, 2000) studying the cellular morphology of the HP employing Golgi impregnation describe two major neuronal classes, spiny projection neurons and nonspiny local interneurons. These two classes may further be classified according to their dendritic and axonal arbors, or locations within the HP of birds. The intrahippocampal distribution of different neuronal populations also offers a basis for identifying circumscribed hippocampal subregions (Montagnese *et al.*, 1996).

Immunohistochemical analyses (Krebs *et al.*, 1991; Erichsen *et al.*, 1991) and pathway tracing studies (reviews in Székely, 1999; Atoji and Wild, 2004) suggest certain equivalence between the avian and mammalian HP subdivisions, on the basis of their peptide and/or neurotransmitter content, as well as their extrinsic or intrinsic connectivity patterns. However, an accurate avian parallel for the trisynaptic loop of the mammalian HP has not yet been discovered.

Both long-term depression (LTD) and long-term potentiation (LTP) of synaptic efficacy, the electrophysiological correlates of synaptic plasticity (i.e., learning and memory), have been found to be prerequisites of memory formation in birds, as in mammals (Wieraszko and Ball, 1991, 1993). However, LTP in the avian HP is not correlated with a change in transmitter release and does not require activation of adenylyl cyclase (Margrie *et al.*, 2000) or NMDA receptors; therefore, an alternate form of synaptic plasticity might be characteristic for the avian HP.

2.12.1.2 Food-Storing Behavior

Middle-scale spatial navigation is an ability to explore an area in search of food, nesting sites, or mates. The majority of animals are capable of learning the exact location of an important site in relation to their environment. Many birds and rodents show the ability to remember the locations of hundreds of food caches and can store and retrieve food using these sites. Food hoarding, or storing, is a widespread behavior, practised by animals to provide a steady food supply during periods of low food abundance, such as in the winter. Food-storing birds, such as titmice, chickadees, jays, magpies, and nutcrackers have been used extensively in behavioral studies of spatial memory. These birds store pieces of food in their local environments and are capable of remembering the locations of over 100 different food caches (Vander Wall, 1990). In laboratory experiments (Clayton and Krebs, 1994; Cristol, 1996), birds are allowed to store and retrieve food items, mostly seeds, in an enclosure decorated with artificial trees. The laboratory contains a variety of spatial and visual cues to be manipulated in order to test whether they influence the ability to remember the locations of food items.

Notably, storing birds may not only rely on their remarkable spatial abilities, but they also have to employ specific sensory and motor skills to store and retrieve hidden food items successfully (Vander Wall, 1990). Most food-storing birds also use visual landmarks, or the sun compass, to locate their caches, and a few storers are able to recover their stores using olfactory cues (Sherry and Duff, 1996). Storing behavior is steadily built on experience as well as learning and practice in juveniles (Clayton and Krebs, 1994; Clayton, 1995).

Food-storers create a cognitive map of the explored environment, which is an abstract representation of the type of food, exact time of storing, and the location of sites contained in their memory ('hippocampal network'). The geometrical

relationships of objects within a cognitive map can be reconstructed from memory and do not have to rely on visual cues.

A cognitive map consists of a number of landmarks that are placed in a geocentric reference frame and then stored in memory. For each landmark to be stored in a cognitive map, it would require a form of vector addition. The location of the landmark with respect to the animal (egocentric vector) plus the location of the animal in the environment (geocentric vector) would generate a vector for each landmark. This would allow each landmark to be set in its correct location within a geocentric framework.

2.12.2 The Organization of the Hippocampal Formation

2.12.2.1 Subdivisions

Traditionally, the definition of hippocampal structures was based on standard histological techniques. In the early twentieth century, subfields of the avian hippocampus were determined on the basis of cytoarchitecture, enzyme reactivity (i.e., acetyl cholinestherase), and neuronal density (cresyl violet staining, review in Székely and Krebs, 1996). Table 1 summarizes all previous, recent, and present suggestions for hippocampal subdivisions (review in Atoji and Wild, 2004). More recently, several investigators have attempted to establish subregions within the HP using various anatomical and physiological techniques. Karten and Hodos (1967) have divided the dorsomedial cortex of pigeons into hippocampus proper and area parahippocampalis (APH), using the parahippocampal sulcus as a landmark between the two subregions. The regional distribution of neurotransmitters and neuropeptides (Krebs *et al.*, 1991; Erichsen *et al.*, 1991) enabled the characterization of seven subdivisions in the pigeon HP. Later, an alternative subregional system of five areas has emerged from the cytoarchitectonical observations in the zebra finch by employing zinc staining (Montagnese *et al.*, 1993) or Golgi impregnation (Montagnese *et al.*, 1996).

Hodological studies of the zebra finch HP (Székely and Krebs, 1996; Székely, 1999) have revealed three areas. Based on their specific projection patterns, the dorsolateral (DL) region was paralleled to the subiculum of mammals with its main projections to the basal ganglia, the limbic archistriatum, the lateral septum, and the paraxial mesodiencephalic centers. The ventral (V) subdivision was suggested to be equivalent to the Ammon's horn of mammals with its commissural projections to the contralateral HP. We thought that, based on its purely intrinsic connectivity, the dorsomedial (DM) region would be a good candidate for an avian dentate gyrus; however, its relative topographical position to the avian Ammon's horn seemed to be opposite to that described for the reptilian or mammalian hippocampi (review in Székely and Krebs, 1996).

Further studies on the connectivity or evoked field potentials of the homing pigeon HP (Hough *et al.*, 2002; Siegel *et al.*, 2002; Kahn *et al.*, 2003) have refined the above system and characterized five subregions including DM, DL and, respectively, the ventral core, VL, and VM of the V subdivision. Nevertheless, the borders between the subregions have been shifted, probably due to the obvious interspecies differences between songbirds and pigeons.

Table 1 Summary of all formerly and recently established subdivisional systems of the avian HP

	Subdivisional nomenclatures					
HP area	*K/H*	*E/B/K*	*B&Co*	*M/K/M*	*Sz/K*	*A/W*
Ventral (or medial)	Hp	Area 1	VL	HCl	V	Lateral blade
		Area 2	VM	HCm		Medial blade
		Area 3	Ventral core	APH, PHc		Tr
Dorsomedial	Hp-APH	Area 4	DM	CF	DM	Po
		Area 5				Ma
		Area 1				Pa
Dorsolateral	APH	Area 3	DL	APH	DL	DM
		Area 6		PHc		
Lateral	APH	Area 7	DL	Cl	SPf	DLd
						DLv

Note that certain topographically created terms (e.g., DL or DM) may refer to different subdivisions in the works of different authors. CF, crescent field; Cl, intermediate corticoid area; HCl, hippocampal complex pars lateralis; HCm, hippocampal complex pars medialis; PHc, central field of para hippocampus; SPf, substance P field. K/H refers to the work of Karten and Hodos (1967); E/B/K refers to Krebs *et al.* (1991) and Erichsen *et al.* (1991); M/K/M refers to Montagnese *et al.* (1993, 1996); Sz/K refers to Székely and Krebs (1996) and Székely (1999); B&Co refers to Hough *et al.* (2002), Siegel *et al.* (2002), and Kahn *et al.* (2003); A/W refers to Atoji and Wild (2004).

Table 2 Summary of the internal and external connectivity of the zebra finch HP following PHAL iontophoresis as described in Székely and Krebs (1996) and Székely (1999)

Target or source	*Intrinsic*		*Extrinsic*	
	Afferents	*Efferents*	*Ipsilateral efferents*	*Contralateral efferents*
DL	V	V	HA, HD/HV	A
	DM		DLC, AP	
			SL, FDB	
			MSt, PVT	
			Tn, AP	
			SCE, ME	
			DMP, SHb	
			GCt, AVT	
DM	V	V	Dorsorostral septum	
	DL	DL	Caudal HA	
V	DL	DL	SM	SM
	DM			V

DLC, dorsolateral cortex; DMP, nucleus dorsomedialis posterior thalami; FDB, fasciculus diagonalis Brocae; MSt, medial striatum; PVT, paleostriatum ventrale; SCE, stratum cellulare externum; SHb, nucleus subhabenularis.

The most recent parcellation of the avian HP has been based on connectivity studies and Nissl staining of the pigeon brain (Atoji and Wild, 2004). The V-shaped structure of the ventral subdivision has been separated into three elements, the lateral and medial blades (ll and ml, respectively) enclosing a triangular area (Tr). The DM subdivision of Székely and Krebs (1996) has been further classified, on the basis of neural density, to Po (cell poor), Ma (magnocellular), and Pa (parvocellular) subdivisions. The rest of HP has been named as DM, and the more lateral areas, demarcated by the parahippocampal sulcus have been termed as DLd and DLv.

2.12.2.2 Connectivity

As described in Székely and Krebs (1996), iontophoretic administration of Phaseolus vulgaris leucoagglutinin in the zebra finch HP have revealed specifically altering projection patterns ascribed to the three subdivisions of the DM cortex (summarized in Table 2), whereas previous tracing studies have discussed projections from the earlier defined two subdivisions in the pigeon brain. The efferent connectivity of the songbird HP shows certain resemblance to the wiring pattern of the mammalian hippocampal complex. Most importantly, there is a medial-to-lateral topographical organization of efferents in relation to the septal complex similar to that described in mammals. Axons arising from the ventral portion (i.e., medialmost aspect of the medial pallium, closest to the septohippocampal junction) will principally innervate the medial septum, whereas, fibers from the more dorsal and lateral aspects of the medial pallium (i.e., hyperpallial junction) will enmesh the lateral septum.

However, some recent investigations (Hough *et al.*, 2002; Siegel *et al.*, 2002; Kahn *et al.*, 2003; Atoji and Wild, 2004) have introduced a more intricate hippocampal circuitry using various tract tracing, lesion, and electrophysiological techniques.

2.12.2.2.1 Intrinsic projections Despite certain differences in terms of parcellation, most investigators agree on the direction of projections within the hippocampal connections (Hough *et al.*, 2002; Siegel *et al.*, 2002; Kahn *et al.*, 2003; Atoji and Wild, 2004). The intrinsic projections of HP are summarized in Table 2.

Generally, the DL aspect projects to the V-shaped layer and gives rise to axons terminating in the paraventricular fiber zones. The ventral strip of DL (DLv) innervates the more medial central field (DM) of HP which, in turn, projects back to the dorsal strip of DL (DLd). Furterhmore, DM is reciprocally connected with the neuronal layers of V together with the interposed triangular (Tr) field, as well as the magnocellular portion (Ma) of the dorsomedial corner of HP. The cellular elements of V form a presumably bisynaptic loop with the cells of Tr, which then give rise to efferents enmeshing DLd, but seem to avoid DLv.

According to a recent hypothesis (Atoji and Wild, 2004), the majority of neurons of the V-shaped layer should be intrinsic to the HP, whereas some cells in a small portion of the medial layer of V, together with the adjacent aspect of the Tr area, send comissural projections to the contralateral HP.

2.12.2.2.2 Extrinsic projections All studies describing 'hippocampal and parahippocampal'

efferents and afferents in detail have so far agreed on the majority of regions connected ipsi-, bilaterally, or even reciprocally to the entity named as HP. However, this consensus seems to fade when the accurate intrahippocampal origins or targets of the projections are discussed. Generally, the fibers leave and/or invade the HP via lateral, rostral, and ventromedial paths. The extrahippocampal connections are summarized in Table 3, on the basis of Atoji and Wild (2004).

The lateral-most aspect of HP, DL, receives intratelencephalic afferents from the ipsilateral hyperpallium, nidopallium, the DL cortex, and from the medial layer–Tr complex of the contralateral HP. Within the septum, afferent fibers arise both from the medial and lateral nuclei (SM and SL, respectively) and the diagonal band of Broca (NDB), as well as from the commissural nucleus adjacent to the pallial commissure and the ventral pallium (VP) further caudally. Diencephalic and brainstem sources of HP afferents include the bilateral nucleus dorsolateralis anterior (DLM) and the ipsilateral nucleus subrotundus of the thalamus and several hypothalamic areas, as well as the ventral tegmental area (AVT), midbrain central gray (GCt), locus ceruleus (LoC), and the contralateral 'raphe nucleus' (i.e., nucleus linearis caudalis). Most of the areas rostral to the level of the midbrain have established reciprocal connections with DL.

DM injections have resulted in a more extensive reciprocal pattern of afferent and efferent connections. Most of the afferents to DM appeared to be arising from ipsilateral sources except for the NDB, the medial layer of contralateral V, DLM, and the medial and lateral hypothalamic fields, as well as the AVT, LoC, and the 'raphe complex'. DM efferents invaded the contralateral HP and were widely distributed in the ipsilateral telencephalon, except for the nidopallium, mesopallium, and the dorsal basal ganglia. Further projections have enmeshed the septum (bilaterally), numerous diencephalic centers including the dorsal thalamic nuclei and the hypothalamus together with the AVT and GCt, but more caudal efferents have not been detected.

Table 3 Summary of the internal and external wiring of the pigeon HP following anterograde and retrograde tracing as described in Atoji and Wild (2004)

Target or source	*Intrinsic*		*Extrinsic*	
	Afferents	*Efferents*	*Afferents*	*Efferents*
DL	Tr Ma	V Tr Ma	HA, HD, NFL, AV CDL, HL, NDB, VP CoS, SM, SL, SRt AVT, GCt, LoC Raphe (LC) Contralateral HP Bilateral DLM	HA, HD, NFL, PoA AV, TnA, CDL, HL NDB, VP, CoS, SM SL, DLM, VLT, SRt LHy, GLv, GCt
DLd	DM DLv	DM DLv		
DLv	DLd	DLd DM		
DM	DLd V Ma DLv	DLd V Ma	HD, HL, NFL, CDL TuO, VP, nCPa, NDB, SM, SL, Poa, TnA PCT, DLM, LHy, AHP, ML, CoS SRt, PMI, raphe (LC), LoC, SCd Bilateral AVT	BO, HA, HD, HL NFL, BSTL, Ac TuO, VP, CDL CoS, NDB, PoA AV, TnA, PCT, SM SL, DMA, DMP LHy, SCE, ML AVT
Ma	DL DM	DL DM		
Tr	V DL	V DL		
V	DL Tr DM	Tr DM	Contralateral HP	Contralateral HP

AHP, area hypothalami posterioris; CDL, area corticiodea dorsolateralis; CoS, nucleus commissuralis septi; LHy, regio lateralis hypothalami; nCPA, nucleus commissurae pallii; NFL, nidopallium frontolaterale; Poa, nucleus posterioris amygdalopallii; PCT, polus caudalis telencephali; SCd, nucleus subceruleus dorsalis; SRt, nucleus subrotundus; TnA, nucleus taeniae amygdalae; TuO, tuberculum olfactorium.

2.12.3 Neuronal Components of the Avian HP

2.12.3.1 Distribution of Neurochemical Markers

2.12.3.1.1 Neuropeptides and transmitters in the pigeon brain The neurotransmitter and neuropeptide content of the avian HP has been most extensively studied in the pigeon brain (Krebs *et al.*, 1990; Erichsen *et al.*, 1991). Choline acetyltransferase-, serotonin-, and tyrosine hydroxylase-like immunostaining has highlighted the subpial fiber tract passing through the septo-hippocampal junction and along the medial wall of HP. The most intensive fibrous labeling was found in the DM area. Glutamic acid decarboxylase-reactive neurons were sparsely scattered throughout the HP.

The distribution of substance P (SP), leucine (leu5-) enkephalin (LENK), vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), neuropeptide Y (NPY), and somatostatin (SS) was found in fiber tracts invading the septo-hippocampal junction and passing along the subpial wall. NPY-, SS-, and VIP-positive fibers were apparent in the pallial commissure. NPY and SS have seemed to co-localize within the hippocampal neurons, while VIP-positive cells were found dorsal to the SS/NPY cell region. CCK-like immunoreactive terminal baskets were seen around cells of the V-shaped structure. One of the most significant findings of the study was that a circumscribed area, containing SP- and LENK-positive neuropil, was found in a dorsal–lateral region, demarcating clearly the lateral border of HP. The suggested subdivisional pattern, emerging from the distribution of the aforementioned neuroactive substances, has been introduced in the previous section.

2.12.3.1.2 Calcium binding proteins and food-storing Calbindin-like (CB) immunocytochemistry (Figures 1a and 1b) has revealed a polymorphic neuronal population embedded in a dense neuropil. CB immunoreactive (CB+) cells have exhibited a specific topographical distribution throughout the hippocampal complex (review in Montagnese *et al.*, 1993), whereas no similar grouping was observed following parvalbumin or calretinin immuncytochemical stainings. CB+ neurons have delineated three subdivisions, DL, DM and V, largely identical to those described for HP as a result of tract tracing in the zebra finch. Large CB+ neurons (Figure 1a) have been found in the dorsal subdivisions of food-storers (marsh tits and magpies), but not in the HP of nonstorers (great tit and jackdaw, Figure 1b). Hence, it has been suggested that CB+ neurons are responsible for certain aspects of memory formation and/or the remarkable spatial abilities of food-storing songbirds.

Also forebrain ischemia seems to leave the CB+ neuronal elements of the HP largely unaffected (Székely, 1999), whilst the parvalbumin containing cells are severely damaged.

2.12.3.1.3 Neuronal nitric oxide synthase in the avian HP There is a rather small population of hippocampal cells (Székely, 1999), which contain neuronal nitric oxide synthase (NOS). The neuronal complex seems to spread from the edge of the lateral

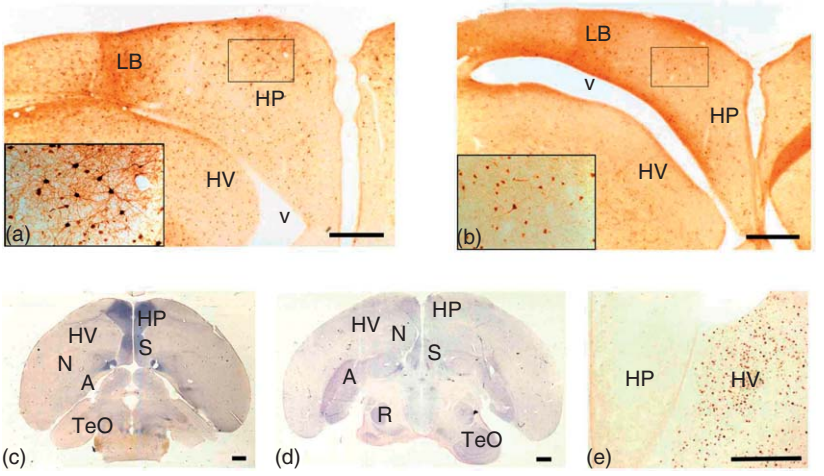

Figure 1 Neurochemical markers of memory formation in the forebrains of storing and nonstoring parids. Calbindin immunocytochemistry in the marsh tit (a) and the great tit (b) telencephalon. The inset in (a) shows a distinct population of immunoreactive neurons located in central HP absent from the nonstorer HP (inset in (b)). NADPH-diaphorase histochemistry gives a high diffuse reactivity within the neuropil of the marsh tit HP and dorsal septum (c), whereas the same staining method results in a significantly weaker neuropil reactivity in the great tit (d). Fos immunoreactive neuronal nuclei in the forebrain of a marsh tit (e); note the low level of labeling in HP, 30 min following cache retrieval. Scale bars: 1 mm.

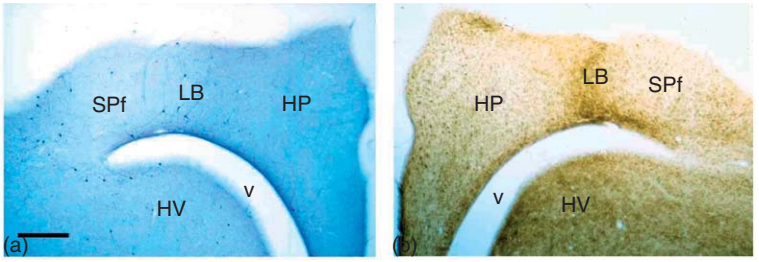

Figure 2 The songbird hippocampal complex demonstrated by: a, NADPH-diaphorase histochemical staining; and b, acetyl cholinestherase histochemistry. Note the prominent lateral boundary (LB). Scale bar: 1 mm.

ventricle to surround the substance P field, thus, clearly demarcating the lateral border of HP (Figure 2a). Within the lateral portion of HP, axonal baskets are found around unlabeled perikarya, with the fibers presumably belonging to the NOS reactive intrinsic cells.

In mammals and reptiles, there is an almost complete co-localization between NOS and GABA within HP, although GABA cells are far more numerous. In birds, the number of NOS reactive neurons is rather low, and only a minor contingent (~25%) contains both neuronal markers.

It is worth noting that in the HP of food-storers (marsh tit, coal tit) the diffuse NOS labeling of neuropil is stronger than in related nonstoring birds (great tit, blue tit, Figures 1c and 1d, respectively); however, no such correlations are apparent in neuronal cell counts (Székely, 1999).

2.12.3.2 Neuromorphological Correlates of Food-Storing Behavior

2.12.3.2.1 Expression of immediate early genes IEGs are thought to be associated with neural plasticity, including memory formation. In a recent account (Smulders and DeVoogd, 2000), the number of cells immunopositive for either of the three IEG products (Fra-1, c-Fos, and ZENK) were counted in order to map neuronal activity in the chickadee HP in relation to food-storing and retrieval. In storing and retrieving chickadees Fra-1-like immunoreactivity seemed to be downregulated in the dorsal HP when compared to controls. According to the present authors, the number of Fos-like immunopositive neurons very probably is associated with the number of items remembered by retrieving birds, while the number of ZENK-like immunoreactive neurons may be a marker of accuracy in cache retrieval. These results imply that neurons expressing different IEG proteins may establish a specific hierarchical order when processing the consecutive stages of food-storing memory. The hypothesis is supported by an earlier study by Székely *et al.* (1992), where hardly any Fos immunolabeled neurons were detected in the HP (Figure 1e) of marsh tits following a single storing session. It is therefore possible that storing experience alone does not trigger the expression of c-fos in HP neurons, but needs reinforcement, that is, retrieval of the cached item using spatial memory.

2.12.3.2.2 Glutamate receptor binding In an earlier study, quantitative receptor autoradiography was used to localize NMDA-sensitive [3H]glutamate, [3H]MK801, and [3H]alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) binding sites (Stewart *et al.*, 1999), in the forebrain of food-storing marsh tits and nonstoring blue tits. Although similarly high levels of labeling were apparent to both NMDA and AMPA receptors in both species, binding to NMDA ion channel sites appeared to be significantly lower (20%) in the HP in food-storers than in nonstorers. Other selected forebrain regions expressed remarkably similar levels of binding in the two species.

A recent investigation (Shiflett *et al.*, 2004) has assessed whether long-term memory for spatial locations requires NMDA receptor-dependent synaptic plasticity in food-storing birds. Black-capped chickadees were given bilateral infusions of the NMDA receptor antagonist AP5 into the HP, and their spatial memory was assessed. Inactivation of NMDA receptor during learning prevented the formation of long-term spatial memories but did not affect short-term memory or retrieval. Most notably, NMDA receptor inactivation immediately following learning did not interfere with the formation of long-term memory, thus suggesting that NMDA receptors may play a role both in the incorporation of new information into an already existing long-term memory, as well as in the formation of unitary long-term memories.

2.12.3.2.3 Adaptive modification of hippocampal size as a result of food-storing According to a widely accepted hypothesis, HP is larger in many birds and mammals that store food than in related

nonstorer species (Krebs *et al.*, 1989; Colombo and Broadbent, 2000; Shettleworth, 2003). The three-way association linking spatial memory, food-storing behavior, and the volumetric changes experienced in HP enable these birds to survive under harsh weather conditions because the duration of time over which they can remember spatial information is largely increased. Thus, the enlarged memory capacity is paralleled by an increase in relative hippocampal volume compared to the rest of the brain. There is, apparently, a certain difference in the HP/ telencephalon ratio between birds specified as large-scale or low-scale storers. Avian species, exhibiting a higher storing activity will have an HP relatively larger than those species which store less food (Healy and Krebs, 1992, 1996; Hampton *et al.*, 1995). Furthermore, the different latitudinal distribution of members of the same species (e.g., black-capped chickadees) seems to influence their storing activities accompanied by a consequent difference in hippocampal volume. Alaska chickadees cache more food and have a significantly larger HP, containing more neurons, when compared with Colorado chickadees (Pravosudov and Clayton, 2002).

The volume of HP shows seasonal alterations in relation to storing or nonstoring periods during the year (Krebs *et al.*, 1995). In the autumn, some food-storing birds spend more time caching, store more items, and leave their caches for longer periods than they do in the spring. Correspondingly, these birds have a larger HP in the autumn than in the spring and, together with the volumetric changes, there is an increase in hippocampal neuronal number. Thus, a higher rate of cell birth, or neurogenesis, has to occur in the autumn when caching activity is highest (Barnea and Nottebohm, 1994).

2.12.3.2.4 Neurogenesis In general, cells are continuously born within the ventricular zone of the avian brain and migrate into the deeper areas to differentiate into neurons. In a study by Patel *et al.* (1997), it has been shown that the rate of cell proliferation within HP and the hyperpallium ventrale (HV) is influenced by behavioral experience. Juvenile birds were allowed to store and retrieve food regularly between days 35 and 56, while controls were prevented from food-storing. Experienced birds have expressed a higher rate of cell proliferation (3.9–10%) and an increase in total cell number, presumably due to the higher rate of neurogenesis. Neuronal recruitment into HP may therefore occur in order to address the memory demands associated with storing and thus the subsequent behavior will be facilitated by hippocampal growth.

It has to be noted that neuronal recruitment seems to vary not only with experience (i.e., aging) but also expresses seasonal changes (Barnea and Nottebohm, 1994) with the different subregions exhibiting different rates of neurogenesis (the rostral-most portion contains most of the new cells).

It is, however, not yet clear whether in the experienced adult animals such changes of spatial memory should also be accompanied by morphological alterations and/or a higher cell proliferation. Cristol (1996) has found no detectable differences between experimental and control groups of adult storing willow tits, in terms of volume, neuron density, or total neuron number within their HP. Hoshooley and Sherry (2004) have reported similar findings for the hippocampal cellular recruitment of black-capped chickadees. Birds, caught from the wild during the winter, were injected with 5-bromo-2′-deoxyuridine and then neurogenesis, apoptosis, neuron number, and hippocampal volumes were determined. Chickadees collected in October, November, January, February, or March did not show alterations in any of the studied aspects of neuronal plasticity. Thus, the findings seem to suggest that increases in neuronal recruitment in the fall do not result from a higher neuron proliferation but, rather, the prolonged survival of previously born 'new' neurons.

Spatial memory performance was found to be inferior in socially subordinate food-storing birds (among mountain chickadees, Pravosudov and Omanska, 2005) whilst no significant differences in volume or the total number of neurons in the HP have been verified between dominant and subordinate chickadees. Nonetheless, subordinate birds had lower cell proliferation rates in the ventricular zone compared to dominants, thus suggesting that social status may affect cell proliferation rates and support the hypothesis that neurogenesis might be involved in memory function also in adult animals.

2.12.4 Evolutionary Considerations

Birds living in environments that produce food in abundance during one season of the year, and relatively little (or none) during others, may cope with the uneven supply in several different ways. One strategy is to leave the area for the periods of shortage, as many birds do in migrating from cold or polar regions to the relatively food-rich and warmer tropical areas for the winter. The perils of migration are accepted in return for the continuity of food supply. An alternate approach is to collect more

food than needed during periods of seasonal abundance for subsequent storage. This strategy, too, has its challenges, the cache may be pilfered while the birds must find enough fuel to maintain their relatively high daytime body temperature and to replenish the fat used during the nights. Food-storing is essential for the survival of winter for most parids, since they do not accumulate large fat reserves like finches. Nevertheless, being slim has a distinct advantage, plump redpolls or purple finches lack the mobility of titmice and are more likely to fall victims to predators.

A learning mechanism is able to detect and store information about casual relationships. Based on this definition, the environment should confer fitness in direct proportion to the assessment of causality by any network in which we would like to evolve the capacity to learn. Evolution should therefore act to set up the machinery which will subsequently allow an association to be formed, and these associations should be formed within the lifetime of a single individual.

Nonetheless, such changes in behavior will certainly need to be accompanied by an enlarged memory capacity to successfully localize the (sometimes) several hundred food caches. In food-storers, the neuronal basis of memory formation (i.e., HP) seems to express an adaptive specialization (increase in relative hippocampal volume) in parallel with the evolutionary changes of behavior. Comparative studies within the parids and corvids suggest two kinds of memory specialization associated with food-storing. Some storer species have a more accurate and enduring spatial memory, together with a strong preference to rely more upon spatial cues than some related nonstorers. Furthermore, some storing parids and corvids appear to be more resistant to memory interference by remembering additional information about the contents and status of cache sites. Still, the question remains as to how the HP is able to address such a complex task alone? Certainly, HP appears to be a key figure (or none at all, as suggested by Macphail and Bolhuis, 2001) of the underlying neuronal structure, a multiconnected neuronal network of several brain areas. It is possible that adaptation to a specialized behavior may lead to the coordinated evolution of several interconnected neural structures resulting in the evolution of the entire brain (Garamszegi and Eens, 2004), thus suggesting that the evolutionary shift from nonstoring toward food-storing might have been accompanied by robust evolutionary changes in brain volume during the long-term phylogenetic history of birds.

In fact, little is known about the role of telencephalic or subtelencephalic centers, apart from that of the HP in mediating food-storing behavior. Shiflett *et al.* (2002) have found septal specialization to parallel hippocampal changes in storing chickadees.

Finally, the question arises whether the concerted evolution of different brain structures leading to volumetric changes has been induced simultaneously but independently in storers or, instead, their behaviors are emerging from common ancestry. The apparently very close phylogenetic relationship between two food-storer families, Paridae and Sittidae (Sibley and Ahlquist, 1990), suggests that storing arose in their common ancestor (Brooks and McLennan, 1990). As food-storing is rather widespread in the animal kingdom, and espesially in Paridae and Sittidae (Vander Wall, 1990), the 'common origin' hypothesis is evolutionarily more feasible than the one of multiple origins (Brooks and McLennan, 1990).

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2.13 Sex and Species Differences in Hippocampal Volume

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Glossary

higher vocal center (HVC)	A region in the songbird brain that is involved directly in song production and indirectly in song learning.
lateral line	Sensory cells arranged either in groups or in rows found on the sides and head of fish, which enable them to detect changes in water pressure and very low frequency sounds (<100 Hz).
mental rotation	An experimental test procedure in which subjects are shown an object for a short period of time and then asked to choose from a number of similar alternatives, which is the same object rotated.
natural selection	The differential survival and reproduction of individuals that vary in a heritable trait. Via natural selection, traits that increase reproductive success will increase in frequency in the population through time.
ontogeny	The process of development from the fertilized egg, to the embryo, through to adulthood.
prepubescence	A juvenile stage, beyond infancy but not yet sexually mature.

The hippocampus in mammals (the dorsomedial forebrain in birds) is considered by many to be the brain region that is heavily involved with processing of spatial information. Much of the traditional support for this hypothesis came from lesion studies, mostly in rodents, which showed that damage to the hippocampus affected the animal's ability to solve cognitive tasks with a spatial component, while not affecting their ability to solve other cognitive tasks without a spatial component.

While there continues to be much debate over the degree to which the considerable lesion data from rodent studies, in particular, provide support for the involvement of the hippocampus in spatial information processing, an alternative avenue is proving useful for investigating hippocampal function. This is the use of another traditional approach, comparative neuroanatomy, framed within an evolutionary context. The underlying assumption of this work is that variation in hippocampal structure, between and, perhaps, within species, is due, to a significant degree, to variation in the selective pressure imposed on those species, via the physical and biological environment in which they live. In other words, natural selection has shaped cognitive regions of the brain just as it has moulded parts of the brain that are concerned solely with specific sensory information (e.g., visual and olfactory areas in primates/mammals: Barton *et al.* 1995; Barton and Harvey, 2000; olfactory bulbs in birds: Healy and Guilford, 1990).

2.13.1 Ecology and Sensory Brain Structures

In the late 1970s and early 1980s, there was a flurry of studies showing that variation in brain size in both birds (see Forebrain Size and Social Intelligence in Birds) and mammals was correlated with variation in the lifestyles of those animals. For example, frugivorous primates have larger brains than do folivorous or insectivorous primates (Harvey *et al.*, 1980). Whole brain size in birds, however, is not correlated with diet but rather with ontogenetic pattern – altricial birds (those that are born with their eyes shut and are totally dependent on parental care for at least the first few weeks of life, e.g., starlings, *Sturnus vulgaris*, sparrows, *Passer domesticus*) have larger brains as adults than do precocial birds (birds that, within a

few hours of hatching, are mobile and semi-independent of their parents, e.g., chickens, grouse, and quail). It soon became clear, however, that using whole brain size as a means of understanding the role of natural selection in shaping variation among species was only the beginning and, as neuroscientists had long known, greater understanding would be achieved only when component parts of the brain were the focus of attention (Mace *et al.*, 1980).

Within the brain it is relatively straightforward both to see and to understand that sensory structures are correlated with the lifestyle of an animal. Animals that live in the dark, for example, have to cope with the difficulty of seeing their surroundings, finding food, mates, home, and so on. This problem has been solved in a variety of ways: many bats detect prey via echolocation, owls use both enlarged eyes and highly sensitive hearing, kiwis (flightless, nocturnal birds from New Zealand) have a highly developed sense of smell, electric fish (as their name implies) use electric fields to find their way around, and, in the complete dark, as is the problem for Mexican cave fish, eyes have disappeared altogether to be replaced by a very sensitive lateral line system (for more details see de Perera and Braithwaite, 2005). In some cases, the system appears to have developed specifically in response to low lighting (e.g., echolocation); in others, there has been an increase in one sensory system at the cost of another (e.g., kiwis, cave fish). The change in brain structure as a result can be striking; for example, a third of a kiwi brain is occupied by the olfactory bulb, in contrast to a diurnal species such as a starling, in which the olfactory bulb is almost nonexistent. It should be noted, however, that even in starlings with such tiny olfactory neural structures, there is a functional sense of smell, enabling these birds, for example, to detect the volatiles given off by plants they use for their antiseptic qualities, to line their nests and thus reduce the parasite load for their nestlings (Gwinner, 1997; Gwinner *et al.*, 2000). The point here is that, although the size of structure can be taken as a measure of the amount of use to which the animal puts that structure, even very small structures are likely to play an important role in the animal's life. There is little evidence that there are brain regions that are vestigial in the way the appendix is thought to be.

2.13.2 Ecology and the Hippocampus

Like the relationship between ecology and the sensory apparatus in the brain, there is growing evidence that the volume occupied by cognitive brain regions can also be explained to a significant degree by an animal's lifestyle. The hippocampus is one of these areas for which there is considerable evidence that the demand for a specific cognitive ability is associated with an enlargement of the relevant brain region (see Evolution of the Hippocampus). In all of the cases to date, animals that have a more than usual need for spatial information processing have a larger hippocampus for their brain size than do closely related species that do not depend so heavily on spatial information. For example, migratory birds have a larger hippocampus than do birds that do not migrate (Healy *et al.*, 1991). The increase in hippocampus size is not related to the distance the birds fly but rather to the fact that they must remember details (e.g., relevant landmarks) of the stopping over places en route and both of the endpoints (the breeding grounds and the overwintering grounds). These differences are apparent within species: migrant dark-eyed juncos, *Junco hyemalis*, have both better spatial memory and more densely packed neurons in the hippocampus than do nonmigratory dark-eyed juncos (Cristol *et al.*, 2003). Older, experienced garden warblers, *Sylvia borin*, have larger hippocampal volumes than do young, inexperienced birds. While age itself contributes to some of this increase in volume, the experience of migration is a major component, as shown by changes in hippocampal volumes in birds kept in captivity for 12 months (Healy *et al.*, 1996).

Frequent, short-distance trips also seem to be spatially demanding. Homing pigeons have larger hippocampal volumes than do breeds of pigeons selected for attractive morphological features (Rehkamper *et al.*, 1988). Pigeons have provided much of the direct evidence for avian hippocampal function: an intact hippocampus is essential for learning the geometrical features of a landscape (Vargas *et al.*, 2004). Recording from cells in the hippocampus has shown that cells in the left hippocampus are responsive to nearby landmarks, whereas those in the right hippocampus respond to global cues around the experimental arena (Bingman *et al.*, 2003). The effects of lesions to each half of the hippocampus are consistent with the cell recording data: pigeons with left hippocampal lesions use global cues to solve a task, whereas right hippocampus-lesioned birds used local landmarks (Kahn and Bingman, 2004). As yet, however, there is no evidence for an equivalent cell type to the place cells found in rodent hippocampus (Bingman *et al.*, 2003).

Like homing pigeons, scatterhoarding birds, by dint of their response to excess food, constantly face a considerable spatial memory task. Species such as the black-capped chickadee, *Poecile*

atricapillus, for example, hide large numbers of food items, often singly, scattered throughout their territory. They may not retrieve this food for several hours, days, weeks, or months. Although considerable numbers of stores are made, these birds use memory to retrieve those stores (e.g., Sherry, 1984). Lesions to the hippocampus of black-capped chickadees result in the birds being unable to use spatial cues, in this case, to relocate food (Sherry and Vaccarino, 1989). As the birds can use color cues to find food, it appears that the hippocampus is involved specifically in the memory for locations.

A number of subsequent studies have shown a relationship between hippocampal volume and food storing. Food-storing species have a larger hippocampus than do nonstorers (Krebs *et al.*, 1989; Sherry *et al.*, 1989; Garamszegi and Eens, 2004; Lucas *et al.*, 2004; see The Hippocampal Formation in Food-Storing Birds). There is also a positive relationship between the degree of food storing and hippocampal volume, such that birds that store more and/or for longer have larger hippocampal volumes (Healy and Krebs, 1992; Hampton *et al.*, 1995; Basil *et al.*, 1996; Healy and Krebs, 1996). The difference in volume is due to an increase in the number of cells in the hippocampus, not to the size of the cells. As in the juncos that differed in migration propensity, black-capped chickadees that differ in dependence on food stores also differ in hippocampal volume: chickadees from Alaska have better spatial memory and larger hippocampus than do chickadees from Colorado, where the conditions the birds live under are significantly less harsh than they are in Alaska (Pravosudov and Clayton, 2002). All of the species tested differ both in the number of items stored and in the duration before they retrieve their food, so it is not clear from the hippocampal morphology whether or not an enlarged hippocampus confers specific spatial cognitive abilities. Some experimental data suggest that for some food storers, at least, an enlarged hippocampus specifically enables them to remember even small amounts of information for longer than can nonstorers (Biegler *et al.*, 2001).

Food storing is also common throughout mammals, although much of it is best described as larder hoarding in which the animals are storing all of their excess food in one or very few places. However, there are some mammalian scatterhoarding species and from the few data that currently exist, it appears that hippocampal volume in these animals is, like the birds, correlated with the presence/absence of food storing (e.g., Dipodomys kangaroo rats: Merriam's and bannertails; Jacobs and Spencer, 1994). It is not yet clear whether these hippocampal differences and ecological differences are also associated with variation in cognitive ability (as assessed experimentally).

2.13.3 Development

In food-storing birds, hippocampal volume increases once the birds have left the nest, which is when they begin to store and retrieve food (Healy and Krebs, 1993; Healy *et al.*, 1994). This has been shown experimentally to be due to the experience of storing and retrieval (Clayton and Krebs, 1994; Clayton, 2001). However, it is also the case that experience of a very similar kind to that of food storing, such as a food finding task that requires memory of those locations for successful return, may also affect hippocampal growth (Clayton, 1995). This effect has, so far, only been found in the food storing species. Nonstorers with the same experience do not achieve an enlarged hippocampus as a result. This may indicate some underlying propensity for response to certain kinds of cognitive experience that has been selected for in the food storers. This could be, for example, the ability to respond with increased neurogenesis or decreased apoptosis (Clayton and Krebs, 1994).

Far more is known about development and changes to hippocampal structure in mammals, particularly rodents. Unlike the interspecific differences in hippocampal morphology seen in birds, it is clear in mammals that actions of gonadal hormones (such as estrogen and testosterone) while the animal is *in utero* have significant and long-lasting effects on both hippocampus and spatial cognition (so-called organizational effects). Neonatal castration of male rats results in spatial cognition performance like that of females, while provision of estradiol (an androgen metabolite) to neonatal female rats enhances their spatial cognition as adults to levels indistinguishable from those of normal males (Williams *et al.*, 1990; Williams and Meck, 1991). Exposure to testosterone *in utero* via placental circulation also enhances the spatial cognitive abilities of female rats born into litters with more males than females, while those females born with more sisters than brothers have poorer spatial cognition (Williams and Meck, 1991). This effect of litter sex ratio has also been found in meadow voles (Galea *et al.*, 1994b) but not in gerbils (Sherry *et al.*, 1996). Experimental application of testosterone to young male and female rats enhances female spatial cognition but appears to impair that of males (Roof, 1993b).

Such ontogenetic effects of gonadal hormones are also apparent in humans: girls exposed to high levels of androgens tested on spatial tasks perform better

than do girls exposed to low levels (Hampson *et al.*, 1998).

The mechanism via which testosterone may act can be investigated by using an aromatase blocker to prevent the breakdown of testosterone into estradiol. Application of aromatase blockers to neonatal male rats results in significantly impaired spatial cognition. In rodents, at least, it appears that estradiol is the active agent controlling spatial cognition. There is little evidence from birds, one way or the other, that there is an organizational effect of gonadal hormones on adult spatial cognition. However, estradiol has been shown to have a small but significant positive effect on spatial cognition in zebra finches (Oberlander *et al.*, 2004).

Concomitantly with the organizational effects of gonadal hormones on spatial cognition are the effects on hippocampal structure. Prepubescent normal male and female rats differ in hippocampal morphology: the dentate gyrus cell layer of the hippocampus is thicker in males than it is in females (Roof, 1993a). There is also a difference between hemispheres: the layer in the right hippocampus is significantly thicker than that in the left, again only in males. The lateralization appears similar to that seen in the pigeons, although it is not clear whether there is a sex difference in the birds. The right hippocampus is larger than the left in human children but there are no differences between the sexes in total hippocampal volume (Giedd *et al.*, 1997). In spite of the rat data described above, this lack of a sex difference in prepubertal mammals is rather common (but see Kerns and Berenbaum, 1991), although still underexplored. Also, there are few data demonstrating sex differences in spatial cognition prior to sexual maturation in any mammal (e.g., Galea *et al.*, 1994a; see also Voyer *et al.*, 1995; Sherry and Hampson, 1997).

Testosterone administration to female rats in the first postnatal week results in male-like hippocampal structure, both in size and in lateralization (Roof and Havens, 1992). Testosterone administration to males does not increase the cell layer; if anything, it is decreased as a result. The increased thickness of the dentate gyrus cell layer is due to an increase in the number of cells and not to cell size. Neonatal castration of male rats results in a decrease in dendritic spine density in the hippocampus relative to those seen in females or intact males (Meyer *et al.*, 1978; Juraska *et al.*, 1988). Dendritic tree morphology is also affected by the environment in which young rats are raised: enriched environments lead to denser dendritic tree branching than do impoverished environments (Juraska *et al.*, 1985). There is also evidence that stressed pregnant rats shift the timing of testosterone delivery to a slightly earlier stage of gestation and that this effect has a significant, negative impact on maze performance in sons born to these females. This effect may be due to a decrease in hippocampal corticosteroid receptors (Szuran *et al.*, 2000). In rhesus monkeys, however, prenatal stress did not affect hippocampal volume of the resulting offspring (Lyons *et al.*, 2001).

2.13.4 Sex Differences

As will be clear from the developmental data described above, gonadal hormones affect hippocampal structure and spatial cognition during early ontogeny and into early postnatal stages in mammals. These effects result in differences between males and females as adults, at least in some species. In meadow voles, *Microtus pennsylvanicus*, and deer mice, *Peromyscus maniculatus*, for example, males have larger hippocampal volumes than do their conspecific females (Gaulin and FitzGerald, 1986, 1989; Galea *et al.*, 1996). Pine and prairie vole males, *M. ochrogaster* and *M. pinetorum*, on the other hand, do not show any sex differences in hippocampal volume. These hippocampal differences are also associated with differences in spatial cognition and in the ecology of the animals: deer mice and meadow voles are polygynous (mate with more than one female) and have home ranges that encompass the ranges of more than one female, whereas pine and prairie voles are monogamous with a home range that matches that of their mate. It is not clear whether the difference in mating system or in home range size has been the selection pressure resulting in these correlated differences, as both are completely confounded in all of the species examined thus far (see Jones *et al.*, 2003, for review of the status of the rival evolutionary theories).

One of the few instances of selection for enhanced female spatial cognition and hippocampal volume comes from birds: nest parasitic brown-headed cowbird females, *Molothrus ater*, which lay their eggs in the nests of other species (like cuckoos), scout out potential nests prior to hosts beginning to lay and return at a propitious moment (e.g., once the host female has laid at least one or two eggs of her own and at a time of day when the host female is away from the nest), to lay their own egg in among those of the host. As the female cowbird may visit more than 40 potential host nests prior to the hosts laying, it is thought that she may have to have quite a good memory in order to go back directly, but sneakily, so that the host remains unaware of the interloper in her nest. The male brown-headed cowbird, meanwhile, does nothing with regard to searching for

host nests and spends his time hanging out with other conspecific males. In this species, it is the female that has the larger hippocampus (Sherry *et al.*, 1993). Shiny cowbird females also scout alone for host nests and they, too, have larger relative hippocampal volumes than do their conspecific males (Reboreda *et al.*, 1996). Interestingly, there is a cowbird species in which both males and females search for nests (the screaming cowbird). There are no differences in hippocampal volume between the sexes but they have larger hippocampal volumes than do male shiny cowbirds and than either male or female baywinged cowbirds, which are not nest parasites. The degree to which the sex differences are specific to particular behaviors can be seen when comparing volumes of song nuclei: in all three species (shiny, screaming, and baywinged), males have larger higher vocal centers than do their conspecific females (Hauber *et al.*, 1999), consistent with the observation that males of all three species use song in sexually selected contexts (i.e., mate choice and territory defense).

2.13.5 Activational Effects of Gonadal Hormones

Although it seems likely that organizational hormone levels contribute to the sex differences seen in these species, there are multiple instances of adult sex differences in spatial cognition and hippocampal structure that are more obviously coupled with variation in circulating plasma gonadal hormones during adulthood (so-called activational effects). In deer mice, for example, the sex differences in spatial cognition and hippocampal structure are seen only in the breeding season (also when range size is sexually dimorphic). Outside the breeding season, the differences no longer exist. Sexual selection, mediated by circulating gonadal hormones (specifically testosterone, in these cases), seems to play a major role in this brain/cognition/behavior relationship (Gaulin and FitzGerald, 1986). It is not clear why this selection pressure should not have had so significant an impact on pine voles.

Seasonal differences in hippocampal volume are also apparent in the cowbirds described above: the sex differences seen in shiny cowbirds during the breeding season are not visible in the nonbreeding season (Clayton *et al.*, 1997). Seasonal variation in hippocampal volume that is not correlated with breeding has been seen in black-capped chickadees. Intensity of food-storing peaks in this species in the autumn, and there are correlated changes in hippocampal structure, both in volume and in the longevity of hippocampal neurons born during that season: the hippocampus is larger and autumn-born neurons live longer than do spring-born neurons (Barnea and Nottebohm, 1994; Smulders *et al.*, 1995). The volume of the septum, which shares reciprocal connections with the hippocampus, changes in a similar seasonal fashion in black-capped chickadees but not in two nonstoring species, blue tits, *Parus caeruleus*, and great tits, *P. major* (Shiflett *et al.*, 2002). These effects do not occur across the brain, as the nucleus of the diagonal band does not change seasonally.

In humans, there is less evidence for circulating testosterone affecting spatial cognition or hippocampal structure than there is that estrogen has an impact, especially on female spatial ability. Women's spatial ability is better (on tests, for example, such as mental rotation) during menstruation, when estradiol levels are lowest (e.g., Hampson, 1990; Hausmann *et al.*, 2000). However, data on changes in hippocampal structure in women are, unsurprisingly, scanty. The data from rats are inconsistent. Some studies have found improved spatial performance with high levels of estrogen (e.g. Healy *et al.*, 1999), others with low levels (Frye, 1995; Warren and Juraska, 1997) and yet others (e.g., Berry *et al.*, 1997) have found no effect. All of the morphological evidence points to a negative effect of estrogen on hippocampal morphology: pregnant rats tend to have smaller hippocampal volumes than do nonpregnant rats and dendritic spine density decreases by as much as 30% on estrous days (Gould *et al.*, 1990). In female meadow voles, hippocampal cell proliferation is lowered when estradiol is high (Galea and McEwen, 1999). It is not yet clear why there is such discrepancy in the rodent data as to the relationship between estradiol, hippocampal morphology, and spatial cognition, although it may be, in part, due to a nonlinear relationship between gonadal hormone level and spatial cognition. It may also be that the task used to assess cognition, or the level of stress the animals are under at test, may also contribute to the observed variation. That estrogens are involved via their receptors in the mammalian hippocampus is clear (e.g., Rissman *et al.*, 2002). It is less clear what role gonadal hormones play in the variation in hippocampal morphology in the bird examples.

2.13.6 Summary

There are a multitude of examples of variation in hippocampal volume, both within and between species. In mammals, the best studied of these is the sex difference, which appears to be primarily caused by

effects of gonadal hormones present when the fetus is *in utero*, although circulating levels during adulthood also have an impact. The particular selection pressure or pressures that have brought about this variation is still under debate. In birds, there is less evidence, as yet, for a role of gonadal hormones in changing hippocampal morphology, although there is considerable evidence for differences that are correlated with variation in demand for spatial cognition. Investigation of hippocampal structure in a wider range of mammalian species and of the effects of gonadal hormones on hippocampal structure and function in birds may help to resolve these issues.

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2.14 Evolution of the Amygdala in Vertebrates

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Glossary

agonistic behaviors Behaviors expressed during confrontation with conspecifics for resources, territory, or mates. This usually leads to aggressive/defensive behaviors different from prey–predator interactions. In territorial species, encounters with adult conspecifics can be either agonistic or reproductive.

conditioning, instrumental (or operant) This is a type of associative learning in which an unlikely motor response to a given clue (such as pressing a lever when the animal sees it) is associated with a reward (e.g., food) or to absence of punishment. The chances of showing the motor response augment due to its association with the reward. This can be interpreted as a stimulus–response association (detection of the lever – pressing it) due to reinforcement (food consumption).

conditioning, Pavlovian (or classical) As opposed to instrumental conditioning, in this kind of associative learning two stimuli called unconditioned (US) and conditioned stimulus (CS), are associated. The US renders an automatic response (usually not voluntary or not conscious), which is part of the repertoire of behaviors specific to that species (for instance, salivation, increased blood pressure). This is called unconditioned response. Repeated presentation of the CS together with the US leads to an

association of the former to the latter that results in a conditioned response, e.g., the CS alone provokes the response that was elicited by the US.

fear and anxiety Fear is an acute reaction to potentially harming stimuli that consists of behavioral (e.g., freezing, potentiated startle), vegetative (e.g., increased blood pressure, tachycardia) and endocrine responses (surge in epinephrine/norepinephrine, adrenocorticotropic hormone, and corticosteroid levels). Anxiety is a sustained reaction in advance to a potentially harming but ill-defined outcome.

hodology Study of pathways. In neurobiology, hodology is used to define the study of the interconnections of brain cells and areas.

homeotic genes A set of genes characterized by a sequence (homeobox) whose product constitutes a DNA-binding homeodomain. This binding to DNA mediates their ability to modulate or control transcription of other genes (in other words, homeotic genes code transcription factors). The expression of homeotic genes during early development determines the main positional coordinates of the different body parts, thus being fundamental for morphogenesis.

pheromone Substance secreted or excreted by an animal that has an intrinsic biological significance for conspecifics, thus inducing an unconditioned behavioral or neuroendocrine response. Although it is assumed that pheromones are mainly detected by the vomeronasal organ, it has not been demonstrated in every instance. Moreover, the vomeronasal organ also detects signals from common predators or preys, which obviously cannot be considered pheromones. Finally, some pheromones have been shown to be detected by the main olfactory system.

reinforcement, reward Etymologically, reinforcement means strengthening. In psychological terms, reinforcement designates the increase in the probability of expressing a given behavior induced by the positive outcome that this behavior renders, in the context of instrumental conditioning. Reinforcers (stimuli-reinforcing behaviors) are usually perceived as pleasant or rewarding, so that in many instances rewarding is used as synonymous with reinforcing.

stimulus–reward association Associative learning in which an initially neutral stimulus that precedes or is presented together with a reinforcer, becomes rewarding (secondary or conditioned reinforcer). After this association, the animal would work for the secondary reward.

topography Physical relationships of regions in the adult brain. For example, *A* is topographically dorsal to *B* if, in histological sections of the adult brain, *A* occupies a location nearer the dorsum than *B*.

topology In terms of anatomy, this refers to physical relationships of regions based upon their developmental history. Thus, when a structure *A* is said to be topologically deep to structure *B*, it is means that *A* and *B* derive from the same region of the embryonic neuroepithelium. Topology and topography may not coincide: a structure may be topologically lateral to another, but, due to a flexure of the medio-lateral axis, appears (topographically) medial to it in the adult brain.

Abbreviations: Mammals

AA Anterior amygdaloid area (AAD: dorsal; AAV: ventral).

AB Accessory basal (or basomedial) nucleus of the amygdala (ABa: anterior; ABp: posterior).

Acb Nucleus accumbens.

AHA Amygdalohippocampal area (or posterior nucleus of the amygdala).

APir Amygdalopiriform transition area.

AStr Amygdalostriatal transition area.

B	Basal (or basolateral) nucleus of the amygdala (Ba: anterior; Bp: posterior; Bv: ventral).
BAOT	Nucleus of the accessory olfactory tract.
BST	Bed nucleus of the stria terminalis.
Ce	Central amygdala.
CeC	Capsular or paracapsular division of the central amygdala.
CeL	Lateral central amygdala.
CeM	Medial central amygdala.
Cl	Claustrum.
COAa	Anterior cortical amygdala.
COApl	Posterior lateral cortical amygdala or periamygdaloid cortex.
COApm	Posterior medial cortical amygdala.
CPu	Caudate putamen (striatum).
CxA	Corticoamygdaloid transition.
DEn	Dorsal endopiriform nucleus.
DG	Dentate gyrus.
EA	Extended amygdala (CeEA: central; MeEA: medial).
I	Intercalated nuclei of amygdala.
IPAC	Interstitial nucleus of the posterior limb of the anterior commissure.
L	Lateral nucleus of the amygdala.
LGP	Lateral globus pallidus.
lot	Lateral olfactory tract.
LOT	Nucleus of the lateral olfactory tract.
Me	Medial amygdala.
MeA	Anterior medial amygdala.
MeP	Posterior medial amygdala (MePV: ventral; MePD: dorsal).
MGm	Medial division of the medial geniculate nucleus.
Pir	Piriform cortex.
PMv	Ventral premammillary nucleus.
SI	Substantia innominata.
SN	Substantia nigra.
st	Stria terminalis.
TR	Postpiriform transition area.
VEn	Ventral endopiriform nucleus.
VMH	Ventromedial nucleus of the hypothalamus.
VTA	Ventral tegmental area.

Abbreviations: Reptiles

ac	Anterior commissure.
Acb	Nucleus accumbens.
ADVR	Anterior dorsal ventricular ridge.
Amb	Nucleus ambiguus.
aot	Accessory olfactory tract.
BAOT	Nucleus of the accessory olfactory tract.
BST	Bed nucleus of the stria terminalis.
BSTl	Dorsolateral (supracommisural) division of the BST.
BSTm	Ventromedial (subcommissural) division of the BST.
DC	Dorsal cortex.
DLA	Dorsolateral amygdaloid nucleus.
dLC	Deep lateral cortex.
DMX	Dorsal motor nucleus of the vagus.
DSt	Dorsal striatum.
GP	Globus pallidus.
LA	Lateral amygdala.
LC	Lateral cortex.
LCc	Caudal lateral cortex.
lfb	Lateral forebrain bundle.
LHN	Lateral posterior hypothalamic nucleus.
lot	Lateral olfactory tract.
MA	Medial amygdala.
MC	Medial cortex.
NAOT	Nucleus of the accessory olfactory tract.
NLOT	Nucleus of the lateral olfactory tract.
NS	Nucleus sphericus.
PB	Parabrachial nucleus.
PDVR	Posterior dorsal ventricular ridge (PDVRv: ventral; PDVRdl: Dorsolateral; PDVRdm: dorsomedial).
PMv	Ventral premammillary nucleus.
S	Septum.
SAT	Striatoamygdaloid transition area (SATm: medial; SATl: lateral).
sm	Stria medullaris.
sol	Nucleus of the solitary tract.
st	Stria terminalis.
VAA	Ventral anterior amygdala.
Vds	Nucleus descendens nervi trigemini.
VMH	Ventromedial nucleus of the hypothalamus.
VP	Ventral pallidum.
VPA	Ventral posterior amygdala.
zl	Zona limitans.

Abbreviations: Birds

AA	Anterior arcopallium (anterior archistriatum).
Acb	Nucleus accumbens (medial aspect of the lobus paraolfactorius).
AD	Dorsal arcopallium (dorsal intermediate archistriatum).
AM	Medial arcopallium (medial archistriatum).

APH	Parahippocampal area.
AV	Ventral arcopallium (ventral intermediate archistriatum).
Bas	Nucleus basorostralis pallii.
BSTl	Lateral bed nucleus of the stria terminalis (called BST or nucleus accumbens depending on the authors).
BSTm	Medial bed nucleus of the stria terminalis.
CDL	Area corticoidea dorsolateralis.
CPi	Cortex piriformis.
E	Entopallium.
FA	Tractus frontoarcopallialis (frontoarchistriatalis).
GP	Globus pallidus (paleostriatum primitivum).
H	Hyperpallium.
Hp	Hippocampus.
INP	Intrapeduncular nucleus.
L	Field L of the NC.
LAD	Lamina arcopallialis dorsalis.
lfb	Lateral forebrain bundle.
LM	Lamina mesopallialis.
LPS	Lamina palliosubpallialis.
LSt	Lateral striatum (paleostriatum augmentatum).
M	Ventral mesopallium (hyperstriatum ventrale).
MSt	Medial striatum (lateral aspect of the lobus paraolfactorius, just medial to the paleostriatum).
N	Nidopallium (neostriatum).
NC	Caudal nidopallium, usually divided into lateral (NCL) and medial divisions (NCM).
OB	Olfactory bulb.
OM	Occipitomesencephalic tract.
OMH	Occipitomesencephalic tract, hypothalamic part.
PoA	Posterior nucleus of the pallial amygdala (posterior archistriatum).
Rt	Nucleus rotundus.
S	Septum.
SpA	Subpallial amygdala (ventral paleostriatum).
TnA	Nucleus teniae of the amygdala.
TPO	Area temporoparieto-occipitalis of the cerebral hemispheres.
tsm	Tractus septopalliomesencephalicus.
VP	Ventral pallidum.

Abbreviations: Amphibians

Aa	Anterior amygdala.
BST	Bed nucleus of the stria terminalis (BSTr: rostral; BSTc: caudal).
Ca	Central amygdala.
Dp	Dorsal pallium.
La	Lateral amygdala.
lfb	Lateral forebrain bundle.
Lp	Lateral pallium.
Ls	Lateral septum.
Ma	Medial amygdala.
Mp	Medial pallium.
PLa	Posterior lateral amygdala.
POA	Anterior preoptic area.
Str	Striatum.

Other Abbreviations

AChase	Acetylcholinesterase.
ChAT	Choline acetyltransferase.
CRF	Corticotropin-releasing factor.
NT	Neurotensin.
SP	Substance P.
SS	Somatostatin.

2.14.1 Introduction

The name amygdala (from the Latin–Greek *amygdala*, almond) was coined by Burdach (cited by Swanson and Petrovich, 1998) to designate an almond-shaped structure deep in the temporal lobe of the human brain. The amygdala is easy to identify macroscopically in the brain of many mammals as a smooth bump in the caudal ventral cerebral hemispheres (e.g., rat, lamb; Figure 1). In both lissencephalic and gyrencephalic mammals the amygdala is ventral to the rhinal fissure and apparently connected with the lateral olfactory tract (*lot*), which reflects the olfactory and vomeronasal function of some of its components.

A closer look at the mammalian amygdala reveals, however, that it is an extremely complex and anatomically heterogeneous structure. Thus, in the first comparative approach to the anatomy of the amygdala of vertebrates, Johnston (1923) proposed that the amygdala includes pallial (basolateral and cortical divisions) and subpallial derivatives (central and medial amygdala), a view demonstrated by Swanson and Petrovich (1998). Moreover, the amygdala is not just a component of the chemosensory (olfactory and vomeronasal) systems, but also includes nonchemosensory areas of diverse embryological origin displaying distinct anatomical and neurochemical features. This has led Swanson and Petrovich to conclude that "terms such as 'amygdala' ... combine cell groups arbitrarily rather than according to the structural and functional units to which they seem to belong. The amygdala is neither a structural nor a functional unit."

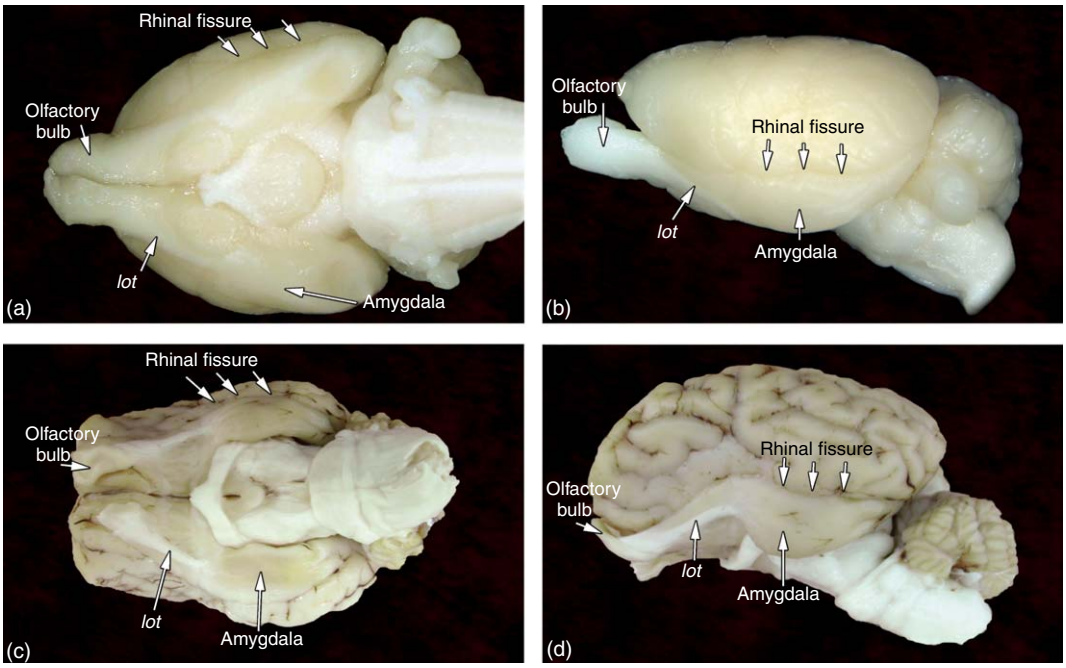

Figure 1 Gross anatomy of the mammalian amygdala. The amygdala can be easily identified in the brains of lissencephalic (e.g., the rat; ventral view (a); lateral view (b)) and gyrencephalic mammals (e.g., the lamb; ventral (c); lateral (d)). In both cases, the lateral olfactory tract (*lot*) can be followed from the olfactory bulbs up to a basal bump in the caudal cerebral hemispheres that corresponds to the amygdala.

The extreme morphological and functional complexity of the mammalian amygdala makes identifying its diverse components in nonmammals a very demanding task. This is further rendered difficult by the strong differences in the anatomical organization of the cerebral hemispheres of mammals and nonmammals, derived from the development of a huge isocortex in the mammalian forebrain. Therefore, a previous step to the comparative study of the amygdala of vertebrates is to characterize the divisions of the mammalian amygdala using developmental approaches (including expression of homeotic genes) as well as neurochemical and hodological data in the adult. Once the mammalian amygdala is fully characterized, we will identify the divisions of the amygdala of reptiles and birds using the same criteria. Then, we will discuss the evolutionary origins of the amniote amygdala and the possible influence that the amygdaloid function might have had on the evolution of the cerebral hemispheres.

2.14.2 Anatomical Heterogeneity of the Mammalian Amygdala

As a previous step to the study of the anatomy of the mammalian amygdala, and to understand its position within the cerebral hemispheres, we briefly discuss the identity and characteristic features of the main divisions of the vertebrate telencephalon: the pallium, striatum, and pallidum (Figure 2).

2.14.2.1 Organization of the Cerebral Hemispheres

In 1975, two groups independently observed that the nucleus accumbens (Acb) and olfactory tubercle showed a set of connections with the midbrain tegmentum that recalled those of the caudate putamen (CPu: Heimer and Wilson, 1975; Swanson and Cowan, 1975). Based on this evidence, Heimer and Wilson (1975) suggested that the Acb, olfactory tubercle, and fundus striatum were just the ventral portion of the striatum, the dorsal one being the CPu. Recent studies have revealed further similarities between the dorsal and ventral portions of the striatum, pertaining to their intrinsic organization, extrinsic connections, and neurochemistry. The common pattern of organization shared by all the striatal structures includes a massive glutamatergic input from areas of the cortex (archicortex – hippocampal formation – for the ventral striatum, isocortex for the dorsal striatum), as well as direct and indirect (through the ventral pallidum (VP) and globus pallidus (GP)) efferent projections to tegmental centers (ventral tegmental area (VTA) and substantia nigra

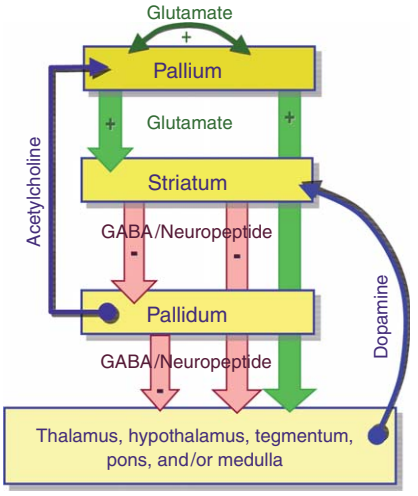

Figure 2 Organization of the vertebrate cerebral hemispheres. The telencephalon of vertebrates is composed of the pallium (cortex and other nuclear pallial centers) and subpallium (striatum and pallidum). The pallium gives rise to glutamatergic (excitatory) intrapallial and descending projections that reach the striatum and several extratelencephalic targets. The subpallium is engaged in a striatopallidotegmentostriatal loop in which the descending projections are GABAergic and peptidergic, whereas the tegmentostriatal pathway is dopaminergic. Both the pallium and subpallium project (directly or indirectly) to extratelencephalic executive centers. Whereas the pallium has an excitatory influence on them, the subpallium probably affects behavior by disinhibition. Based on Swanson and Risold (1999) and Lanuza *et al.* (2002).

(SN)), arising from spiny stellate, GABAergic (and peptidergic) cells. In turn, the tegmental targets of the striatum give rise to dopaminergic projections back to their striatal input areas (Figure 2).

Recent studies have revised and expanded this view. Thus, Swanson and Risold (1999) have reinterpreted the lateral septum as a portion of the striatum that they call the medial striatum (MSt), following a suggestion by Ramón y Cajal (1901). Like the striatum proper, the lateral septum receives a dense glutamatergic input from parts of the cortex (hippocampal formation *sensu lato*). Moreover, the spiny stellate cells of the lateral septum originate a GABAergic projection to pallidal (medial septum and diagonal band complex, which is thus considered the medial pallidum) and tegmental structures (VTA) from which they receive, in turn, a dopaminergic afferent. This scheme has been further extended to the cerebral hemispheres of all amniote vertebrates (Lanuza *et al.*, 2002). As indicated in Figure 2, the corticostriatopallidal pathway (including the striatotegmental loop) seems to be the basic circuit of the cerebral hemispheres. In addition to the hodological and neurochemical features mentioned above, this circuit is further characterized by the presence of dense (glutamatergic) corticocortical connections including, in some cases, commissural projections. Cortical connections also include descending projections to the thalamus, hypothalamus, pons, tegmentum, and/or brainstem that bypass the striatopallidum. Finally, a sparse population of cholinergic cells (and some GABAergic ones; Kohler *et al.*, 1984; Zaborszky *et al.*, 1999; Sarter and Bruno, 2002) located in the striatopallidum provides a feedback to the cortex.

The organization of the mammalian amygdala and its diverse anatomical components can be studied by using this simple model of the cerebral hemispheres that considers them to be composed of pallial structures, derived of the embryonic pallium, and subpallial ones, which are the adult derivatives of the ganglionic eminences. Although tangential migration during embryonic development (Marin and Rubenstein, 2003) might have partially blurred this scheme, it is still a useful framework to describe the anatomical heterogeneity of the amygdaloid complex.

2.14.2.2 The Pallial Amygdala

The definition of the pallial territories in the adult brain is a complex issue, as it is to delineate its different regions or compartments. This is so because not all the pallial territories use the same developmental program. Parts of the pallium develop in a very organized way, according to which those neurons produced in different times migrate following a neurogenetic gradient, either inside-out (isocortex) or outside-in (e.g., hippocampal fascia dentate; Jacobson, 1991). This leads to the formation of a cortex, a superficial structure showing a layered cytoarchitectonic organization that makes it easy to identify. Other pallial derivatives, however, the claustrum (Cl) and the endopiriform nucleus being good examples, show not a cortical but rather a nuclear organization, with no apparent stratification. We call them nuclear pallial structures. Since the amygdala occupies the lateroventral edge of the pallium, the nuclear pallial derivatives of the amygdala are adjacent to subpallial ones that are equally nonlayered, so that the palliosubpallial boundary becomes especially difficult to trace within the amygdaloid complex.

Two kinds of additional data can be helpful to delineate the palliosubpallial boundary. First, the pallium and subpallium express different sets of homeotic genes during intermediate embryonic development. Thus, the maps of expression of these genes in embryos are used to cartograph the pallial and subpallial territories of the amygdala (Puelles *et al.*, 2000; Medina *et al.*, 2004). However, during late embryonic stages, the

amygdala undergoes a topologically complex development that makes it difficult to interpret the fate of the embryonic labeled territories. This may be further complicated by tangential migration similar to that reported to occur from subpallial to pallial territories (Marin and Rubenstein, 2003).

Therefore, data on the neurochemistry and neuronal morphology of the adult brain are also helpful in this respect. As Swanson and Petrovich (1998) pointed out, pallial structures are characterized by originating excitatory (mostly glutamatergic) extrinsic projections to the subpallium and brainstem (as well as intrapallial projections), whereas subpallial structures give rise to descending GABAergic projections. Whereas labeling of GABAergic cells is relatively easy by using immunohistochemistry for GABA or glutamic acid decarboxylase (GAD), and/or *in situ* hybridization for the detection of GAD mRNA, the histochemical mapping of glutamatergic cells is not as easy. Nevertheless, it is well known that a subpopulation of the glutamatergic cells of the cerebral hemispheres are rich in zinc (Frederickson *et al.*, 2000) and can be visualized using a variety of histochemical techniques. The maps of the expression of GAD (Pare and Smith, 1993; Swanson and Petrovich, 1998) and of the distribution of zinc-rich (glutamatergic) cells in the amygdala of rats (Christensen and Geneser, 1995; Brown and Dyck, 2004) are nicely complementary and delineate quite a clear pallio-subpallial boundary. These data indicate that the subpallial amygdala includes the medial and central nuclei of the amygdala and the BST (at least its intra-amygdaloid portion). The remaining nuclei, including all the cortical amygdala and the AHA, as well as the whole basolateral division of the amygdala (lateral, basal, or basolateral and basal accessory or basomedial nuclei) are pallial derivatives (Figures 3 and 4). The presence of zinc-laden cells in the amygdalostriatal area (Brown and Dyck, 2004) suggests that some pallial cells may have migrated into putative striatal territories.

2.14.2.2.1 The cortical and basolateral divisions of the amygdala The pallial amygdala is composed of two kinds of structures. Some of them are superficial and show a layered organization, thus constituting the cortical amygdala. Topologically deep to these structures (and to the adjacent piriform cortex, see below) one finds a series of cell groups with nuclear configuration that conform the basolateral division of the amygdala. Some additional nuclei, not included in the basolateral amygdala, such as the AHA are also deep nuclear pallial structures.

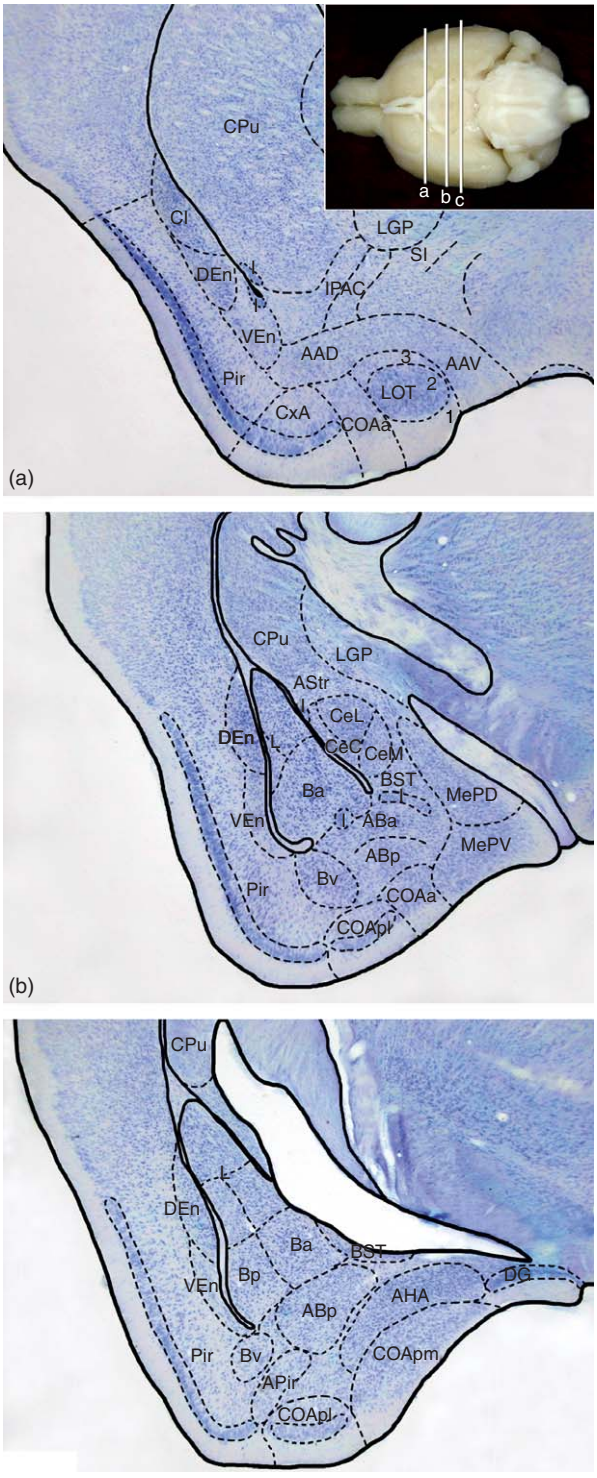

Figure 3 Cytoarchitecture of the amygdala of the mouse. Nissl-stained frontal sections through the left cerebral hemisphere of a mouse at about commissural (a); anterior postcommissural (b); and caudal levels of the telencephalon (c). The approximate levels of the sections are indicated on a ventral view of the brain in the inset. The cytoarchitectonic boundaries of the different amygdaloid nuclei and adjoining structures are delineated on the sections (for abbreviations, see 'Glossary').

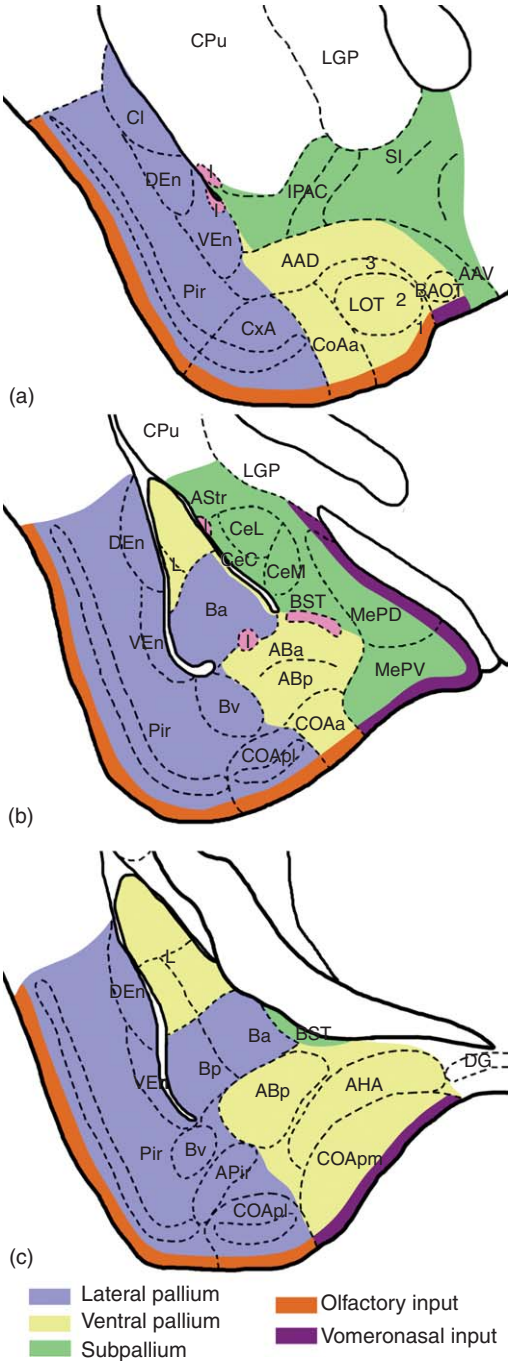

Figure 4 Pallial and subpallial territories of the mammalian amygdala. A schematic diagram of the mammalian amygdala, based on the Nissl-stained sections shown in Figure 3, shows the palliosubpallial boundary and the extent of the latero- and ventropallial territories within the pallial amygdala. The main olfactory bulb projects to entire superficial lateral pallium plus a small portion of the ventral pallium. In contrast, as indicated, the accessory olfactory bulbs project exclusively to ventropallial and subpallial structures. The intercalated cell masses (pink) and the amygdaloid capsule connect the deepest (L) with the more superficial (AB, COApm) parts of the ventropallial amygdala. This gives topological congruence to the proposed picture, since the deep lateral pallium (Ba) is separated from the subpallium (Ce and intra-amygdaloid BST) by a ventropallial bridge.

The cortical amygdala is composed of several areas on the ventral surface of the caudal cerebral hemispheres. These include the nuclei of the accessory (BAOT) and lateral olfactory tract (LOT), as well as the anterior cortical amygdala (COAa) rostrally, and the posterior cortical amygdala (the medial part, COApm, and the lateral part, COApl; also called periamygdaloid cortex by some authors) caudally. To these structures, some authors add rostral (corticoamygdaloid, CxA) and caudal (amygdalopiriform, APir; postpiriform, TR) transitional areas. All these structures show a similar (though not equally neat) layering with a (molecular) layer 1 that receives a superficial projection from the main (LOT, COAa, COApl) or accessory olfactory bulb (BAOT, COApm) and a deeper commissural-associative afferent (McDonald, 1998), thus resembling the piriform cortex.

The nuclear pallial amygdala includes at least the three nuclei that conform the basolateral division of the amygdala: the basal (B, or basolateral), accessory basal (AB, or basomedial) and lateral (L) nuclei. In addition, some of the structures rostral and caudal to these nuclei and deep to the cortical amygdala and/or the piriform cortex also belong to the pallial noncortical amygdala. Thus, the AHA (included in the posterior nucleus of the amygdala by Swanson and collaborators; Canteras *et al.*, 1992a) seems to be a caudomedial continuation of the AB. On the other hand, the dorsal portion of the anterior amygdaloid area (AA), which is immediately deep to the COAa and LOT, seems a rostral continuation of the AB.

2.14.2.2.2 Compartments of the pallium: Lateropallial and ventropallial portions of the mammalian amygdala Pallial derivatives occupy the dorsal surface of the cerebral hemispheres from the midline to the lateral ventricular sulcus. It is generally assumed (although this division is not based on solid experimental evidence) that the cortex is composed of three wide areas, derived from the medial, dorsal, and lateral pallia. In mammalian neuroanatomy, this roughly fits the traditional classification of the cortex into three cytoarchitectonically distinct regions: from medial to lateral, (1) the archicortex with a single cell layer sandwiched between two plexiform layers (hippocampal formation); (2) the isocortex (or neocortex) with 5–6 cell layers plus a molecular layer on top of the white matter; and (3) the paleocortex (olfactory cortex), characterized by a superficial molecular layer (I) and two cell layers (plus the endopiriform nucleus). Since the boundaries between these three areas are quite fuzzy, the existence of transitional cortical areas should be

taken into consideration. It is evident that the lateralmost regions of the pallium impinge on the amygdala.

The molecular and developmental bases for such a division of the pallium are not yet clear. Nevertheless, the expression of developmental genes in vertebrate embryos reveals an unexpected heterogeneity within the lateral aspect of the pallium (Puelles *et al.*, 2000; Medina *et al.*, 2004) that has led to the identification of a fourth pallial region, the ventral pallium (previously considered by Smith-Fernandez *et al.*, 1998, as an intermediate zone between the pallium and the subpallium). The ventral pallium was defined by a pattern of genetic expression that includes several pallial markers (Tbr-1 and a juxtaventricular rim of Pax-6) but excludes Emx-1 (Puelles *et al.*, 2000). *In situ* hybridization in 15-day-old mouse embryos indicates that the amygdala includes ventropallial derivatives together with portions of the lateral pallium (Puelles *et al.*, 2000; Puelles, 2001). Medina *et al.* (2004) further refined this analysis and demonstrated that during late embryonic development the lateral and ventral pallial territories display a differential pattern of genetic expression (lateral pallium: *Cadherin 8* and *Emx-1*; ventral pallium: *Dbx-1*, *Neurogenin*, and *Semaphorin 5A*).

From a comparative viewpoint, assigning each one of the areas and nuclei of the pallial amygdala to either the lateral or ventral pallium constitutes a key issue. Namely, derivatives of the embryonic ventral pallium of mammals can only be homologous to ventropallial structures of nonmammals, and the same is valid for the lateropallial derivatives. Data on the expression of homeotic genes during embryonic development, derived from the above-cited studies (Puelles, 2001; Medina *et al.*, 2004), indicate that the only lateropallial derivatives of the amygdala are the basal nucleus (B) and posterolateral cortical amygdala (COApl). In addition, it is likely that the transitional territories located between the cortical amygdala and the piriform/entorhinal cortex (CxA, APir, TR) also belong to the lateral pallium.

An analysis of the topology of the mammalian amygdala indicates that the remaining areas and nuclei of the pallial amygdala are ventropallial, since they are adjacent to striatal territories. Thus, the ventropallial division of the cortical amygdala consists of the LOT, COAa, BAOT, and COApm. In addition, the L and AB constitute the deep ventropallial amygdala. Thus, the L is adjacent to the striatal derivatives such as the CPu, the striatoamygdaloid transition, and the central amygdala. On the other hand, the AB and maybe its rostral (dorsal AA) and caudal neighbors (AHA) are adjacent to the Me (anterior or posterior divisions) and to the intra-amygdaloid portion of the BST. Topology demands that the B (lateropallial) and the Ce (striatal) be separated by a rim of ventropallial territory. This is likely constituted by the amygdaloid capsule and the adjoining posterior paracapsular intercalated cell masses of the amygdala (Medina *et al.*, 2004).

This renders a scheme of the mammalian pallial amygdala (Figure 4) in which every nucleus or area belongs to a compartment depending on its superficial or deep position as well as its ventropallial or lateropallial nature.

2.14.2.3 The Subpallial Amygdala

As discussed above, the subpallial telencephalon develops from two structures of the embryonic cerebral hemispheres, namely the lateral and medial ganglionic eminences. It is generally assumed that the pallidum of the adult telencephalon (including the GP, VP, and medial pallidum or medial septum, according to Swanson and Risold, 1999) derives from the embryonic medial ganglionic eminence. On the other hand, the Acb, olfactory tubercle, and CPu (striatal structures) are supposedly the adult derivatives of the lateral ganglionic eminence.

2.14.2.3.1 Striatal and pallidal compartments within the subpallial amygdala In the caudal cerebral hemispheres the striatal or pallidal identity of the subpallial structures becomes confused. These include the BST (both their intra- and extra-amygdaloid portions), the central amygdala (Ce), and the medial amygdala (Me) and several adjoining structures. The Ce is composed of three subnuclei, namely the medial (CeM) and lateral (CeL) divisions, plus the lateral-most cell group in touch with the amygdaloid capsule, the capsular or paracapsular central amygdala (CeC). Some authors consider that the CeC includes a ventral portion of the caudate, recognized by other authors as an independent structure called the amygdalostriatal transition (Cassell *et al.*, 1999). The Me is usually divided into anterior (MeA) and posterior parts, the latter divided in turn into a dorsal (MePD) and a ventral division (MePV). With regard to the extra-amygdaloid BST, it includes the supracapsular part plus a myriad of subnuclei within the BST proper, most of which receive topographical names. A detailed description of the BST is beyond the scope of this review. The interested reader is referred to Moga *et al.* (1989) and Dong *et al.* (2001). To sum up, an anterior and a posterior division of the BST are generally recognized. The anterior BST is

composed of several cell groups surrounding the anterior limb of the anterior commissure caudal to the Acb. The posterior division consists of several cell groups caudal to the anterior commissure, which apparently impinge on the preoptic hypothalamus.

Data on the neurochemistry, connections, neuronal morphology, and the pattern of expression of homeotic genes during embryonic development should be used to trace the pallidostriatal boundary at the level of the subpallial amygdala. Swanson and Petrovich (1998) delineated the palliosubpallial boundary by studying the expression of GAD mRNA. Topology requires that those subpallial structures that are in contact with pallial ones be striatal derivatives. Thus, these researchers proposed that the medial and central amygdaloid nuclei constitute the caudal tip of the (ventral) striatum. In addition, it is generally agreed that the BST constitutes a part of the pallidal complex related to the septal or amygdaloid formations (Swanson and Risold, 1999), since it derives from the medial ganglionic eminence. Taking these considerations into account, the projections from the basolateral division of the amygdala to the central and medial amygdala (Dong *et al.*, 2001) should be interpreted as corticostriatal projections (palliostriatal, glutamatergic), whereas the well-known projections from the central and medial amygdaloid nuclei to the anterior and posterior (roughly) portions of the BST (Dong *et al.*, 2001) would represent striatopallidal projections (GABAergic). However, as discussed in the next section, a closer analysis of the available evidence indicates a more complex panorama.

2.14.2.3.2 The extended amygdala: A striatopallidal structure or a third subpallial compartment? On the one hand, the basolateral amygdala projects not only to the Ce and/or Me (corticostriatal intra-amygdaloid pathways), but also portions of the BST (Adamec, 1989; Dong *et al.*, 2001). This projection is rich in zinc (Perez-Clausell *et al.*, 1989), thus glutamatergic, so that it represents a corticopallidal glutamatergic pathway that would contravene the scheme proposed for the organization of the cerebral hemispheres (but see Naito and Kita, 1994). On the other hand, the subpallial amygdala, including the BST, displays bidirectional intrinsic connections: as expected, the putative striatal compartments (Me and Ce) project to presumptive pallidal structures (anterior and posterior BST), but there are also important projections from the BST back to the Ce and Me (Ottersen, 1980; Coolen and Wood, 1998; McDonald *et al.*, 1999; Shammah-Lagnado and Santiago, 1999; Dong *et al.*, 2000; Shammah-Lagnado *et al.*, 2000; Dong and Swanson, 2003, 2004a, 2004b), which would represent pallidostriatal pathways. Therefore, the above-described general scheme on the organization of the cerebral hemispheres does not fit the hodological and histochemical features of the subpallial amygdala.

The exceptional properties of this region of the cerebral hemispheres have generated the idea that they belong to a third area of the subpallial telencephalon known as the extended amygdala (EA) (Alheid and Heimer, 1988; Olmos and Heimer, 1999; Shammah-Lagnado *et al.*, 1999), usually considered to be composed of two divisions, the central and medial EA (Alheid *et al.*, 1995). The medial EA is composed of the Me and the portions of the BST with which the Me is interconnected (roughly the posterior BST), plus a few intervening cell groups within the sublenticular substantia innominata and supracapsular BST (Shammah-Lagnado *et al.*, 2000). On the other hand, the Ce plus the anterior BST, together with a ring of additional cell groups linking these two structures both above (supracapsular BST) and below the internal capsule (fundus striatum or interstitial nucleus of the posterior limb of the anterior commissure (IPAC), within the sublenticular substantia innominata) make up the central EA. Data are more abundant and convincing for the existence of the central rather than the medial EA.

Although the internal capsule divides the EA into two apparently unconnected poles (Ce/Me amygdala and BST proper), the connective and histochemical properties of both poles of the EA are similar, thus suggesting a functional unity and a structural continuity (Roberts *et al.*, 1982). Thus, the Ce and those divisions of the anterior BST with which it is interconnected show a similar pattern of distribution of cells co-expressing different neuropeptides, such as corticotropin-releasing factor (CRF), neurotensin (NT), and enkephalin or substance P (SP)/somatostatin (SS) (Shimada *et al.*, 1989; Day *et al.*, 1999). In turn, the two main components of the medial EA, the Me and posteromedial BST, possess similar populations of vasopressinergic cells that are sexually dimorphic and display similar projections (Wang *et al.*, 1993).

Indeed, if they are interpreted as striatal or pallidal structures, the medial and central EA are also atypical in other respects besides the existence of intrinsic bidirectional connections and the presence of massive palliopallidal glutamatergic projections. Thus, the EA only receives a scarce tegmental innervation (compared with other

striatal compartments) that reaches presumed pallidal territories as well (BST; Hasue and Shammah-Lagnado, 2002). Moreover, the EA (at least its central division) displays long descending projections directed to the hypothalamus, periaqueductal gray, monoaminergic cell groups in the midbrain and brainstem, parabrachial region, and dorsal vagal complex (see below). This contrasts with the rest of the striatopallidal system, whose descending projections reach specifically the tegmental cell groups that originate their dopaminergic innervation.

The existence of the EA, or the usefulness of the concept of EA, is at the center of an intense debate (Canteras *et al.*, 1995; Swanson and Petrovich, 1998), from which two alternative views for the nature of the subpallial amygdala emerge: either it is viewed as a third subpallial compartment (neither striatal, nor pallidal), the EA, or it is envisaged as a patchwork of distinct striatal (Me and Ce) and pallidal territories (most of the BST). However, McDonald (2003) has recently put forward a third hypothesis that somewhat reconciles both views. According to his view, a certain mixing up of the striatal and pallidal cells has occurred in the caudal cerebral hemispheres. For instance, during embryonic development cells derived from the medial and lateral ganglionic eminences migrate tangentially (as has been shown to happen for the GABAergic cells in the pallium; Marin and Rubenstein, 2003), thus generating a series of structures where the striatopallidal boundaries are very fuzzy. This mixing up of striatal and pallidal cells may have generated special properties for the resulting cell groups, which constitute the above-mentioned distinctive features of the EA.

In line with this view, Cassell *et al.* (1999), using data on the cellular morphology, neurochemistry, and connections of the Ce, have proposed that it is composed of a striatal portion with a core-shell configuration (paracapsular and lateral divisions) and a pallidal one (the medial Ce). McDonald studied the morphology of Golgi-impregnated neurons in the Ce (McDonald, 1982b) and BST (McDonald, 1983). In both nuclei, he found a mixture of typical striatal medium-sized, spiny stellate cells (more abundant in the lateral aspect of both nuclei), with neurons displaying a pallidal morphology (long, thick, sparsely branched dendrites with few or no spines) more abundant in the medial Ce and posterior lateral BST. McDonald (2003) interprets these data as suggestive of a striatal nature of the lateral Ce and dorsolateral BST and of a pallidal or pallidostriatal nature of the rest of both nuclei. It is interesting to note that his drawings (McDonald, 1982a, 1983) show several cases of dendrites arising from cell bodies in the putative striatal compartment of the central EA that cross the boundaries to extend into the presumed pallidal (or striatopallidal) compartment (and vice versa). This dendritic exchange explains why the pallial and striatal projection fields overlap extensively within the central EA. A similar intermingling of striatal and pallidal cells has been suggested for nucleus X of the subpallium of songbirds (Perkel *et al.*, 2002).

This view is easy to test, since it predicts that inputs from pallial regions to the EA (e.g., zinc-positive fibers from the basolateral amygdala) should synapse onto (spiny) dendrites of striatal cells, whereas intrinsic connections within the EA should arise from striatal neurons (medium-sized spiny stellate cells) and terminate on pallidal ones.

2.14.3 Functional Neuroanatomy of the Mammalian Amygdala

Current ideas on the role of the mammalian amygdala in physiology and behavior are based on deep knowledge of its connections and on solid experimental evidence using techniques of functional neuroanatomy (lesion experiments, electrophysiology, and the study of the expression of immediate-early genes). In summary, it is generally accepted that the amygdala receives sensory information from many sources (brainstem, thalamus, olfactory bulbs, and different areas of the cortex) and gives rise to three main output pathways by which it modulates different aspects of behavior and physiology. First, the descending pathways of the central EA to hypothalamic and brainstem centers are involved in the generation of fear/anxiety responses (Davis, 2000; Ledoux, 2000), including motor, vegetative, and endocrine components (although the medial amygdala may also be involved in the endocrine responses to emotional stressors; Dayas *et al.*, 1999). Second, projections from the medial EA (and portions of the pallial amygdala) to hypothalamic centers probably constitute the pathway that mediates the neuroendocrine (and maybe some behavioral) responses to chemical cues (e.g., pheromones) in relation to reproduction (Newman, 2002; Halpern and Martinez-Marcos, 2003) but also to agonistic behaviors (Meredith and Westberry, 2004). Finally, the massive amygdalostriatal projections arising from the basolateral division of the amygdala and terminating in the ventral (but also in the dorsal) striatum seem involved in reward-related processes (Baxter and Murray, 2002). In this

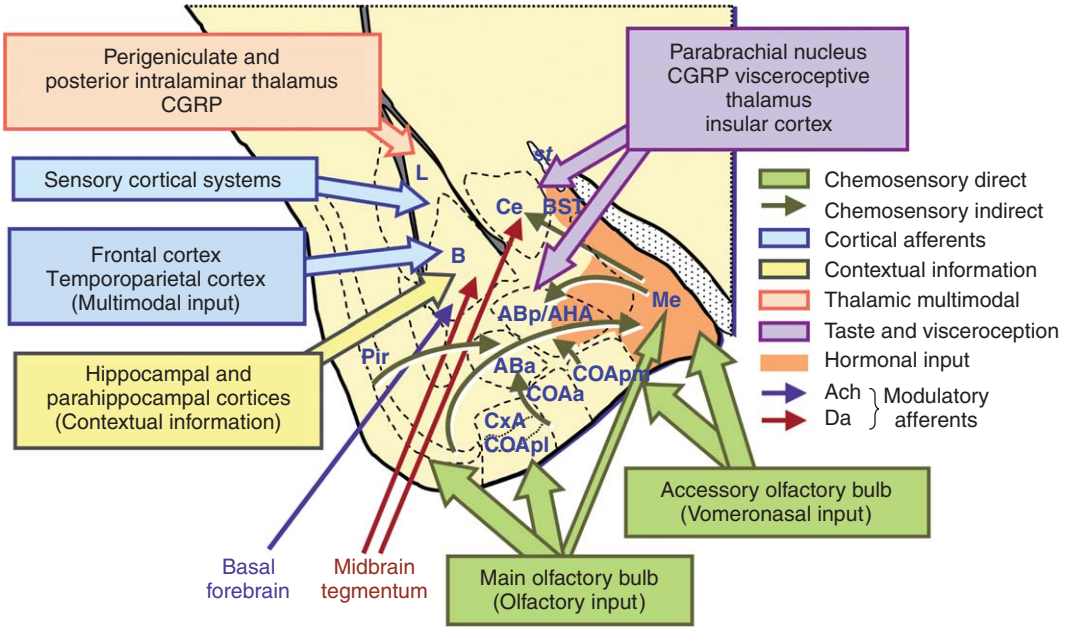

Figure 5 Inputs to the mammalian amygdala. Besides the olfactory and vomeronasal inputs to the corticomedial amygdala from the olfactory bulbs, the deep nuclei of the amygdala are targeted by brainstem, thalamic, and cortical afferents. The cortical inputs arise from different portions of the isocortex, including secondary and tertiary sensory areas, the frontal and temporal associative isocortex, the archicortex (hippocampal formation, *sensu lato*) and the paleocortex (see green arrows arising from the piriform cortex). The latter projection, together with the intra-amygdaloid projections from the corticomedial amygdala, makes the basolateral amygdala an olfactory–vomeronasal-associative center. As detailed in the text, most of the nonolfactory afferents reach both the basolateral and the central amygdala. Among them, it should be noticed that part of the thalamic and brainstem (parabrachial) sensory afferents contains the peptide CGRP. Several modulatory afferents reach the amygdala from many different sources, among which the cholinergic and dopaminergic ones (arising from the basal forebrain and the ventral tegmentum, respectively) converge on the B nucleus.

context, the intricate intrinsic circuitry of the amygdala apparently provides a basis for convergence of different stimuli onto amygdaloid neurons (especially in the basolateral amygdala, Pitkanen, 2000), which by means of synaptic plasticity (Blair *et al.*, 2001) would lead to the establishment of learned responses (e.g., conditioned fear or secondary reward).

In order to review the functional anatomy of the amygdala we will first describe the neural pathways that convey sensory information to the amygdala. Other afferents with a less clear sensory significance but having a modulatory role will be described. Moreover, different cell groups in the amygdala express receptors to steroid hormones, thus providing endocrine inputs to the amygdala, which will also be reviewed. Finally, the outputs of the amygdala will be analyzed on the context of their role in the expression of behavior and physiologically related processes.

2.14.3.1 Inputs to the Amygdala

In addition to its direct olfactory and vomeronasal inputs, sensory information reaches the amygdala from three different relay stations (Figure 5). First, some brainstem centers receiving relatively direct projections from the sensory organs project to the amygdala. Second, nuclei of the dorsal thalamus provide the amygdala with unimodal or multimodal sensory afferents. Finally, several parts of the cortex convey highly processed sensory information to the amygdala.

2.14.3.1.1 The amygdala as part of the olfactory and vomeronasal systems The amygdala is the recipient of several afferents from primary or secondary chemosensory centers. The most direct and massive ones arise from the main and accessory olfactory bulbs and terminate in the cortical and medial amygdala. Thus, olfactory information reaches directly the LOT, CxA, COAa, and COApl (although, according to Scalia and Winans (1975), the LOT shows only fiber but not terminal degeneration after lesions of the main olfactory bulbs). On the other hand, the accessory olfactory bulbs provide a direct vomeronasal input to the BAOT, COApm, Me, and portions of the BST (Broadwell, 1975; Scalia and Winans, 1975). Thus, whereas the

main olfactory system includes several cortical areas (parts of the cortical amygdala plus the piriform and entorhinal cortices), there is a single vomeronasal cortex, the COApm. In fact, not only does it receive a dense input from the accessory olfactory bulb but also it projects back to it (Canteras *et al.*, 1992a; Martinez-Marcos and Halpern, 1999). Other connections of the COApm include projections to olfactory centers (piriform cortex and endopiriform nucleus) and to its contralateral counterpart, via the anterior commissure (Canteras *et al.*, 1992a).

However, the amygdala is also a tertiary olfactory center. In fact, the piriform cortex projects to the cortical amygdala (a projection that recalls the associative connections within the olfactory cortex), to parts of the AB and to the Me (McDonald, 1998). Moreover, other cortical areas receiving olfactory projections, such as the CxA (Shammah-Lagnado and Santiago, 1999), and the COApl (or periamygdaloid cortex; Majak and Pitkanen, 2003) do project to the deep pallial amygdaloid structures, such as the anterior AB, posterior B and L nuclei. In addition, the LOT projects massively and bilaterally to the B and parts of the L (Santiago and Shammah-Lagnado, 2004). There is an additional projection, which has important functional implications, from the COApl to the COApm (Canteras *et al.*, 1992a; Majak and Pitkanen, 2003).

The data reveiwed above suggest that the Me is an associative olfactory–vomeronasal center. This associative role of the Me is in agreement with the existence of direct projections from the main olfactory bulb to the anterior Me reported in several mammals, including rabbits, rats, and several species of opossum (Scalia and Winans, 1975; Shammah-Lagnado and Negrao, 1981), as well as the Madagascan hedgehog tenrec (Kunzle and Radtke-Schuller, 2000). Therefore, there is anatomical evidence for an olfactory–vomeronasal convergence in both the Me and COApm. The latter has been confirmed electrophysiologically (Licht and Meredith, 1987).

In a similar way, the amygdaloid nuclei receiving projections from the accessory olfactory bulb also give rise to intrinsic amygdaloid projections. Thus, the Me projects to parts of the Ce and to the medial part of the BST, but also to parts of the basolateral amygdala, including the posterior AB, the AHA, and parts of the L (Gomez and Newman, 1992; Canteras *et al.*, 1995). In addition, the COApm displays a set of projections to the subpallial amygdala, including the Me and portions of the BST, but also to pallial regions such as the AB (Canteras *et al.*, 1992a) and the posterior part of the B (our unpublished results in mice). Finally, the AHA receives a strong input from the BAOT (in addition to the projections already described from parts of the Me).

In conclusion, although the amygdala is usually considered to contain distinct, nonoverlapping olfactory and vomeronasal territories, most of the vomeronasal amygdala (MeA and COApm) receives convergent inputs from the main and accessory olfactory bulbs and it projects, in turn, to several secondary olfactory centers. In addition, intra-amygdaloid connections (superficial to deep), including an intricate set of interconnections within the basolateral division of the amygdala (Pitkanen *et al.*, 1997), allow the association of both modalities of chemosensory information in discrete nuclei and subnuclei of the basolateral division of the amygdala.

2.14.3.1.2 Brainstem sensory afferents: The amygdala as part of the gustatory, viscerosensory, and nociceptive systems Other kinds of chemosensory information also reach the amygdala quite directly. Thus, brainstem gustatory/visceroceptive centers, namely the nucleus of the solitary tract and parabrachial pons (Ricardo and Koh, 1978; Saper and Loewy, 1980; Cechetto and Saper, 1987; Halsell, 1992), as well as the gustatory/visceroceptive thalamus (parvicellular division of the ventroposterior nucleus, central medial, interanteromedial, and paraventricular thalamic nuclei; Turner and Herkenham, 1991) project to different parts of the amygdala. These projections not only terminate in the pallial (nuclear) amygdala (mainly AB and COAa) but also in the subpallial amygdala (CeL and parts of the BST; Zardetto-Smith and Gray, 1987; Volz *et al.*, 1990; Turner and Herkenham, 1991).

It is interesting to note that part of the projection from the parabrachial nucleus to the amygdala contains calcitonin gene-related peptide (CGRP) in humans (de Lacalle and Saper, 2000) and rodents (Schwaber *et al.*, 1988), where this peptide has been shown to coexist with SP (Yamano *et al.*, 1988b) and NT (Yamano *et al.*, 1988a). This projection rich in CGRP/NT/SP, seems to convey specifically nociceptive stimuli (Bernard *et al.*, 1993). In this respect, the CGRPergic innervation of the amygdala consists of a dense fiber plexus in the CeL and CeC, which extends to the so-called amygdalostriatal transition (Figure 6e) and to most of the anterior BST (central EA), but not to the medial EA (Kawai *et al.*, 1985; Kruger *et al.*, 1988; Yasui *et al.*, 1991). As we will see, part of the thalamus also seems to contribute to the CGRP innervation of the central EA and neighboring areas (Yasui *et al.*, 1991).

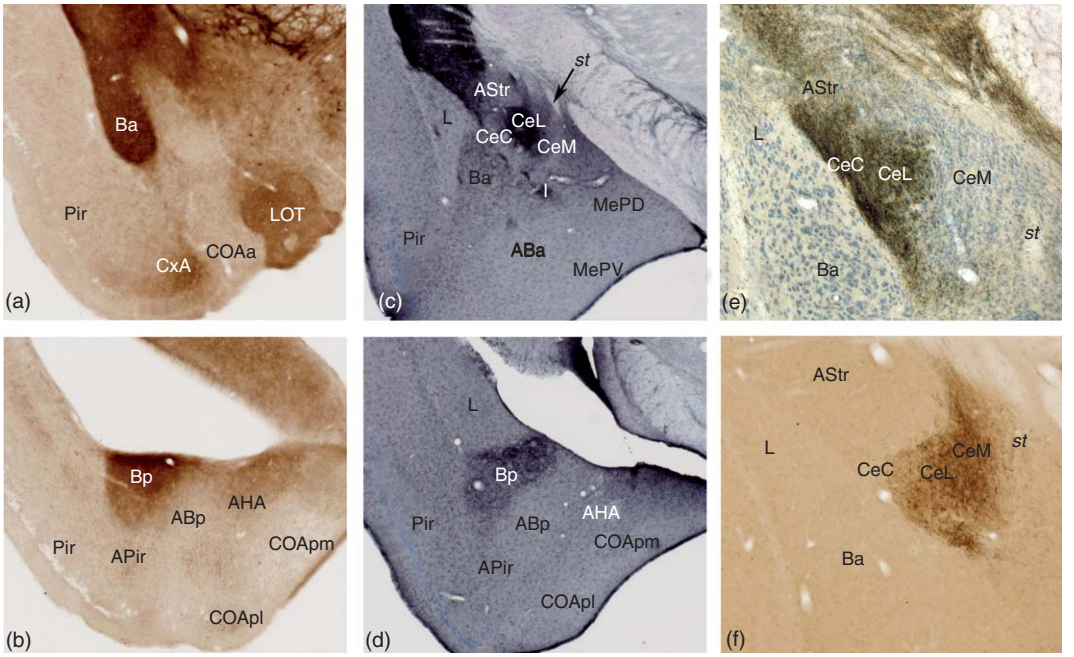

Figure 6 Histochemical features of the amygdala of mammals. a and b, Histochemical detection of AChase reveals a dense cholinergic innervation of the basal nucleus (both anterior, Ba; and posterior parts, Bp), as well as the LOT. c and d, The B (especially the Bp) also receives a distinctive dopaminergic innervation, as revealed by the immunohistochemical detection of dopamine transporter. e and f, The immunohistochemical detection of the peptides CGRP (e) and CRF (f) reveal the presence of two main compartments within the Ce. Thus, the capsular (CeC) and lateral (CeL) parts of the central amygdala display a dense innervation by CGRP-immunoreactive fibers (e). In contrast, most of the CRFergic cells are located in the medial aspect of the nucleus (CeM), although the CeL also displays a few CRFergic cells and fibers (f).

2.14.3.1.3 Thalamic inputs to the amygdala The amygdala is the target for several ascending pathways from the thalamus. As we have already described, the parvicellular division of the ventroposterior nucleus, central medial, interanteromedial, and paraventricular thalamic nuclei relay gustatory and viscероceptive information to the amygdala. In addition, there are reports of afferents to the amygdala from the magnocellular (medial and dorsal) portions of the medial geniculate nucleus, posterior intralaminar, suprageniculate, subparafascicular, and parataenial nuclei (Turner and Herkenham, 1991; Doron and Ledoux, 1999). The posterior intralaminar thalamus is the relay station for the main somatosensory pathways (dorsal column and spinothalamic pathways; Kunzle, 1994) and also receives visual and auditory information from the superior and inferior colliculi (LeDoux *et al.*, 1987; Linke, 1999; Linke *et al.*, 1999). Therefore, the posterior intralaminar thalamus conveys a mixture of somatosensory (mainly nociceptive and thermoceptive), visual, and auditory information to the central and basolateral amygdala (Bordi and Ledoux, 1994). This projection reaches both the pallial (L; Doron and Ledoux, 1999) and subpallial amygdala (Ce plus amygdalostriatal transition; Turner and Herkenham, 1991). Although most of the thalamic projections to the amygdala are glutamatergic (LeDoux and Farb, 1991), part of the projection from the posterior intralaminar thalamus also contains CGRP (Yasui *et al.*, 1991).

2.14.3.1.4 Highly processed sensory inputs: The cortical afferents to the pallial and subpallial amygdala All three main areas of the cortex project to the amygdala: (1) isocortex; (2) paleocortex (piriform cortex and adjacent areas); and (3) archicortex (hippocampus or Hp). For a thorough review of the corticoamygdaloid connections, the reader is referred to McDonald (1998). The pallial amygdala stands as the endpoint of a cascade of projections from the sensory areas of the isocortex. As a rule, projections to the amygdala from primary sensory cortical areas are scarce, if at all present, whereas secondary and associative sensory cortical areas display massive projections to the amygdala. This is true for the auditory temporoperirhinal cortex (Vaudano *et al.*, 1991; Mascagni *et al.*, 1993; Romanski and Ledoux, 1993; Shi and Cassell, 1999) and for the somatosensory parietal posterior insular cortex (Shi and Cassell, 1998a). The

gustatory visceroceptive anterior–posterior insular cortex (Shi and Cassell, 1998b) is exceptional, since both primary and secondary associative areas display important projections to the amygdala. Visual cortical areas also show a cascade-like projection to the amygdala, which is especially clear in cats and primates, where the bulk of the visual inputs to the amygdala arise from visual areas in the inferior temporal lobe, involved in complex higher-order visual processing (Shi and Davis, 2001). Cortical sensory inputs mainly reach the basolateral amygdala (L and B nuclei), though many of the cortical areas also show relatively minor projections to the lateral Ce (McDonald, 1998).

In addition, high-order associative areas of the cortex, such as the hippocampal and parahippocampal cortices (including the subiculum and entorhinal areas) project heavily to the amygdala (Brothers and Finch, 1985; Aggleton, 1986; Canteras and Swanson, 1992; McDonald and Mascagni, 1997). These connections are usually interpreted as providing the amygdala with information about the spatial and temporal context in which events take place (Ergorul and Eichenbaum, 2004). This is demonstrated by the fact that hippocampal lesions impair the capacity of acquiring fear to a context (test cage) where a foot shock is systematically given to the animals, but not to a pure tone with which the foot shock is paired (Phillips and Ledoux, 1992). Hippocampal/parahippocampal projections target the basolateral amygdala (mainly the L, B, and AHA). In addition, parts of the hippocampal and parahippocampal cortex (especially the entorhinal cortex; McDonald and Mascagni, 1997) also project massively to the CeL, Me, and intra-amygdaloid BST (Canteras and Swanson, 1992).

Finally, with minor differences, all the mammals studied (monkeys, cats, and rats; McDonald, 1998) display robust, topographically organized prefronto-amygdaloid projections. These include not only the aforementioned inputs from the anterior insular cortex, but also a set of projections from the medial (prelimbic, infralimbic, and anterior cingulate) and ventrolateral (orbitary) prefrontal areas (Carmichael and Price, 1995; McDonald *et al.*, 1996; Ghashghaei and Barbas, 2002). The prefrontal afferents terminate massively in the basolateral amygdala (B, AB, and L nuclei) but also in parts of the cortical (COAa and COApl) and the subcortical amygdala (CeL, MeA, and parts of the BST). Convergence of all the prefrontal afferents seems to occur, especially upon the B (and to a lesser extent the AB). Instead of providing information on the spatial, temporal, or spectral configuration of specific stimuli, or on the chemical nature of odorants (and pheromones), the prefrontal cortex seems to convey information on the outcomes of detecting incoming stimuli in terms of reward/aversion. In other words, the connections of the prefrontal cortex and amygdala are the substrate for emotional tagging of incoming stimuli by establishing, maintaining, or modifying associations of stimuli with reward (Gaffan *et al.*, 1993; Rolls, 2000) or aversion (Garcia *et al.*, 1999; Morrow *et al.*, 1999). In this context, it has been shown that lesions of the medial prefrontal cortex of rats interfere with the extinction of conditioned fear to a tone (Morgan *et al.*, 1993; Lebron *et al.*, 2004). This indicates that extinction of conditioned fear is an active process that involves modulation of the activity of neurons in the amygdala by prefrontal inputs (Milad and Quirk, 2002).

2.14.3.1.5 Redundant sensory pathways to the amygdala? All these anatomical data indicate that sensory information reaches the amygdala using two different gateways, namely thalamic (plus direct brainstem) afferents and cortical pathways. In this respect, conditioned fear to a simple tone is acquired by rats with either lesions of the medial division of the medial geniculate body (MGm) or of the auditory cortex (including temporal and perirhinal areas), but not by rats with combined cortical and thalamic lesions (Romanski and Ledoux, 1992). This suggests that the thalamic and cortical sensory pathways to the amygdala are redundant (for an alternative interpretation, see Shi and Davis, 2001). Nevertheless, it is likely that both pathways convey different kinds of information. Thus, the thalamic route provides the amygdala with crude sensory information that, nevertheless, can be biologically meaningful, such as loud noises, big moving objects or shadows (e.g., looming objects), pain, visceral sensations or sweet, salty, or bitter taste. In contrast, the cortical sensory pathway provides the amygdala with highly processed sensory information mediating recognition of stimuli with complex spatial (visual stimuli; Tanaka, 1996) or spectrotemporal configuration (e.g., species-specific vocalizations; Wang and Kadia, 2001), multimodal contextual information (hippocampal and parahippocampal (APH) areas), or information about the possible outcomes (reward/aversion) of the incoming stimuli (prefrontal cortex). Although both pathways can be used to elicit responses to simple stimuli (a pure tone), responding to complex stimuli surely requires the intervention of the cortical loop.

This is supported by physiological studies in different species indicating that neurons in the amygdala robustly respond to vocalizations of conspecifics and to biologically relevant sounds emitted by related species (Sawa and Delgado, 1963; Kling *et al.*, 1987), as well as to meaningful visual stimuli such as faces from conspecifics or closely related species (AB nucleus of monkeys; Leonard *et al.*, 1985) or sexually arousing images (humans; Hamann *et al.*, 2004). Interspecies differences are expected on the kind of sensory cortical processing performed (thus, in the cortical areas involved). Thus, primates are specialized in using visual and auditory stimuli for intra- and interspecies recognition, whereas most of the remaining mammals would use auditory or olfactory/vomeronasal cues instead.

2.14.3.1.6 Modulatory afferents: Cholinergic projections from the basal forebrain and tegmental monoaminergic afferents Afferents to the amygdala also include several neurochemically identified afferents. This has both functional and comparative implications. On the one hand, these afferents are surely playing a modulatory role of the amygdaloid function. On the other hand, using (immuno)histochemical tools, the terminal fields of these afferents are easily revealed and help to identify and delineate some of the amygdaloid nuclei (chemoarchitecture). Concerning this, the cholinergic and dopaminergic afferents are especially useful from a comparative viewpoint.

The innervation of pallial derivatives by basal forebrain cholinergic cell groups (Ch1–Ch4, according to the classification by Mesulam *et al.*, 1983) is part of the fundamental circuit of the cerebral hemispheres of, at least, tetrapod vertebrates (Medina and Reiner, 1994; Marin *et al.*, 1997c; Lanuza *et al.*, 2002) and seems to be present in some teleost species (Rodriguez-Moldes *et al.*, 2002; Mueller *et al.*, 2004). Therefore, cholinergic innervation is a neurochemical feature with an added value for comparative neuroanatomy. In this respect, the mammalian amygdala is richly innervated by cholinergic fibers (Amaral and Bassett, 1989), immunoreactive for choline acetyltransferase (ChAT), and positive for acetylcholinesterase (AChase histochemistry). The densest patches of cholinergic innervation are found in the B nucleus and the LOT (Figures 6a and 6b), which represent dense afferents from the Ch4 cholinergic cell group, namely the nucleus basalis–substantia innominata complex (Hecker and Mesulam, 1994).

Second, all the vertebrates studied show important ascending projections to the forebrain from dopaminergic tegmental cell groups (Smeets and Reiner, 1994). This consists of tegmentostriatal projections arising from groups A9 (SN) and A10 (VTA) that mainly terminate in the dorsal and ventral striatum respectively. Indeed, some of the putative striatal components of the mammalian amygdala, such as the Ce (Figure 6c) and the anterior and posterolateral BST (Freedman and Cassell, 1994; Freedman and Shi, 2001) display dopaminergic fibers. These fibers arise from the dorsocaudal A10 group, e.g., dopaminergic cells caudal to the VTA and medial to the SN, within the cytoarchitectonic boundaries of the dorsal raphe nucleus and periaqueductal gray (Hasue and Shammah-Lagnado, 2002). In addition, portions of the pallium are specifically innervated by dopaminergic tegmental cell groups, as is the case for the prefrontal cortex (mesocortical pathway) and the basolateral amygdala. Specifically, a dense plexus of dopaminergic fibers, mainly arising from A10, innervates the caudal aspect of the B nucleus (Fallon *et al.*, 1978; Brinley-Reed and McDonald, 1999; Figures 6c and 6d).

In addition, the amygdala displays distinct serotonergic and adrenergic innervations (Emson *et al.*, 1979; Sadikot and Parent, 1990; Canteras *et al.*, 1992a; Asan, 1998) arising from the raphe complex, and the locus coeruleus and A1/C1 A2/C2 medullary adrenergic cell groups respectively (Myers and Rinaman, 2002). Some of the adrenergic projections from the A1 group also contain neuropeptide Y (Zardetto-Smith and Gray, 1995).

Finally, the amygdala receives projections from diverse hypothalamic nuclei, such as the ventromedial (VMH; Canteras *et al.*, 1994) and anterior nuclei (Risold *et al.*, 1994), the lateral hypothalamic area (a projection that contains dynorphin; Zardetto-Smith *et al.*, 1988) and the ventral premammillary nucleus (PMv; Canteras *et al.*, 1992b). These nuclei receive strong inputs from parts of the amygdala (see below). The VMH displays diffuse projections to the medial, central, and basolateral amygdala whereas the PMv is interconnected with the principal nucleus of the BST, the MePD, as well as the AHA and adjacent portions of the COApm.

2.14.3.1.7 Hormonal inputs to the amygdala Besides receiving inputs from numerous sensory and modulatory centers of the brain, the amygdala is also the target for the action of steroid hormones. In fact, together with the hypothalamus, the medial EA (Me and posteromedial BST) is the area of the brain with the highest density of cells concentrating both estrogens and androgens (Pfaff and Keiner, 1973; Warembourg, 1977).

Immunohistochemical and *in situ* hybridization studies on the distribution of androgen and (α and β) estrogen receptors (Simerly *et al.*, 1990; Lu *et al.*, 1998; Mitra *et al.*, 2003; Perez *et al.*, 2003) reveal that sexual steroids influence not just the medial EA but also the remaining telencephalic centers projecting to the medial preoptic and ventrolateral aspect (shell) of the ventromedial hypothalamus. Thus, the ventral lateral septum and the AHA are among the nuclei displaying the highest concentration of steroid-sensitive cells in the forebrain. Since, at least in rodents, the VMH and medial preoptic nucleus are known to control feminine (Blaustein and Erskine, 2002) and masculine (Hull *et al.*, 2002) sexual behaviors, the receptors to gonadal steroids in the amygdala and septum are thought to be part of the mechanism for the endocrine control of copulatory behavior. However, other parts of the amygdala that do not project substantially to these hypothalamic centers also show a high (COAa) or moderate density (COApl, and, to a lesser degree, the COApm) of neurons expressing sexual steroid receptors. This suggests additional roles of sexual steroids in the modulation of amygdala-mediated behavioral and/or physiological processes.

Corticosteroids may also influence amygdaloid function. In fact, most of the peptidergic cells in the central EA display receptors to corticosteroids (Cintra *et al.*, 1991; Honkaniemi *et al.*, 1992). This is supposed to mediate stress-induced changes in the expression of peptides by the projection cells of the central EA, as part of the stress-adaptive changes (Palkovits, 2000). The medial EA is also rich in neurons expressing glucocorticoid receptors (Honkaniemi *et al.*, 1992) and may participate in stress responses (Dayas *et al.*, 1999). In addition, the basolateral amygdala displays low levels of receptors to glucocorticoids for which a role in modulating memory acquisition has been proposed (Roozendaal and McGaugh, 1997).

2.14.3.2 Outputs of the Amygdala

The influence of the amygdala on behavior is mediated by amygdaloid projections to diverse neural centers (Figure 7). Attaining their sites of

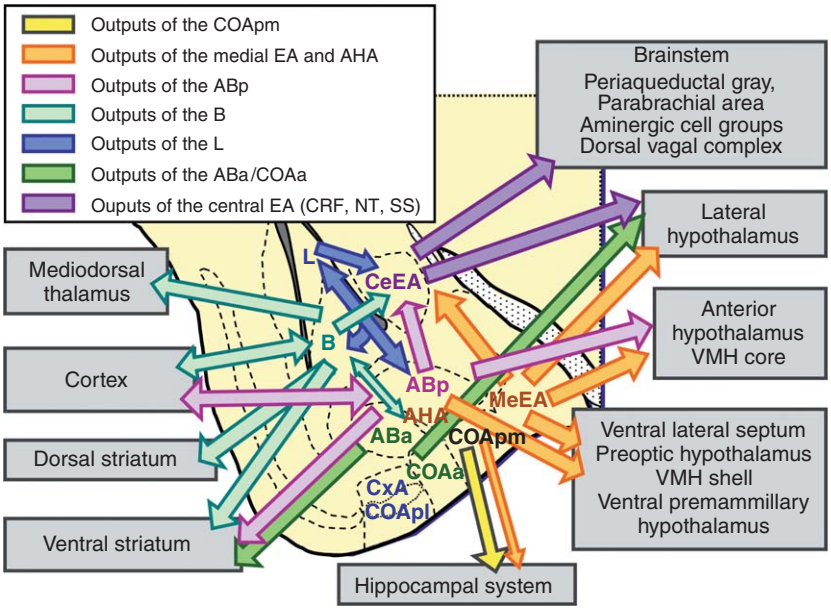

Figure 7 Schematic view of the main outputs of the mammalian amygdala. As a part of the pallium, the basolateral amygdala is engaged in connections with the rest of the pallium, the striatum, and the thalamus. In this respect the B nucleus constitutes the main (but not the only) source of outputs from the amygdaloid pallium. In contrast, the L is almost exclusively engaged in intra-amygdaloid connections. Since it is targeted by many sensory cortical and subcortical inputs, it is usually considered the sensory interface of the basolateral amygdala. The CeEA gives rise to long-distance descending projections to different centers of the hypothalamus, tegmentum, pons, and medulla. These projections seem to constitute the anatomical substrate of fear/anxiety reactions. Massive projections from the basolateral to the central (extended) amygdala allow fear reactions to different stimuli. The medial EA, together with the AHA, project to a sexually dimorphic forebrain system, including parts of the septum and the preoptic tuberal and premammillary hypothalamus. This seems to allow reproductive/agonistic behaviors elicited by vomeronasal-detected pheromones. In addition, the medial EA and AHA project to other parts of the hypothalamus (anterior nucleus and VMH core) involved in the expression of defensive behaviors. The basolateral amygdala, more specifically the posterior part of the AB, also projects to the circuit of defensive behavior.

origin and of termination, amygdaloid projections can be classified into five main pathways. Most of the pallial regions of the amygdala, and some subpallial centers, give rise to projections to striatal territories, including the dorsal and ventral striatum (*sensu stricto*) and parts of the lateral (striatal) septum. Second, parts of the pallial amygdala project to the ventromedial hypothalamus through the stria terminalis, and to the lateral hypothalamus through the ansa lenticularis. Third, the EA also projects to the several hypothalamic centers using both strial and nonstrial pathways. In addition, the central EA gives rise to long-distance projections directed to the pons and medulla (long descending projections of the amygdala). Finally, the cortical afferents to the pallial amygdala are reciprocated by a series of amygdalocortical pathways. This is complemented with projections from the pallial amygdala to the discrete regions of the dorsal thalamus.

2.14.3.2.1 Projections of the pallial amygdala to the basal telencephalon Virtually all the nuclei in the pallial amygdala project to striatal territories. The main amygdalostriatal pathway originates in the basolateral division of the amygdala and terminates in a continuum of structures within the ventral striatopallidum, which extends from the Ce caudally, to the shell of the Acb rostrally, and roughly defines the central EA. This projection mainly arises from the B and AB (Kelley *et al.*, 1982; Brog *et al.*, 1993; Petrovich *et al.*, 1996; Wright *et al.*, 1996; Dong *et al.*, 2001).

In addition, the basal nucleus (B), and, to a much lesser extent, the AB and L, are the origin of a massive projection to the dorsal striatum (CPu; Kelley *et al.*, 1982; Wright *et al.*, 1996). In contrast to the amygdaloid projection to ventral striatal territories, the projection from the B to the CPu is bilateral and is organized so that each portion of the amygdala projects to equivalent points of the striatum in both hemispheres.

Projections from the basolateral amygdala to the ventral striatum have been implicated in stimulus–reward associations (Everitt *et al.*, 1999) and, in fact, the basolateral amygdala is a focus for self-stimulation (through implanted electrodes) with a low-intensity threshold (Kane *et al.*, 1991a, 1991b).

The AHA displays a distinctive pattern of projections to the basal telencephalon (Canteras *et al.*, 1992a). Thus, in contrast to the B and AB, the AHA does not project to the central EA but rather to the medial EA (Me and posteromedial BST). This projection continues further rostrally to reach the ventral lateral septum and, to a lesser degree, the shell of the Acb, the olfactory tubercle, and the substantia innominata.

2.14.3.2.2 Projections of the extended amygdala to the striatopallidal telencephalon One of the features of the EA, in which it differs from the striatopallidum, is the presence of important reciprocal connections between the BST and the intra-amygdaloid EA (Me and Ce; see above). The Me gives rise to additional projections to the basal forebrain. First, the Me and the posteromedial BST also project to two striatal territories, the medial shell of the Acb and parts of the olfactory tubercle, as well as to the ventral aspect of the lateral septum (Canteras *et al.*, 1995). These projections mainly arise from the MeA. The projection of the Me to the lateral septum is known to contain vasopressin and shows a clear sexual dimorphism (Wang *et al.*, 1993).

As expected, the Me is interconnected with the posteromedial BST, a connection that defines the medial EA. However, the ventral aspect of the MeA also projects to the central EA (Canteras *et al.*, 1995; Shammah-Lagnado *et al.*, 1999; Dong *et al.*, 2001). This connection from the medial to the central EA is unidirectional. In fact, although the Ce shows minor intra-amygdaloid projections (Jolkkonen and Pitkanen, 1998), it projects neither to the Me nor to the posteromedial BST (Dong *et al.*, 2001). The functional significance of these connections is not properly understood. Nevertheless, since the Me is dominated by chemosensory inputs (mainly vomeronasal), its projections to the central EA and lateral septum are likely involved in orchestrating fear/anxiety (Ce) and other behavioral reactions (ventral lateral septum) to chemical cues derived from conspecifics (agonistic encounters and territorial behavior: Compaan *et al.*, 1993; Kollack-Walker *et al.*, 1997) or from predators (Meredith and Westberry, 2004). This is further discussed at the end of this article.

2.14.3.2.3 Projections from the pallial amygdala to the hypothalamus The second major output pathway for the amygdala is the stria terminalis. Although this is usually viewed as a single, homogeneous tract, it is indeed composed of fibers from different pallial and subpallial centers. The pallial component of the stria terminalis arises from cells within the boundaries of the posterior AB (ABp) and the AHA (Price *et al.*, 1991). The pallial stria terminalis in fact includes two different projections. The ABp projects to the core of the VMH (Petrovich *et al.*, 1996). On the other hand, the AHA (plus maybe the deepest parts of the COApl) projects the

anterior and preoptic hypothalamus (mainly to the medial preoptic nucleus), to the shell of the VMH and ventral lateral hypothalamic area in the tuberal hypothalamus, and to the PMv (Canteras *et al.*, 1992b). The pallial components of the stria terminalis are characteristically positive for zinc (Haug, 1973; Perez-Clausell *et al.*, 1989; Howell *et al.*, 1991), thus indicating their glutamatergic nature.

The pallial amygdala gives rise to additional projections to the hypothalamus. Thus, the anterior AB (ABa) and the COAa send a major projection to the lateral hypothalamic area (Price *et al.*, 1991; Canteras *et al.*, 1995; Petrovich *et al.*, 1996). This allows quite a direct influence of olfactory stimuli (received by the cortical amygdala) on physiology and behavior (probably reproductive and/or ingestive).

2.14.3.2.4 Projections from the extended amygdala to the hypothalamus The two main components of the subpallial amygdala are the sites of origin of projections directed to the BST that continue to the hypothalamus. Thus, the Me mainly projects to the posteromedial BST (a projection that delineates the so-called medial EA) and the whole medial EA projects to the preoptic hypothalamus, the anterior hypothalamic nucleus, the VMH, and the PMv (Kevetter and Winans, 1981; Price *et al.*, 1991; Canteras *et al.*, 1995; Dong and Swanson, 2004b). In contrast, the whole central EA projects to the posterior lateral hypothalamus (Price *et al.*, 1991; Bourgeais *et al.*, 2001) and the paraventricular nucleus (Gray *et al.*, 1989; Dong and Swanson, 2003, 2004a).

Projections from the amygdala to the hypothalamus may allow an influence of different stimuli (received directly and indirectly through the diverse pathways we have previously described), including pheromonal and olfactory ones (Me), in the expression of reproductive and agonistic behaviors (Canteras *et al.*, 1994, 1995). In addition, the direct and indirect projections from the amygdala (Ce) to the preoptic and paraventricular hypothalamus may be involved in the control of neuroendocrine responses associated with sexual and agonistic behaviors, as well as in responses associated with fear and stress (see below).

The central EA displays one of the densest populations of peptidergic cells of the cerebral hemispheres (Shimada *et al.*, 1989; Day *et al.*, 1999). Specifically, the Ce and the anterior and posterolateral BST contain cells immunoreactive for CRF (Figure 6f), NT, SP, and SS. Some of these peptides coexist in the same cells. Specifically, Shimada *et al.* (1989) described a population of cells co-expressing CRF and NT, and a second population in which SP and SS coexist. Peptidergic cells seem to be the projection neurons of the EA. For instance, the pathways from the Ce and Me to the BST, as well as the long-distance pathways directed to the lateral hypothalamus, are rich in SS/SP (Sakanaka *et al.*, 1981), NT (Allen and Cechetto, 1995), and CRF (Sakanaka *et al.*, 1986).

2.14.3.2.5 Amygdaloid projections to the brainstem Projections from the medial amygdala also reach portions of the periaqueductal gray, the VTA, and the midbrain raphe (Canteras *et al.*, 1995). In contrast the Ce displays much more abundant and long-distance projections that target centers in the midbrain and brainstem (Krettek and Price, 1978; Cassell *et al.*, 1986; Petrovich and Swanson, 1997; Bourgeais *et al.*, 2001). These include projections to most of the monoaminergic cell groups of the midbrain and brainstem, such as the dopaminergic cells in the VTA (A10), SN (A9), and retrorubral field (Gonzales and Chesselet, 1990; Vankova *et al.*, 1992; Dong and Swanson, 2003, 2004a), the adrenergic cells of the locus coeruleus (A6), as well as the noradrenergic and adrenergic cells in the nucleus of the solitary tract (C2/A2) (Wallace *et al.*, 1989). In addition, the Ce projects to the parabrachial nucleus and the NTS (Danielsen *et al.*, 1989; Petrovich and Swanson, 1997; Dong and Swanson, 2003, 2004a). Many of the neurons giving rise to these projections of the central EA are peptidergic (like the amygdalohypothalamic cells; see above). Thus amygdalonigral projections are rich in Met-enkephalin, dynorphin, and NT (Vankova *et al.*, 1992), whereas a low proportion of the Ce neurons projecting to the parabrachial region and nucleus of the solitary tract contain NT, SS, SP, or CRF (Veening *et al.*, 1984). In spite of the low proportion of projecting cells in the central EA containing this peptide, CRF-immunostaining depicts a nice amygdalofugal pathway, which is especially useful for comparative purposes. Thus, CRFergic fibers arising from the immunopositive cells in the CeL and CeM (Shimada *et al.*, 1989; Day *et al.*, 1999) and dorsolateral anterior BST course within the medial forebrain bundle and the periventricular system to innervate the substantia innominata, the medial and lateral preoptic areas, lateral hypothalamic area, central gray (Gray and Magnuson, 1992), laterodorsal tegmental nucleus, locus coeruleus (Van Bockstaele *et al.*, 2001), parabrachial nucleus, dorsal vagal complex, and regions containing the A1 and A5 catecholamine cell groups (Swanson *et al.*, 1983; Sakanaka *et al.*, 1987).

There is compelling evidence indicating that neuropeptide-rich descending projection systems arising from the central EA constitute the anatomical substrate for different fear reactions elicited by diverse stimuli under different experimental conditions (Ledoux *et al.*, 1988; Hitchcock and Davis, 1991; Rosen *et al.*, 1991; Walker and Davis, 1997; Kalin *et al.*, 2004; Sullivan *et al.*, 2004). In this context, CRF plays a double role in modulating fear, anxiety, and stress as both a neurohormone and a central neurotransmitter in the main descending projection of the central EA.

2.14.3.2.6 Amygdalocortical and amygdalothalamic projections Besides displaying projections to executive areas of the brain (striatum, hypothalamus, midbrain, and brainstem), the amygdala also originates ascending projections that target several areas of the cortex and projections to the thalamus that can influence cortical function.

As a rule, connections between the cortex and the pallial amygdala are reciprocal. Those areas of the pallial amygdala that receive the bulk of the cortical input, namely the L, B, and the anterior portion of the AB (Krettek and Price, 1978; Porrino *et al.*, 1981; Amaral and Price, 1984; Petrovich *et al.*, 1996), give rise to projections to the cortical fields that provide the most important inputs to the amygdala. Thus, amygdalocortical pathways mainly terminate in the prefrontal cortex (infralimbic, prelimbic, and anterior insular areas), the posterior insular and perirhinal cortices, as well as portions of the entorhinal cortex. Although there are several differences in the pattern of cortical projections of all three areas of the amygdala, probably the most striking one is the fact that the B (at least its anterior part) projects bilaterally to the cortex (Granato *et al.*, 1991). In addition to these projections, the AHA seems to be the interface area in the connections with the hippocampal formation (Canteras *et al.*, 1992a).

Amygdalocortical pathways constitute a likely substrate for modulation of memory storage within the cortex and of attention. Thus, stimuli related to emotional situations (such as fear, anxiety, or attraction and pleasure) are easily recalled. Beta-adrenergic transmission plays an important role in fear and anxiety enhancement of memory processes (Cahill and McGaugh, 1998). Therefore, it is likely that projections of the central EA (apparently mediating the emotional response of fear and anxiety; see above) to brainstem adrenergic cell groups (including locus coeruleus) are responsible for some of these effects. In addition, an action of corticosteroids on key brain structures such as the Hp also seems to account for fear and stress enhancement of memory acquisition (Blank *et al.*, 2003a, 2003b; Roozendaal *et al.*, 2003). Nevertheless, other emotional responses such as those elicited by rewarding stimuli, or by stimuli associated with reward, are probably mediated by amygdaloventral striatal pathways arising from the basolateral amygdala (Everitt and Robbins, 1992). Since the same areas that project to the ventral striatum are engaged in massive amygdalocortical projections (AB and B), it is tempting to suggest that corticosteroids may also play a role in memory enhancement by rewarding events. In addition, the amygdaloid input to the prefrontal (at least to the orbitofrontal) cortex has been shown to be necessary for maintaining in this structure an active representation of reward-predictive information (Schoenbaum *et al.*, 2003).

On the other hand, projections from the amygdala to the cortical areas providing sensory information can be regarded within the cortical circuitry as a feedback loop similar to the ones present within the cortex itself. Feedback pathways allow modulation of sensory processing in low-level sensory areas by higher-order ones, thus directing (for instance) attention to specific details of the sensory fields. This makes the amygdala a key structure within the circuitry of the isocortex.

Finally, the amygdala also projects to specific portions of the thalamus. Reardon and Mitrofanis (2000) have analyzed the amygdalothalamic pathways of the rat. Their results indicate that the amygdala displays reciprocal connections with the midline (e.g., paraventricular, parataenial) and intralaminar thalamus (including the medial division of the medial geniculate nucleus), to which all the divisions of the amygdala contribute (central, medial, olfactory, and basolateral). In addition, the basolateral amygdala (and, to a lesser extent, the medial and central amygdaloid divisions) projects to the mediodorsal nucleus and to the rostral zona incerta. Since the mediodorsal thalamus projects massively to portions of the prefrontal cortex (Porrino *et al.*, 1981), this constitutes an additional, indirect pathway for connections between the amygdala and prefrontal cortex. Nevertheless, both pathways to the prefrontal cortex may be functionally very different. McDonald (1987) demonstrated that the amygdaloid projection to the prefrontal cortex arises from class I cells, most of which are glutamatergic (McDonald *et al.*, 1989). In contrast, neurons within the basolateral amygdala projecting to the mediodorsal thalamus belong to class II cells (nonpyramidal), and the great majority of them did not exhibit glutamate or aspartate

immunoreactivity (McDonald, 1996). This is further supported by the lack of histochemically detectable zinc in the mediodorsal thalamus (Mengual *et al.*, 2001). The fact that the Ce and Me nuclei, which probably lack glutamatergic cells (Christensen and Geneser, 1995; Brown and Dyck, 2004) but are rich in GABAergic neurons (Swanson and Petrovich, 1998) also project to the mediodorsal thalamus supports the view that the amygdaloid projection to this thalamic nucleus is something more than a simple relay to the prefrontal cortex.

2.14.4 The Mammalian Amygdala: A Summary

The amygdala of mammals is composed of pallial and subpallial structures. The pallial amygdala (Table 1) is a mixture of lateropallial and ventropallial derivatives. The lateral pallium includes superficial (cortical) olfactory centers, including the COApl and maybe transitional areas such as the piriform (APir) and entorhinal cortices (TR). The only deep lateropallial structure of the amygdala is the B nucleus, which is also characterized by a dense cholinergic (AChase-positive) innervation and a less dense dopaminergic input from the midbrain tegmentum (mainly terminating in its caudal pole). Besides being involved in a complex intraamygdaloid circuitry, the B gives rise to a bilateral projection targeting both the dorsal and ventral striatum that constitutes the bulk of the amygdalostriatal pathway. Moreover, the B is bilaterally interconnected with the LOT and parts of the isocortex (mostly the prefrontal cortex), and projects to the mediodorsal thalamus.

The ventropallial amygdala also includes cortical superficial and deep nuclei. The cortical ventropallial amygdala is composed of olfactory areas, such as the COAa and LOT, and vomeronasal cortices, namely the BAOT and COApm. The COAa is especially rich in receptors to sexual steroids and, by means of its direct and indirect (through the anterior AB) projections to the lateral hypothalamus, constitutes a link for the transfer of olfactory information to the hypothalamus. The LOT is interconnected with the B, with which it shares a dense cholinergic and dopaminergic innervation as well as important contralateral connections through the anterior commissure. The COApm stands as a specialized vomeronasal cortex that projects to other secondary vomeronasal centers and back to the AOB, to olfactory centers (piriform and endopiriform), and to its contralateral counterpart.

The deep ventropallial amygdala is composed of the L, AB, and AHA nuclei. The former is usually considered as the sensory interface of the basolateral amygdala, since it is the target of most cortical and thalamic inputs, but shows projections virtually restricted to the remaining nuclei of this amygdaloid division. The AB projects to the ventral striatum and cortex. Moreover, it shows a double projection to the hypothalamus. Its anterior part (ABa), together with the overlying COAa (from which it receives a dense input), originates a projection to the lateral hypothalamus via the ansa lenticularis. The posterior AB, together with the AHA, gives rise to the pallial portion of the stria terminalis, and provides a glutamatergic and zinc-positive projection to the BST and medial hypothalamus (VMH). Finally, the AHA should be considered a deep pallial vomeronasal center since it is interconnected with the vomeronasal amygdala (mainly with the BAOT and medial EA), with which it shares a common pattern of afferents and efferents and the presence of receptors to sexual steroids.

The deep pallial amygdala gives rise to a massive projection to the central EA. This projection arises from all the nuclei of this amygdaloid division, with the exception of the anterior B and the AHA, the latter projecting to the medial EA instead.

The subpallial amygdala (Table 2) consists of two main divisions, namely the medial and the central EA. The medial EA is composed of the Me (with its different subdivisions that receive topographic names) and the posteromedial BST, which are deeply interconnected. It is dominated by cascade-like input from the AOB that includes direct and indirect projections through the COApm and AHA, and it is interconnected with the olfactory system. The whole vomeronasal amygdala (medial EA, AHA, COApm) is rich in receptors to steroid hormones. Its main outputs reach parts of the ventral striatum (mainly the olfactory tubercle), and different portions of the hypothalamus, including the preoptic, tuberal, and premammillary hypothalamus.

In addition, the medial EA shows projections to the basolateral amygdala, mainly targeting the ABp and L, as well as to the CeM. This interconnection allows the interplay between the medial and central EA, probably needed for the signaling of the attractive/aversive properties of conspecific chemical cues (such as pheromones) and associated stimuli.

The central EA (Table 2) is composed of the Ce (with medial, lateral, and capsular divisions) and the anterior and posterolateral BST, which are deeply interconnected. In contrast to the medial, the central EA does not express a high level of receptors to

Table 1 Summary of the characteristic features of the different nuclei of the mammalian pallial amygdala. In the columns describing the main inputs and outputs, the term 'intrinsic' has been used to refer to the existence of connections with other pallial amygdaloid nuclei. Most of these projections arise from the cortical amygdala and reach the basolateral (deep pallial) nuclei. In addition, there is an intricate set of interconnections among the nuclei in the basolateral amygdala

Pallial origin	*Topological position*	*Nucleus*	*Main afferents*	*Main efferents*	*Neurochemistry and other features*
Lateral pallium	Superficial	COApl	Main olfactory bulb	Intrinsic	Rich in receptors to sexual steroids
				Hippocampus (CA1, ventral subiculum)	Also called periamygdaloid cortex (see Majak and Pitkanen, 2003)
				Cortex	
	Deep	B	Intrinsic	Intrinsic	Cholinergic innervation
			NLOT (bilateral)	NLOT	Dopaminergic innervation
			Cortex	Cortex (bilateral)	Main output to the prefrontal cortex and striatum
				Ventral striatum (Acb, Tu; bilateral)	
				Dorsal striatum (CPu; bilateral)	
				Mediodorsal thalamus	
Ventral pallium	Superficial	NLOT	Main olfactory bulb	Main olfactory bulb	Dense cholinergic innervation
			B (bilateral)	B (bilateral)	
				Commissural	
				Ventral striatum	
		COAa	Main olfactory bulb	Lateral hypothalamus	Very rich in receptors to sexual steroids
					Olfactory link to the hypothalamus
		COApm	Accessory olfactory bulb	Accessory olfactory bulb	Vomeronasal cortex
			Commissural	Commissural	
			Intrinsic	Cortex	
			Piriform and endopiriform	Hippocampus (CA1)	
			Me	Intrinsic (especially to AB)	
				Medial EA	
	Deep	L	Intrinsic	Intrinsic (B, AB, and central EA)	Sensory interface of the basolateral division of the amygdala
			Thalamus		
			Cortex		
		AB	Intrinsic	Intrinsic (including central EA)	ABa: anterior part, deep and functionally related to COAa
			Cortex	Cortex	ABp: posterior part, functionally related to AHA
				Ipsilateral accumbens	
				Lateral hypothalamus (ABa)	
				Ventromedial hypothalamus (ABp)	
		AHA	Intrinsic (Me, COA)	Intrinsic (including medial EA and COApm)	Very rich in receptors to sexual steroids
			Hippocampus (CA1, ventral subiculum)	Hippocampus (CA1, ventral subiculum)	According to Canteras *et al.* (1992a), it is part of the posterior amygdala
			Ventral premammillary nucleus	Lateral septum (ventral)	Together with the ABp gives rise to the pallial portion of the stria terminalis
				Cortex	
				Ventral striatum	
				Ventromedial hypothalamus	
				Ventral premammillary nucleus	

Table 2 Summary of the characteristic features of the different nuclei of the mammalian subpallial amygdala (extended amygdala)

Division	*Nucleus*	*Main afferents*	*Main efferents*	*Neurochemistry and other features*
Medial EA	MeAD, MeAV, MePD, and MePV	Accessory olfactory bulb (AOB) Posteromedial BST COApm Secondary olfactory centers Subiculum Ventral premammillary hypothalamus AHA? Main olfactory bulb (only partially)	AOB COApm AB, L Lateral entorhinal cortex Posteromedial BST Ventral striatum (olfactory tubercle) CeM Lateral septum (ventral) Hypothalamus: medial preoptic, ventromedial, and ventral premammillary nuclei	• Vomeronasal subpallial amygdala • Very rich in receptors to sexual steroids • Sexually dimorphic population of vasopressinergic cells (projecting to septum and habenula)
	Posteromedial BST	Accessory olfactory bulb (only partially) Me COApm COApl Amygdalopiriform transition (APir) AHA Ventral premammillary hypothalamus	Me ABp and AHA CeM (only the Tr) Lateral septum (ventral) Hypothalamus: medial preoptic, ventromedial, and ventral premammillary Periaqueductal gray (PAG) Midbrain tegmentum (VTA, RR) Parabrachial/pericoerulear area (including Barrington nucleus)	• Very rich in receptors to sexual steroids • Sexually dimorphic population of vasopressinergic cells (projecting to septum and habenula)
Central EA	CeM, CeL, and CeC	Infralimbic and insular cortices CA1 Basolateral amygdala (L, ABa, ABp, Bp) Amygdalopiriform transition (APir) Postpiriform transition area (TR) Anterior and posterolateral BST MeAV Paraventricular, ventromedial, and subparafascicular thalamus Parabrachial pons	Anterior and posterolateral BST Substantia innominata Lateral hypothalamus Paraventricular and mediodorsal thalamus Midbrain tegmentum (VTA, SN, RR) PAG Parabrachial and pericoerulear pons NTS–dorsal vagal complex	• Receptors for corticosteroids • NT/CRF and SP/SS projecting cells in the CeL and CeM (not in the CeC) • CGRP innervation of CeC and CeL (parabrachial and thalamic origin)
	Anterior and posterolateral BST	Basolateral amygdala (ABa, ABp, Bp) Ce Amygdalopiriform transition (APir) Postpiriform transition area (TR) Parabrachial pons	Ce Substantia innominata Lateral hypothalamus Paraventricular and mediodorsal thalamus Midbrain tegmentum (VTA, SN, RR) PAG Parabrachial and pericoerulear pons NTS–dorsal vagal complex	• NT/CRF and SP/ SS projecting cells (lateral aspect). At least the NT/CRF population extends rostrally into the nucleus accumbens • CGRP innervation that extends rostrally into the nucleus accumbens

sexual steroids but it does to corticosteroids. It receives a cascade of sensory inputs (viscerceptive, nociceptive, gustatory, somatosensory, auditory, and maybe visual) directly and indirectly from the brainstem (mainly the parabrachial area and nucleus of the solitary tract), thalamus and cortex (perirhinal, insular, infralimbic), and basolateral amygdala (mainly L, AB, and posterior B), the latter constituting its main input. Many of the projection cells of the central EA are GABAergic and express neuropeptides (CRF, NT, SS, SP, enkephalin). The distribution of peptides may be of interest for comparative purposes. Most of the peptidergic cells are located in the CeL (plus CeM) division, whereas the CGRPergic input (visceroceptive nociceptive) from the parabrachial pons and thalamus terminates in the CeC and, to a lesser extent, in the CeL, and extends to the amygdalostriatal transition. Therefore, the Ce displays a medial aspect rich in peptidergic cells and a lateral one defined by CGRP innervation that partially overlaps with the former.

The projections of the central EA reach a huge variety of structures, including the paraventricular and lateral hypothalamus, periaqueductal gray, midbrain reticular formation, including dopaminergic cell groups (VTA, SN, retrorubral field), locus coeruleus and pericoerulear area, parabrachial area, nucleus of the solitary tract, and dorsal vagal complex. This allows a coordinated control of the somatomotor, vegetative, and endocrine components of fear/anxiety reactions.

This summary reveals the existence of two parallel systems in the amygdala of mammals. One is a multimodal system, composed of the basolateral amygdala (B, L, and AB) and the central EA and seems involved in the generation of two kinds of basic emotional reactions. The fear–anxiety reactions are produced through the long descending outputs of the central EA. Moreover, the basolateral nuclei (mainly the AB and B) massively project to the ventral striatum, and this may mediate the generation of reactions of attraction/reward elicited by incoming stimuli.

The second system of the mammalian amygdala is mainly composed of the secondary vomeronasal centers, since it includes the vomeronasal cortex (COApm) and the medial EA, plus a deep pallial nucleus, the AHA. Their pattern of connections with the preoptic, tuberal, and premammillary hypothalamus and septohippocampal system, as well as the presence of receptors to sexual steroids in most of the centers of this circuit, suggests that this system is involved in the control of reproductive and agonistic behaviors elicited by conspecific chemical signals (mostly pheromones). Both systems are interconnected. Most of these connections arise from the medial EA or COApm and terminate in the basolateral amygdala and central EA. These connections are seemingly providing a substrate for eliciting reactions to conspecific vomeronasal-detected chemicals (e.g., pheromones) such as fear/anxiety against a competitor (territorial behaviors) or attraction/reward, induced by probable mates.

2.14.5 The Amygdala of Reptiles

Identifying the amygdala of nonmammals constitutes a true challenge. One of the first attempts was due to Johnston (1923), who assumed that the amygdala was found in the caudal and basal cerebral hemispheres, in close association with the LOT. In fact, in a ventral view of the brain of most reptiles (Figure 8 shows the brain of the Old World lizard *Podarcis hispanica*), the *lot* is seen to arise from the olfactory bulbs, very developed when compared with mammals, and apparently terminating halfway within the cerebral hemispheres. The structures caudal to the *lot* are good candidates for the reptilian amygdala.

However, to identify the reptilian amygdala with certainty, we will apply to the reptilian brain the same criteria that define the different parts of the mammalian amygdala. Thus, we will explore the reptilian pallium and subpallium to try to delineate the reptilian pallial and subpallial (extended) amygdala.

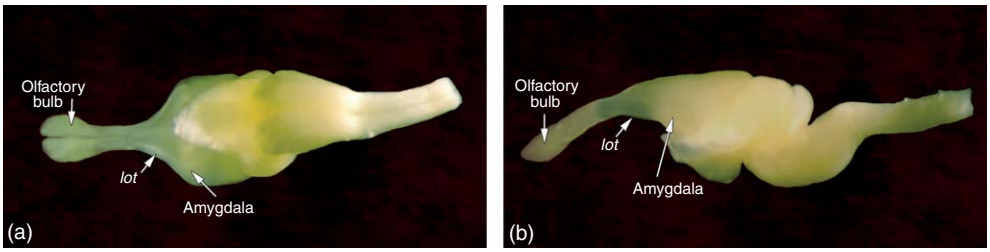

Figure 8 The amygdala in the brain of reptiles. In a ventral (a) and a lateral (b) view of the brain of the lizard *Podarcis hispanica*, the lateral olfactory tract (*lot*) is seen to leave the huge olfactory bulbs to terminate in the caudobasal cerebral hemispheres, where the presumed amygdala is located.

2.14.5.1 A Topological View of the Reptilian Pallial Amygdala

In order to delineate the pallial amygdala of reptiles we will use three main criteria. First, the afferents from the olfactory bulbs will be used to identify the different areas of the presumptive (superficial) cortical amygdala. Second, structures topologically deep to the cortical, olfacto-recipient amygdala would constitute the basolateral division of the amygdala. Finally, we will use topological data together with data on the expression of homeotic genes during development to delineate the lateral versus the ventral pallial derivatives. All of these data allow us to make a proposal of homologies between the reptilian and mammalian amygdalae, which will be further explored using connectional and histochemical data (Figure 9).

2.14.5.1.1 The olfacto-recipient pallial amygdala of reptiles There are several studies of the projections of the main and accessory olfactory bulbs in different reptiles, including squamate reptiles (lizards and snakes: Ulinski and Peterson, 1981; Martinez-Garcia *et al.*, 1991; Lohman and Smeets, 1993; Lanuza and Halpern, 1998) and turtles (Reiner and Karten, 1985). In all reptiles, the main olfactory bulbs project to the outer half of the molecular layer of what is usually called the LC, throughout its rostrocaudal axis. They also project to more ventral cell groups associated with the *lot*, named nucleus of the *lot* (NLOT) (Martinez-Garcia *et al.*, 1991; Figures 9a and 10a) or external amygdala (Lanuza and Halpern, 1998). At caudal levels,

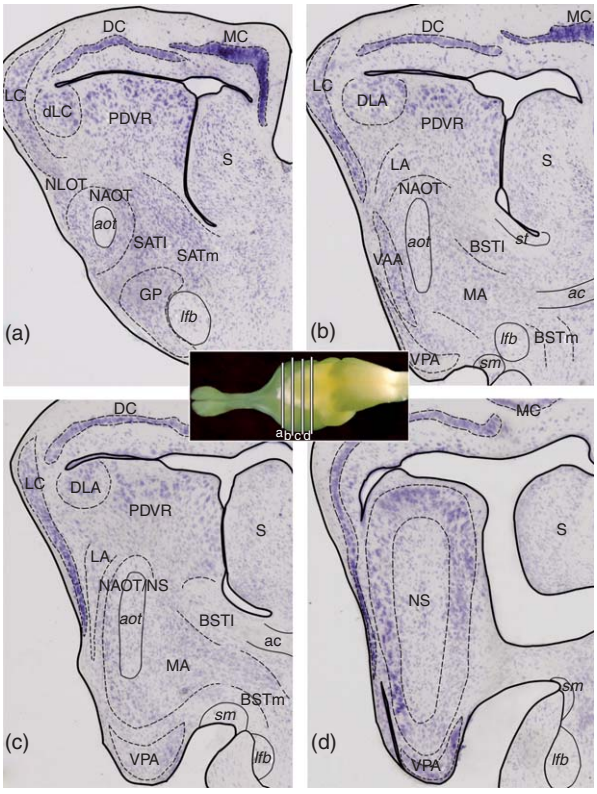

Figure 9 Cytoarchitecture of the amygdala of the lizard *Podarcis hispanica*. Nissl-stained frontal sections through the left cerebral hemisphere of a lizard at slightly precommissural (a); commissural (b); anterior postcommissural (c); and caudal levels of the telencephalon (d). The central inset shows the approximate location of the sections on a ventral view of the brain. The different nuclei and cortical areas of the amygdala and adjoining telencephalic structures are delineated using thin discontinuous lines, whereas thin solid lines indicate the main fiber tracts.

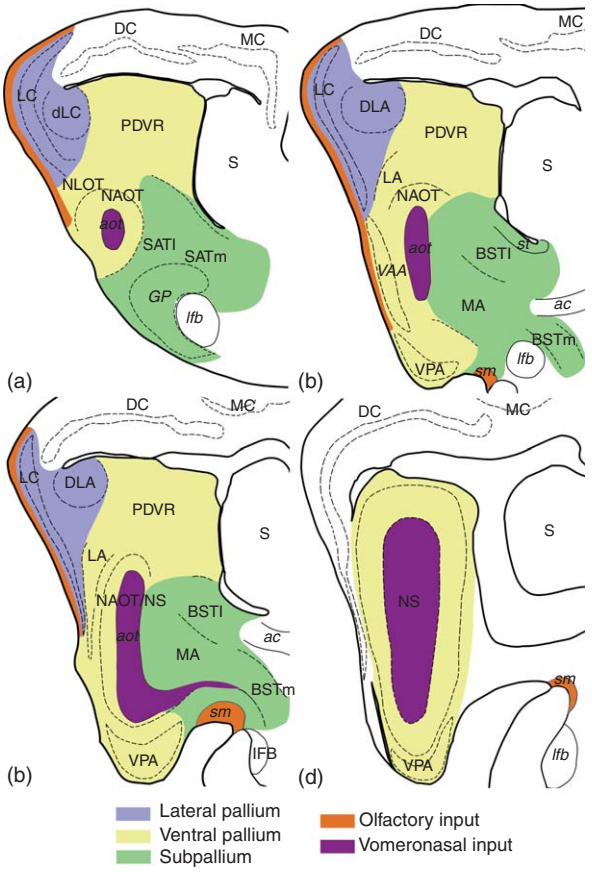

Figure 10 Pallial and subpallial territories within the reptilian amygdala. This diagram, which is based on the Nissl-stained sections of Figure 9, shows the palliosubpallial boundary as well as the lateral and ventral pallial territories in the amygdala of reptiles. The termination areas of the projections from the main (orange) and accessory olfactory bulbs (violet) are also indicated. This reveals that, as in mammals, the olfactory projection reaches mainly superficial lateral and ventropallial regions, whereas the vomeronasal one reaches ventropallial (NS) and subpallial regions (MA). In the brain of squamate reptiles the accessory olfactory tract is internalized (not superficial) and this results in an invagination of the vomeronasal cortex, the NS, which therefore shows an inverted lamination. On the other hand, the olfactory projection courses through the stria medullaris, just superficial to the MA, and reaches the contralateral hemisphere via the habenular commissure.

the LC extends further ventrally (LCc; Figures 9b and 10b). At these levels, MOB fibers extend beneath the surface of the ventrolateral cerebral hemispheres to enter the stria medullaris. In their way, they innervate a layered cell group, caudal to the NLOT, known as the VAA (Figures 9b and 10b; Martinez-Garcia *et al.*, 1991; Lanuza and Halpern, 1998).

In the rostral telencephalon, the fibers arising from the accessory olfactory bulb deepen into the cerebral hemispheres making up the accessory olfactory tract. The accessory olfactory tract is surrounded by a group of cells called bed nucleus of the accessory olfactory tract (NAOT), which is just deep to the boundary between the LCc and the VAA. The NAOT is rostral to and continuous with the main secondary vomeronasal center of squamate reptiles, the NS, which at caudal levels occupies the whole subventricular telencephalon (Figures 9c and 9d). In spite of its subventricular position, the NS displays a neat laminar organization (Ulinski and Kanarek, 1973), with most cells arranged in a single layer (mural layer) sandwiched between two molecular strata called marginal (juxtaependymal) layer and the hilus, where the accessory olfactory tract terminates (Figures 10c and 10d). This is suggestive of a cortical nature. In agreement with this interpretation, like other cortical structures the NS shows most of the GABAergic cells in the molecular layers and only a few of them in the mural layer (Martinez-Garcia *et al.*, 2002a). Perez-Clausell (1988) compared the distribution of zinc between the LC and NS and concluded that the NS is a cortical field whose lamination has become inverted. In fact, in a series of frontal sections through the caudal telencephalon of a squamate reptile, it becomes clear that, as the accessory olfactory tract deepens into the cerebral hemispheres, there is an invagination of the cortical amygdala to which it innervates. Consequently, the hilus constitutes the outer molecular layer, whereas the marginal layer is, in fact, the inner molecular layer. Therefore, in reptiles the vomeronasal cortical amygdala is typically invaginated in the form of an NS. The AOB also projects to a region of the striatopallidal forebrain, just medial to the rostral NS (Figures 10b and 10c) that is usually named as medial amygdala and/or BST (see below).

Considering together all these data, the olfactory cortical amygdala of reptiles seems to be composed of the NLOT, the VAA, and, presumably, parts of the LC. Although the LCc occupies a position compatible with its consideration as part of the caudalmost olfactory cortical amygdala, this is not clearly supported by hodological data (Hoogland and Vermeulen-Vanderzee, 1995; see below). In addition, reptiles possess two vomeronasal cortical structures, the BAOT and the NS.

2.14.5.1.2 The deep pallial amygdala: The reptilian basolateral amygdala Like its mammalian counterpart, the basolateral division of the reptilian amygdala should occupy a topological position deep to the cortical (olfacto-recipient) amygdala. This probably includes the PDVR plus two adjoining cell groups named lateral amygdala (LA) and DLA. Thus, dextranamine injections in the ependymal layer of the medial PDVR in *Podarcis* result in extensive labeling of the glial processes that could be followed up through the deep PDVR and rostral LA, to their contact with the pial surface at the level of the NLOT (Martinez-Garcia *et al.*, 2002a). On the other hand, the rostral LA looks deep to the VAA (Figure 9b). This allows us to consider the PDVR, LA, VAA, and NLOT as neurogenetically related structures. On the other hand, the distribution of radial glia in lizards (Monzon-Mayor *et al.*, 1990; Yanes *et al.*, 1990; Guirado *et al.*, 2000) indicates that the DLA, a cell group just below the lateral sulcus of the lateral ventricle at these caudal levels (Figures 9b and 9c), is topologically deep to the LCc.

In addition, there is a small cell group ventral to the rostral-to-intermediate NS, which, because of the inverted lamination of the NS, should be interpreted as deep to it. This is called VPA. The VPA is contiguous to the LA (Figure 9c) and caudal to the VAA. Some authors consider the VPA a caudal portion of the external amygdaloid nucleus, but this does not seem appropriate for a deep nucleus.

2.14.5.1.3 Lateropallial and ventropallial territories in the amygdala of reptiles From a comparative viewpoint, it is interesting to know the territory of the pallium to which each one of these structures belongs. Since the projections from the olfactory bulb terminate in the superficial layers of the lateral and ventral pallia, we will specifically analyze the ventropallial or lateropallial nature of the abovementioned structures (Figure 10). In this respect, it is sensible to consider the DLA and the LCc, apparently superficial to the former, as lateropallial structures. On the other hand, in their original definition of the ventral pallium, Puelles *et al.* (2000) indicated that it was composed of structures deep to the LOT. Therefore, it is reasonable to suggest that the LOT and its caudal continuation, namely the VAA and NS, are ventropallial derivatives. Finally, the structures deep to these cortical areas, the PDVR and LA and VPA, are also very likely ventropallial. This proposal is supported by data on the expression

of homeotic genes during the development of turtles. Indeed, Smith-Fernandez *et al.* (1998) used *Emx-1* and *Dlx-1* as markers of early telencephalic regionalization and concluded that the dorsal ventricular ridge (probably including its posterior part) is part of the intermediate territory (situated between the pallium and subpallium) renamed by Puelles *et al.* (2000) as ventral pallium.

2.14.5.1.4 A proposal of homologies for the reptilian and mammalian pallial amygdala This allows us to make a proposal of homologies between the reptilian and mammalian amygdala based mainly on topology (superficial-to-deep; ventral or lateral pallium) and on the projections from the olfactory bulb, which is presented in Table 3. According to this proposal, the LC would include the homologues of the COApl plus maybe the transitional areas with the piriform (APir) and entorhinal areas (TR). In turn, the deep lateropallial DLA is the best candidate for the homologue of the B. In the ventral pallium, the LOT and BAOT would be homologues to their mammalian homonyms; the olfactory cortical areas of mammals (COAa) and reptiles (VAA) would be homologous, as would be the vomeronasal cortices of mammals (COApm) and reptiles, the NS. In addition, the reptilian deep ventropallial nuclei, PDVR, and LA are the most likely candidates for the homologues of the mammalian L and AB.

Finally, being a caudal cell group deep to the vomeronasal cortex (NS), the VPA occupies a topological position comparable to that of the mammalian AHA (which indeed is just deep to the COApm). This is further supported by the continuity shown between the reptilian LA and VPA that recalls the relationship between the posterior AB and the AHA of mammals.

This proposal of homologies would be further explored and detailed by analyzing the available literature on the connections and histochemistry of the reptilian caudal cerebral hemispheres.

2.14.5.2 Connections and Histochemical Properties of the Reptilian Pallial Amygdala: Comparison with Mammals

2.14.5.2.1 Cortical amygdala Besides their afferents from the main or accessory olfactory bulb, the cortical amygdaloid areas of reptiles also share many connectional features with their mammalian counterparts, which we analyze in detail below.

2.14.5.2.1.(i) The olfactory cortical amygdala In their study of the connections of the LC of the gecko, Hoogland and Vermeulen-Vanderzee (1995) made restricted injections of lectins in different parts of the LC. According to their results, the dorsal rostral LC and the LCc display exclusively efferent projections to the hippocampal and parahippocampal cortices (medial and dorsal cortical areas). This had been previously reported in *Podarcis* by Martinez-Garcia *et al.* (1986) and confirmed in the snake *Thamnophis sirtalis* (Martinez-Marcos *et al.*, 1999). In contrast, the rostral ventral LC is closely related to the amygdaloid complex, since it projects to the external amygdala (presumably the VAA; from which it receives afferents; Martinez-Garcia *et al.*, 1986), PDVR, and LA (Lanuza *et al.*, 1998; Martinez-Marcos *et al.*, 1999). This was further supported by the results of deep injections in the ventral rostral LC (deep LC according to Novejarque *et al.*, 2004). This suggests that in reptiles and mammals the olfactory areas of the lateral pallium are differently compartmentalized. Whereas in mammals there is a number of cortical areas that project to the Hp and amygdala (COApl, APir, TR, entorhinal cortex), in reptiles the areas of the LC giving rise to these two projection systems are anatomically segregated.

The connections of the VAA (anterior part of the external amygdala, depending on the nomenclature) have been specifically studied in the context of amygdalohypothalamic pathways in lizards (Bruce and Neary, 1995a, 1995b; Lanuza *et al.*, 1997) and snakes (Martinez-Marcos *et al.*, 1999). The results of these studies indicate that the VAA projects mainly to the lateral hypothalamus and, apparently, to a cell population lateral and caudal to the ventromedial hypothalamus, called by Lanuza *et al.* (1997) lateral tuberomammillary nucleus (LTM), that might include ventral premamillary nuclei. A similar situation is observed in *T. sirtalis* (Martinez-Marcos *et al.*, 1999). Since the injections on which this projection was defined in lizards also included the VPA, it is difficult to ascertain whether the VAA and VPA display differential projections to the hypothalamus. New evidence suggests, however, that the projection to the lateral hypothalamus arises from the VAA, whereas the one directed to the LTM/ventral premammillary hypothalamus arises from the VPA (see below).

In conclusion, the available data on the connections of the olfactory cortical amygdala in reptiles suggest that the lateropallial olfactory cortices are differently compartmentalized in reptiles and mammals, so that specific homologues to the COApl and the transitional cortices of mammals are not found in reptiles. In contrast, within the ventral pallium the VAA seems the most likely reptilian homologue for the mammalian COAa (Table 3).

Table 3 Proposal of homologies between the mammalian, reptilian, and avian amygdaloid nuclei and areas

Embryological origin	*Topological position*	*Mammalian nucleus/area*	*Reptilian nucleus/area*	*Avian nucleus/area*	*Properties*
Lateral pallium	Superficial	COApl (APir, TR)	Parts of the LC?	Parts of the CPi?	Mainly intra-amygdaloid and to hippocampal formation
	Deep	B	DLA	Lateral NC TPO AD Lateral PoA	• Bilateral projections to striatum and NLOT • Dopaminergic + cholinergic innervation
Ventral pallium	Superficial	LOT	NLOT	AA	• Connected to the deep lateropallial amygdala • Cholinergic innervation
		COAa	VAA	Rostral olfacto-recipient area of the arcopallium, ventral to CPi	• Projections to striatum • Projections to lateral hypothalamus
		COApm	NS		Vomeronasal cortex
	Deep	L	PDVRdm (ADVR?)	Medial NC (field L, intermediate and frontal N, E, Bas?)	• Sensory interface • Intra-amygdaloid projections • Few extra-amygdaloid projections (to the striatum)
		ABa	Deep VAA	Rostrolateral AV deep to the olfacto-recipient arcopallium	• Projections to striatum • Projections to lateral hypothalamus
		ABp	PDVRv + LA	Posterior AV, nonolfacto-recipient	• Projections to VMH • Minor projections to striatum
		AHA	VPA	Ventromedial PoA / Caudal edge of the AV	• Projection to ventrolateral septum • Projections to preoptic hypothalamus • Interconnected with the ventral premammillary hypothalamus • Receptors to steroid hormones[a]
Subpallium		Central EA	SAT, BSTl	SpA, BSTL (subpallial AM?)	• Long-distance descending projections (e.g., lateral hypothalamus, parabrachial area, dorsal vagal complex) • CRF, NT cells (medial aspect)[b] • CGRP innervation (lateral aspect)
		Medial EA	MA, BSTm	TnA, BSTM (subpallial AM?)	• Massive projections to preoptic, lateral tuberal (ventromedial), and premammillary hypothalamus • High levels of receptors to sexual steroids • Vasopressin/vasotocin-containing projecting cells

[a] A cell group expressing receptors to sexual steroid is lacking in birds.
[b] The distribution of CRF and NT has not been properly studied in reptiles.

2.14.5.2.1.(ii) The vomeronasal cortical amygdala The connections of the NS have been studied in detail in the snake *T. sirtalis* (Lanuza and Halpern, 1997) and the lizard *P. hispanica* (Lanuza *et al.*, 1997; Novejarque *et al.*, 2004). The NS of squamate reptiles shows projections back to the AOB and to the remaining vomeronasal centers (putative medial amygdala and BST), as well as its contralateral counterpart through the anterior commissure. It also projects to olfactory structures such as the LC and displays intrinsic amygdaloid projections (mainly to deep nuclei; PDVR, DLA). Additionally, it displays important projections to the ventral striatum (olfactostriatum; Martinez-Marcos *et al.*, 2005) and projects to parts of the dorsal (hippocampal) cortex. This perfectly fits the pattern of projections from the mammalian COApm (Table 1), thus strongly supporting the homology between both structures.

2.14.5.2.2 The reptilian basolateral amygdala The basolateral amygdala of mammals receives sensory inputs from different sources (brainstem, thalamus, and cortex) and neurochemically identified modulatory afferents. In turn, its components show differential projections to the hypothalamus, to the dorsal and ventral striatum, and to portions of the cortex. As we discuss in detail, most of these same features are met by the proposed homologues in the reptilian brain.

2.14.5.2.2.(i) The deep lateropallial amygdala: The DLA as the reptilian homologue to the B nucleus of mammals The projections from the caudal cerebral hemispheres to the striatal territories in reptiles were neglected until recently (Gonzalez *et al.*, 1990), although there was some evidence suggesting that they existed in lizards (Voneida and Sligar, 1979; Martinez-Garcia *et al.*, 1993), turtles (Siemen and Kunzle, 1994), and snakes (Perez-Santana *et al.*, 1997). We reinvestigated this issue in the lizard *P. hispanica* (Novejarque *et al.*, 2004) using a combination of retrograde and anterograde tracing. Our results demonstrate that the DLA provides a massive and bilateral projection to the dorsal striatum (Figure 11). Moreover, the pallial amygdala gives rise to a massive projection to a continuum of

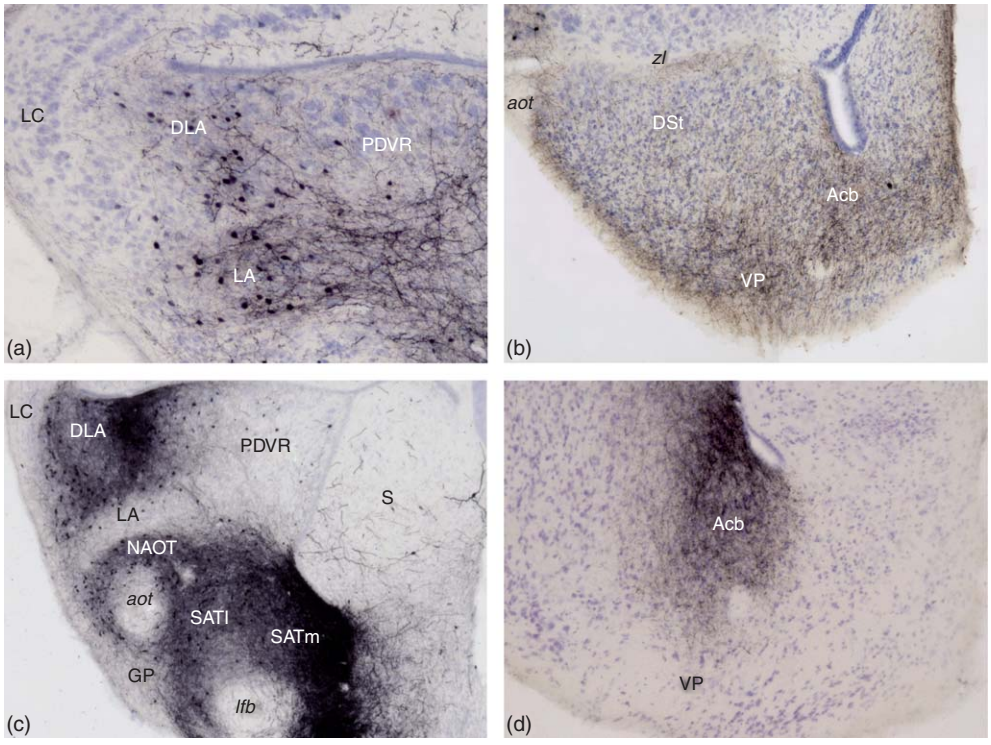

Figure 11 Amygdalostriatal connections in lizards. a and b, The DLA of lizards projects bilaterally to both the dorsal and ventral striata. Thus, retrograde transport is observed in the DLA (and part of the LA) in a lizard that received a tracer (dextranamine) injection encompassing the ipsilateral dorsal striatum (a). In addition, tracer injections into the DLA anterogradely label the dorsal and ventral striatum in the ipsilateral (b) and contralateral (not shown) telencephalon. This amygdaloid projection also reaches parts of the pallidum (VP). c and d, The PDVRdl of lizards projects exclusively to the ipsilateral Acb. Retrograde transport after injections in the Acb (c) indicates that the bulk of this projection arises from the DLA and the PDVRdl, whereas the PDVRvm and LA do not contribute substantially to it. Tracer injections restricted to the PDVR (d) result in anterograde labeling of the Acb but not in the dorsal striatum.

structures in the ventral striatum connecting the striatoamygdaloid transition area (SAT) and the Acb (rostrally). This projection arises mainly from the DLA, the dorsolateral aspect of the PDVR (PDVRdl), the VAA, VPA, and NS, and the deep LC (dLC). Among all these nuclei, only the DLA projects substantially to the contralatateral Acb.

Therefore, the DLA stands as the main source of amygdalostriatal pathways since it projects massively and bilaterally to the dorsal and ventral striatum. This strongly supports our proposal that the DLA is the reptilian homologue of the basal nucleus of the mammalian amygdala. In addition, like the mammalian B, the DLA projects bilaterally to the region of the NLOT (Novejarque *et al.*, 2004).

In the mammalian brain, the pallial amygdala is the target for two well-characterized ascending projections, namely the cholinergic input from the basal forebrain and the dopaminergic input from the dorsocaudal group A10. Both projections converge at the level of the B nucleus. Using appropriate (immuno)-histochemical techniques these projections can also be delineated in reptiles, and the results fully agree with the proposed homologies. Thus, the distribution of both ChAT (Medina *et al.*, 1993) and acetylcholinesterase (Lanuza *et al.*, 1997) reveals a dense cholinergic innervation of portions of the NLOT (Figure 12a) and of the DLA (Figure 12b). In addition, immunohistochemical detection of markers of dopaminergic fibers reveals a dense innervation of the DLA in the lizards *Psammodromus* (Andreu *et al.*, 1994) and *Podarcis* (Figure 12c).

2.14.5.2.2.(ii) Deep ventropallial nuclei: The PDVR and LA In squamate reptiles, the PDVR is composed of a juxtaventricular zone rich in cell groupings called glomeruli, plus a central core that shows no clear boundaries with the LA. In *Podarcis* the PDVR does not seem homogeneous, but is composed of cytoarchitectonically different zones: the ventromedial PDVR (PDVRvm) shows small glomeruli, the dorsomedial PDVR (PDVRdm) shows larger glomeruli, and the dorsolateral PDVR (PDVRdl), next to the DLA, display giant glomeruli. As we will see, the three areas display different connections that allow us to refine our proposal of homologies.

2.14.5.2.2.(ii).(a) The PDVRvm and LA constitute the reptilian homologue to the ABp of mammals The ABp of the mammalian amygdala gives rise to a part of the pallial component of the stria terminalis that terminates in the core of the VMH (Petrovich *et al.*, 1996). The amygdalohypothalamic projections have been fully characterized in different reptiles using retrograde and anterograde tracing techniques (Bruce and Neary, 1995a, 1995b; Lanuza *et al.*, 1997; Martinez-Marcos *et al.*, 1999). In all the species studied, the PDVRvm and LA (Figures 13a and 13b) project to the ventral and medial anterior tuberal hypothalamus (including the retrochiasmatic area and VMH core; Figure 13c). This pathway courses through a compact bundle that leaves the amygdala dorsal and caudal to the anterior commissure, and is called stria terminalis. From a comparative viewpoint, this seems quite a correct name in terms of both its origin (deep pallial amygdala) and its termination site (ventromedial hypothalamus). This tract contains zinc-rich fibers (Perez-Clausell, 1988; Smeets *et al.*, 1989), thus

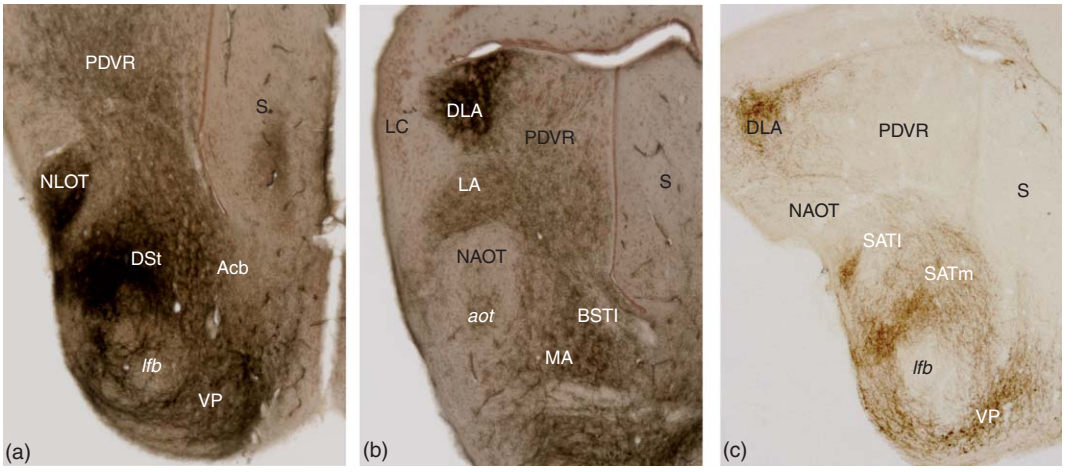

Figure 12 Chemoarchitecture of the amygdala of *Podarcis*. The (immuno)histochemistry for AChase (a and b) and tyrosine hydroxylase (TH, c) reveals a convergent cholinergic/dopaminergic innervation of the DLA. The NLOT also shows a dense AChase reactivity. Compared to the dorsal striatum proper (DSt), the subpallial amygdala shows a scarce AChase reactivity and a low density of TH-immunoreactive fibers.

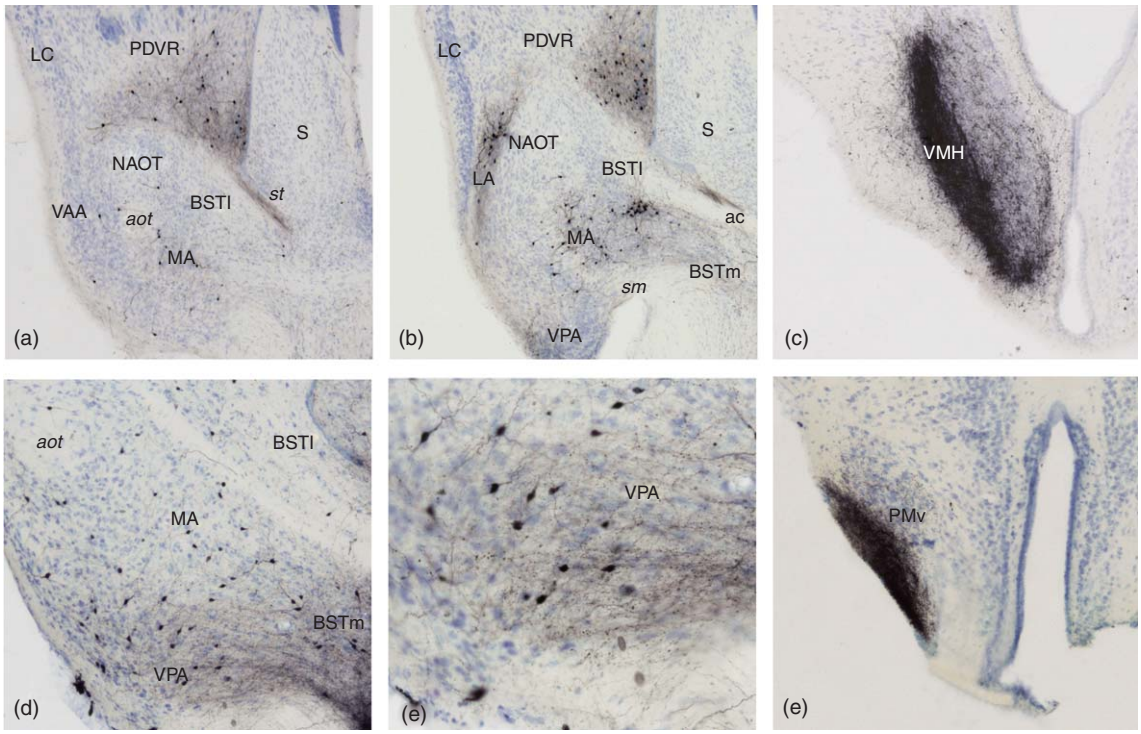

Figure 13 Amygdaloid projections to the hypothalamus in lizards. a and b, Rostral (a) and caudal (b) sections through the amygdala of a lizard that received an injection of dextranamine in the core of the ventromedial hypothalamic nucleus. Cells projecting to the VMH core are seen in the PDVRvm and LA as well as in most of the MA and portions of the BSTm. The pallial and subpallial bundles of the stria terminalis join at preoptic levels. c, The same projection is revealed by anterograde labeling after a tracer injection restricted to the PDVR. Labeling is seen in the VMH core. d and e, Labeling found in the amygdala of a lizard that received an injection of dextranamine in the premammillary hypothalamus, which did not involve the VMH core. Although the labeling in the subpallial amygdala is also located in the MA and BSTm, in the pallial amygdala, labeling is mainly observed in the VPA. At higher magnification (e), both retrograde and anterograde labeling is visible in the VPA, thus indicating that this nucleus is reciprocally connected with the premammillary hypothalamus. f, In a lizard that received an injection involving the VAA and VP, the presumed ventral premammillary hypothalamus shows a dense anterogradely labeled terminal field. More rostrally, labeled fibers avoid the VMH core but innervate the lateral shell (see Lanuza *et al.*, 1997).

confirming the glutamatergic nature (pallial origin) of some of its fibers. This strongly suggests that the ABp finds its homologue in the PDVRvm/LA of the reptilian ventral pallium (Table 3).

2.14.5.2.2.(ii).(b) The dorsomedial PDVR as the sensory interface of the reptilian amygdala As we have already seen, the PDVRdl projects to the ventral striatum (Figure 11c) and the PDVRvm contains the cells of origin of the pathway to the ventromedial hypothalamus (Figures 13a and 13b). In contrast, the PDVRdm seems only involved in intra-amygdaloid connections. Thus, tracer injections in the PDVR result in massive anterograde and retrograde labeling in the LA and DLA (Figure 14a). Moreover, in our material the PDVRdm only shows retrogradely labeled neurons after tracer injections involving most of the SAT, which, as we will discuss below, is the most likely candidate for the reptilian central amygdala.

The afferents to the PDVR were traced for the first time by Lanuza *et al.* (1998) in the lizard *P. hispanica*. Their findings suggest that the dorsal ventricular ridge of reptiles is anatomically and functionally heterogeneous. Its anterior aspect (anterior dorsal ventricular ridge: ADVR) receives discrete nonoverlapping auditory, somatosensory, and visual thalamic inputs. In contrast, the PDVR is the target of the medial posterior and posterocentral thalamus, which receives a convergent set of afferents from auditory, visual, and somatosensory midbrain and brainstem structures. The PDVR also receives direct afferents from the parabrachial region, probably conveying nociceptive, viscerosensitive, and/or gustatory information, and from the nucleus of the lateral lemniscus (auditory). In addition, highly processed sensory inputs arising from the dorsal (hippocampal) cortex (multimodal contextual), the ventral rostral LC (olfactory), and the three sensory areas of the ADVR (auditory, somatosensory, and visual) also reach the PDVR. Finally,

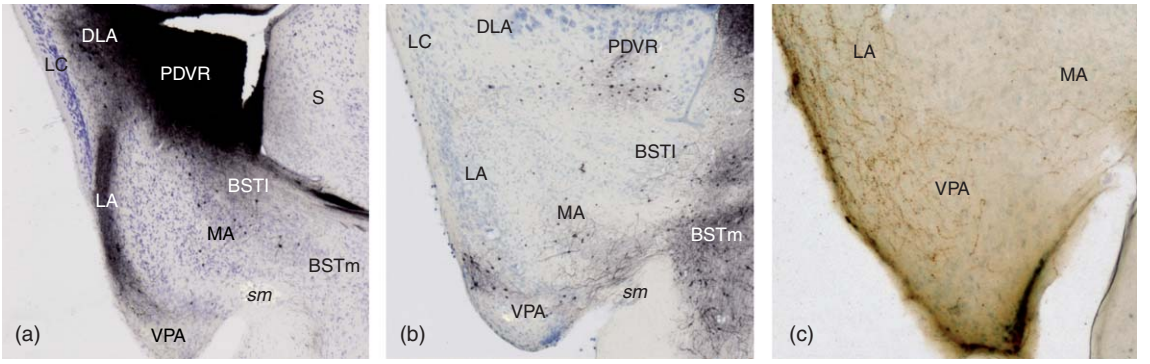

Figure 14 Intrinsic, preoptic, and hippocampal connections of the reptilian amygdala. a, Injections of dextranamine in the PDVR reveal an intricate set of interconnections within the basolateral amygdala of lizards. Thus, both the DLA and the LA show retrograde and anterograde labeling. In addition, the BSTI and SAT (Figure 15c), which constitute the central extended amygdala of lizards (Table 3), show anterograde labeling indicative of abundant basolateral-to-central intra-amygdaloid projections. The MA also seems to project to the basolateral amygdala (see retrogradely labeled cells). b, Retrograde labeling in the amygdala after a tracer injection involving the BSTm and the medial preoptic area. The presence of labeled cells in the PDVRvm should be attributed to the involvement of a part of the pallial stria terminalis in the injection site. In addition, strong retrograde labeling in the MA and VPA indicates a projection of both nuclei to the BSTm/preoptic area. c, Tracer injections (*Phaseolus vulgaris* leucoagglutinin) in the caudal dorsal (hippocampal) cortex of lizards reveal a projection to the basolateral amygdala, including the DLA (not shown), and LA as well as to the VPA and MA.

the PDVR is engaged in intra-amygdaloid afferents (with the DLA, LA, and MA; Figure 14a). Additional afferents to the PDVR arising from the caudal VTA, several hypothalamic nuclei (including the VMH), and the basal forebrain depict a complex pattern of sensory and modulatory afferents that fits with its homology with the basolateral amygdala of mammals.

In summary, the PDVRdm is the ventropallial amygdaloid center that receives massive sensory inputs and is only involved in projections to regions of the basolateral amygdala and to the central EA. In other words, it is the sensory interface of the basolateral amygdala of reptiles, thus being connectionally comparable to the mammalian L. However, when comparing reptiles and mammals, it becomes evident that the mammalian amygdala displays a more elaborated amygdaloid circuitry in which sensory interface and output centers are clearly separated (and interconnected), whereas in the reptilian amygdala both centers are somewhat mixed up.

After their studies of the projections to the hypothalamus in the gecko, Bruce and Neary (1995a, 1995b, 1995c) proposed that the anterior DVR, which receives direct sensory afferents from the thalamus (Ulinski, 1983) and projects to the PDVR (Lanuza *et al.*, 1998) but not to the hypothalamus, is the reptilian homologue of the mammalian L. This hypothesis is also supported by the ventropallial nature of the ADVR (Smith-Fernandez *et al.*, 1998). The amygdaloid nature of the ADVR was questioned by Lanuza *et al.* (1998) on the basis of its unimodal thalamic afferents, which contrasts with the polimodal and limbic thalamic afferents attributed to amygdaloid centers. However, this view needs to be revised in the light of new evidence suggesting that the principal sensory nuclei of the dorsal thalamus of reptiles (medial, posteromedial, posterocentral), including the nucleus rotundus (Guirado *et al.*, 2000), might indeed be homologues to the posterior/intralaminar thalamus of mammals (Davila *et al.*, 2000).

Another important difference between the mammalian L and the ADVR of reptiles that is very significant from a comparative viewpoint is the pattern of projections to the striatum. Whereas the ADVR projects massively and topographically to the dorsal (but not to the ventral) striatum, the mammalian L shows projections to the ventral (and scarce projections to the dorsal) striatum (our unpublished results; Pitkanen, 2000). Nevertheless, the comparative significance of the reptilian dorsal striatum (e.g., its homology with the mammalian CPu) is also a debatable issue (Lanuza *et al.*, 2002). Therefore, the possible homology of the ADVR of reptiles (and birds; see below) with part of the mammalian LA cannot be ruled out, although alternative views should be carefully considered (Striedter, 1997; Puelles, 2001).

2.14.5.2.2.(ii).(c) Amygdalocortical projections
Our proposal of homologies between the reptilian and mammalian amygdala predicts that the DLA, PDVR, and LA should project to the dorsal pallium, thus reciprocating the projection from the dorsal pallium to the amygdala (Hoogland and

Vermeulen-Vanderzee, 1989; Lanuza *et al.*, 1998). This would be the reptilian counterpart of the corticoamygdalocortical loops that in the mammalian brain involve the B and AB on the one hand, and the frontotemporal cortex on the other. However, in contrast to mammals, the amygdala of reptiles apparently display few projections directed to the dorsal pallium. This fact probably indicates that the presence of projections to the dorsal pallium is an acquired trait of the mammalian amygdala, related to the important changes that the dorsal pallium underwent during the early evolutionary history of mammals.

In contrast, all the studied reptiles display important interconnections with the ventropallial areas that provide nonchemosensory information to the amygdala. Thus, the LA projects back to the ADVR, thus reciprocating the strong input from the ADVR to the PDVR/LA (Bruce and Butler, 1984). The comparative significance of this pathway awaits further data on the nature of the ADVR.

2.14.5.2.2.(iii) The VPA shows many similarities with its putative mammalian homologue, the AHA The study of the amygdalohypothalamic pathways of reptiles has revealed the presence of a second projection within the pallial component of the stria terminalis, besides the one arising in the PDVRvm/LA. This projection arises in the ventral amygdala (VPA and VAA), leaves the cerebral hemispheres through the so-called lateral amygdalofugal tract (Lanuza *et al.*, 1997; Figure 13d), and terminates in the VMH shell, the LTM, and premammillary hypothalamus (Figure 13f). Injections of tracers in the premammillary and mammillary hypothalamus, which did not involve the VMH at all (and, as a consequence, show no labeled cells in the PDVR and LA), resulted in the presence of abundant, intensely labeled neurons in the VPA (Figures 13d and 13e). This strongly suggests that the VPA is the origin of the projection to the LTM and premammillary hypothalamus whereas the VAA originates a projection directed at the dorsal lateral hypothalamus.

In mammals, the amygdala also projects to the region of the posteromedial BST and the medial preoptic hypothalamus. This projection arises from both the EA (namely, the medial amygdala; Canteras *et al.*, 1995) and the AHA in the pallial amygdala (Canteras *et al.*, 1992a). The forebrain of reptiles displays a similar projection that arises from the VPA (Figures 14b and 14c). As in mammals, in lizards this connection with the preoptic hypothalamus is reciprocal.

Therefore, the VPA is connected with medial preoptic hypothalamus, the shell of the VMH, and the premammillary hypothalamus. These hodological properties strongly support its homology with the mammalian AHA, based on a similar topological position within the posterior ventral pallium and deep to the cortical vomeronasal amygdala.

Other hodological and histochemical properties of the VPA further support this homology. Thus, like the AHA of mammals (Canteras *et al.*, 1992a), the VPA projects to the ventral lateral septum (Font *et al.*, 1998) and receives afferents from the portion of the Hp (CA1 in mammals) that projects to the ventral lateral septum (Figure 14c). In addition, like the AHA of mammals (Simerly *et al.*, 1990), the VPA is the most prominent pallial cell group of the reptilian telencephalon, expressing high levels of receptors to sexual steroids. The distribution of receptors to sexual steroids has been studied with different techniques in different reptiles, such as the whiptail lizard (Young *et al.*, 1994), the geckonids *Gecko* (Tang *et al.*, 2001) and *Eublepharis* (Rhen and Crews, 2001), in *Sceloporus* (Moga *et al.*, 2000), in the green anole (Rosen *et al.*, 2002), and in the garter snake (Halpern *et al.*, 1982). In lizards steroid-sensitive cells are observed in a cell group called external amygdala that seems to correspond to the VPA, as defined here. In snakes, Halpern *et al.* (1982) described a group of steroid-concentrating neurons located in the ventral telencephalon just rostral to the ventral NS, named ventral amygdaloid nucleus, that seems also to correspond to the VPA (Lanuza and Halpern, 1998).

All these data indicate that the VPA, like its mammalian homologue (AHA), is engaged in a circuit composed of vomeronasal centers (medial EA) and steroid-sensitive structures in the forebrain (ventrolateral septum, medial EA, premammillary hypothalamus), and expresses itself as receptors to sexual steroids. This strongly suggests that the VPA is a nucleus of the pallial amygdala specialized in the control of agonistic and reproductive behavior, as has been suggested for its mammalian counterpart.

2.14.5.3 The Reptilian Subpallial Amygdala

The caudal pole of the striatopallidal telencephalon of reptiles, adjacent to the above-defined pallial amygdaloid centers, includes a number of nuclei that are generally considered as the reptilian subpallial amygdala. There, nuclei have been named using a mixture of topographical and comparative

terminology, such as the medial amygdala, the BST, central amygdala, or striatoamygdaloid transition area. Nevertheless, the delineation and identification of these nuclei are not clear in the literature due to both the use of inappropriate criteria to define them and to the presence of interspecies differences. In addition, the use of topographical terminology might cause confusion since it is suggestive of homologies with the mammalian amygdala that might be erroneous (Box 1).

Box 1 The vomeronasal system and the variability in the organization of the reptilian pallial amygdala

Our proposal of homologies for the reptilian pallial amygdala is mainly based on data from Lacertidae lizards and fit some of the published literature in teiidae (*Varanus* and *Tupinambis*; Hoogland, 1977; Voneida and Sligar, 1979). However, some of the published data in other squamate reptiles apparently do not fit well with the view proposed here. Thus, for instance, in geckonid lizards the massive projections from the amygdala to the striatum have not been described. This is probably due to slight differences in the organization of the pallial amygdala rather than to the absence of these projections in geckonids. For instance, the main source of amygdalostriatal projections in *Podarcis*, the DLA, can be identified in other reptiles by means of the histochemical markers referred to above. Thus, the caudal cerebral hemispheres of geckonids displays an area innervated by cholinergic/AChase fibers (*Gecko gekko*: Hoogland and Vermeulen-Vanderzee, 1990; *Tarentola mauritanica*: unpublished observations) that also shows a rich dopaminergic innervation (Smeets *et al.*, 1986b). Therefore, the DLA of geckonid lizards seems to show a relatively ventral location within the cerebral hemispheres compared with the lizards of the family Lacertidae (such as *Podarcis* and *Gallotia*).

These differences in the organization of the caudal cerebral hemispheres are probably related to the different development of the vomeronasal system that is revealed by the size of the NS, which is much smaller in Geckonidae than in Lacertidae. This is supported by the finding of a more ventral situation of the AChase/tyrosine hydroxylase-innervated amygdaloid area (probably the DLA) in anoles, where the vomeronasal system is nearly absent (Figures B1a and B1b).

On the other hand, comparison of the data in Lacertidae with those of snakes, where the vomeronasal system probably reaches its highest degree of development, also reveals some apparent differences. These pertain to the presence of the projections from the DLA to the hypothalamus in snakes (Martinez-Marcos *et al.*, 1999). Although this may reflect real differences between lizards and snakes, it may also be due to a misdefinition of the DLA of snakes. In fact, the structure named as DLA by Martinez-Marcos *et al.* (1999, see figure 1 in this article) does not seem deep to the lateral cortex (thus lateropallial) but a cell group interposed between the PDVR and the LA, located next to the NS (thus very likely ventropallial). In this respect, it is interesting to note that the DLA cells identified as projecting to striatal territories (olfactostriatum) in *Thamnophis* (see figures 7c and 8c in Martinez-Marcos *et al.*, 2005) occupy a position that is clearly more rostral than those projecting to the hypothalamus. Dealing with this, the study of the distribution of AChase and tyrosine hydroxylase (TH) reveals convergent catecholaminergic and cholinergic innervations (Figures B1c and B1d) of a cell group apparently displaying the same location as the cells projecting to the olfactostriatum (Martinez-Marcos *et al.*, 2005) but clearly rostral to the ones projecting to the hypothalamus (Martinez-Marcos *et al.*, 1999). Therefore, it seems that, when properly defined using reliable histochemical markers, the DLA of snakes turns out to be a cell group projecting bilaterally to the striatum, but not to the hypothalamus.

Although data in crocodiles and turtles are scarcer, in general they agree with the view we propose. For instance, in turtles the region of the posterior DVR shows a similar pattern of multimodal thalamic inputs (Belekhova and Chkheidze, 1992) and intratelencephalic (ADVR-PDVR) sensory afferents (Belekhova and Chkheidze, 1991; Chkheidze and Belekhova, 1992). In addition, the posterior DVR region of turtles also displays abundant projections to the striatum (Siemen and Kunzle, 1994) comparable to the ones described in lizards by our group (Novejarque *et al.*, 2004). Moreover, there is evidence of the existence of massive projections to the hypothalamus arising from the caudal cerebral hemispheres (PDVR and the so-called basal DVR) in turtles (see figure 9 in Cordery and Molnar, 1999).

In a similar way, Pritz and Stritzel (1992) described a projection to the posterior DVR of crocodiles from a thalamic cell group in the neighborhood of the auditory relay, the nucleus reuniens pars diffusa. As discussed in detail by Lanuza *et al.* (1998), this cell group seems part of the multimodal posterior

thalamus, thus suggesting an amygdaloid nature of the PDVR and adjacent areas also in crocodiles. Moreover, in the caudal cerebral hemispheres of the caiman (Brauth and Kitt, 1980), there are two patches intensely reactive for AChase in the NLOT and the lateral edge of the caudal DVR, which projects to the striatum, a situation that recalls the DLA. Data on the anatomy of the cerebral hemispheres of crocodilians are urgently needed, especially since they represent the closest living relatives of the stem reptiles that gave rise to birds (Whetstone and Martin, 1979). In fact, the study of the distribution of glial fibrillary acidic protein reveals a similar organization of the cerebral hemispheres of crocodiles (Kalman and Pritz, 2001) and chicken (Kalman *et al.*, 1998) that may be very useful to understanding the evolution of the amygdala.

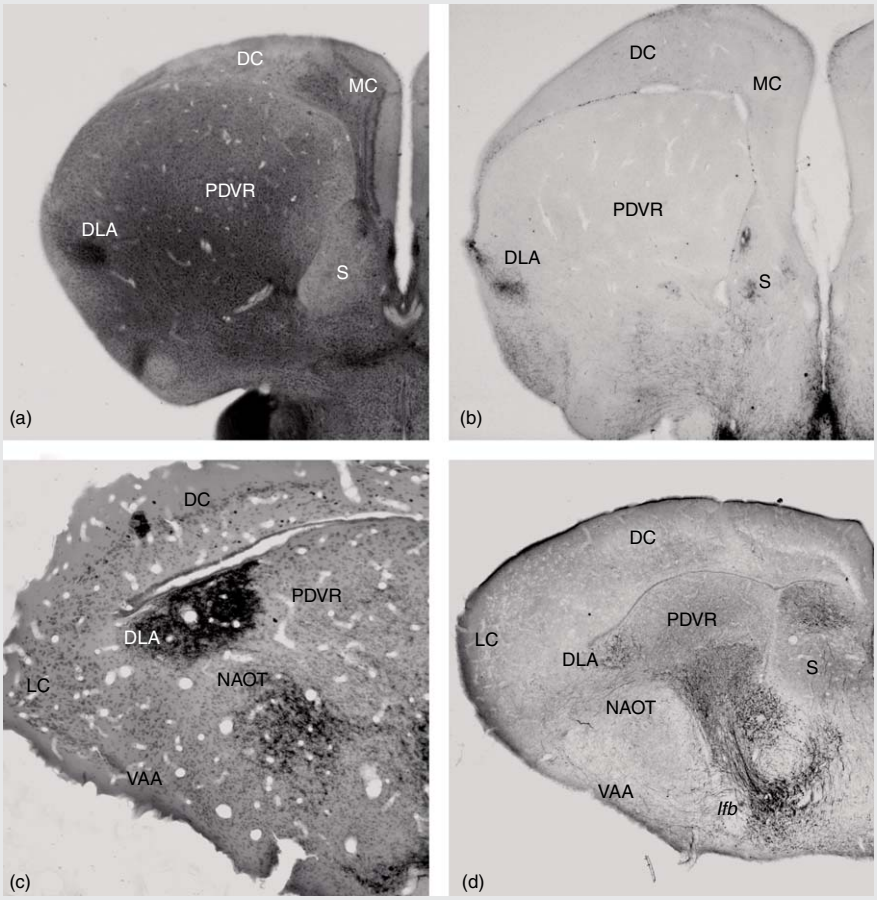

Figure B1 The amygdala of microsmatic and macrosmatic squamate reptiles. a and b, The strong reduction of the olfactory and vomeronasal system in the lizard *Anolis carolinensis* has introduced important changes in the organization of its brain. Nevertheless, the DLA can be identified by the convergent innervation of AChase (a) and TH (immuno)reactivity (b). The so-defined DLA seems displaced ventrally relative to the position it occupies in *Podarcis* (Figure 12), probably due to the lack of a nucleus sphericus in *Anolis*. The DLA of *Anolis* recalls the dorsal arcopallium of birds (Figure 19). c and d, This contrasts with the situation in the snake *Thamnopis sirtalis,* where the presence of a huge nucleus sphericus occupying the whole caudal half of the cerebral hemispheres has displaced the DLA rostralwards, as indicated by the AChase (c) and TH (immuno)reactivity (d). The pictures of *Thamnophis* have been kindly donated by Drs. Alino Martinez-Marcos and Mimi Halpern.

In this section, we try to clarify the architecture of the subpallial amygdala of reptiles, mainly using data of squamate reptiles. In this respect, we will identify the medial and central EA of reptiles by studying the features that define them in mammals.

2.14.5.3.1 The reptilian extended amygdala: The identity and divisions of the bed nucleus of the stria terminalis As we have seen above, in mammals the internal capsule separates the amygdala proper from the extra-amygdaloid BST (with the supracapsular BST and the sublenticular EA

connecting both structures). In reptiles, and in general in nonmammals, an internal capsule is absent (the lateral forebrain bundle is reduced to a compact tract that runs through the basal telencephalon; Figure 9). Therefore, in the subpallial amygdala of reptiles (and this is also valid for birds), the centromedial amygdala and the BST are not separated, but conform a single cell mass in the basal caudal cerebral hemispheres. This has generated a confusing and contradictory nomenclature of this area of the caudobasal cerebral hemispheres, which we are trying to clarify here.

The name 'bed nucleus of the stria terminalis' designates cells that are intermingled among the fibers of the stria terminalis and are targets for them. Our material reveals that, in lizards, the stria terminalis is composed of two main tracts (Figures 13a and 13b) that join at preoptic levels. The dorsal stria terminalis is a compact tract that leaves the PDVR medially, just ventral to the ventral sulcus of the lateral ventricle, and enters the hypothalamus caudal to the anterior commissure. The ventral stria terminalis (which arises from the VPA and MA; see below) is a less compact fiber tract that enters the preoptic area ventral to the anterior commissure. Both components of the stria terminalis have very short intratelencephalic trajectories, and the cell groups with which they are associated should be considered the two main parts of the reptilian BST, namely the lateral (accompanying the dorsal stria terminalis, BSTl) and the medial BST (BSTm).

Comparing this view with previous literature on the architecture of the reptilian forebrain, we conclude that the BSTl as defined here is equivalent to the structures of the brain of the gecko, named as nucleus of the anterior commissure by Smeets *et al.* (1986a), and to the caudomedial SAT according to Bruce and Neary (1995b). In turn, the BSTm is comparable to the BST of the gecko according to the terminology of Smeets *et al.* (1986a), the interstitial amygdala of the gecko according to Bruce and Neary (1995b), and the nucleus interstitialis of the whiptail lizard (Young *et al.*, 1994). The latter terminology was also adopted by our group in some papers on the anatomy of the brain of *Podarcis* (Font *et al.*, 1997; Lanuza *et al.*, 1997).

2.14.5.3.2 The medial extended amygdala of reptiles In most studies of the projections from the AOB, a more or less prominent terminal field has been observed just medial and ventral to the transition between the rostral NS and the BAOT. This structure was named ventromedial amygdala (Martinez-Garcia *et al.*, 1991), medial amygdala (MA; Lanuza and Halpern, 1998; this name was used in the gecko by Bruce and Neary (1995a), and has finally been adopted by us) or central amygdaloid nucleus (Lohman and Smeets, 1993) depending on the authors and/or species under study. In all of these studies, the projection from the AOB is seen to extend further medially into the ventrolateral aspect of the BSTm (Figure 10c). At these levels, the fibers from the main olfactory bulb running within the stria medullaris are just superficial to the MA. This situation recalls the TnA of birds (see below).

There are no studies devoted specifically to the study of the connections of the MA and BSTm. (In a series of experiments designed to unravel the neural basis of tongue-flick (a behavior that delivers chemicals into the vomeronasal organ), Martinez-Marcos *et al.* (2001) analyzed in detail the amygdaloid projections to the hypoglossal nucleus of the snake *T. sirtalis*. Their results indicate that the medial amygdala projects indirectly to the hypoglossal nucleus using a relay in the LHN, but directly to other centers of the dorsal medulla. However, if one compares the retrograde labeling they find after injections into the LHN (see figure 4g in Martinez-Marcos *et al.*, 2002) or their injections into the medial amygdala (see their figure 4a), with the projections from the AOB (see figures 1e and 1f in Lanuza and Halpern, 1998), it becomes evident that most of the cells projecting to the LHN are out of the chemosensory subpallial amygdala (MA), but in a location dorsal to the anterior commissure that probably corresponds to the SAT of snakes.) However, data derived from tracer injections in other areas of the brain of lizards and snakes indicate that, from a comparative point of view, the names MA and BSTm seem appropriate for these structures. As we have described above, the BSTm is crossed by axons from MA cells on their way to the hypothalamus and therefore, very likely, receives projections from the MA. In addition, tracer injections in the preoptic hypothalamus (Lanuza *et al.*, 1997; Martinez-Marcos *et al.*, 1999) suggest that the MA and the BSTm are reciprocally connected with the medial preoptic hypothalamus (Figure 14b). Moreover, both the MA and the BSTm project to the VMH (Figures 13a and 13b) and premammillary hypothalamus (Figures 13d and 13e; Bruce and Neary, 1995b; Lanuza *et al.*, 1997; Martinez-Marcos *et al.*, 1999). This clearly recalls the situation in mammals and suggests that the MA and BSTm conform to the reptilian medial EA. In fact, as

we will see, they share most of their connections and histochemical properties.

Like the mammalian medial EA, the reptilian MA and BSTm provide a feedback projection to the AOB (Martinez-Garcia *et al.*, 1991; Lanuza and Halpern, 1998) and receive a direct projection from the vomeronasal cortex (NS; Lanuza and Halpern, 1997; Lanuza *et al.*, 1997; Novejarque *et al.*, 2004). Moreover, like the medial EA of mammals, the BSTm of lizards projects to the ventral aspect of the lateral septum (Font *et al.*, 1997). Although there is no direct evidence using double-labeling experiments, immunohistochemical data in several reptiles suggest that this projection is rich in one of the forms of the reptilian vasopressin (vasotocin), like its mammalian counterpart (Wang *et al.*, 1993). Thus, the BST (very likely the BSTm plus portions of the MA) displays cells immunoreactive for vasotocin in lizards (Stoll and Voorn, 1985; Thepen *et al.*, 1987), snakes, and turtles (Smeets *et al.*, 1990), and in all the reptiles studied the ventral lateral septum is innervated by vasotocin-immunoreactive fibers. In agreement with the situation in mammals (Wang *et al.*, 1993), this projection system displays sexual dimorphism in reptiles (Stoll and Voorn, 1985; Smeets *et al.*, 1990).

This proposal of homology is strongly supported by the fact that the MA and BSTm of lizards (together with the ventral lateral septum, with which they are connected), display the most remarkable population of sexual steroid-sensitive cells in the whole subpallial telencephalon (Young *et al.*, 1994; Moga *et al.*, 2000; Rhen and Crews, 2001; Rosen *et al.*, 2002). A similar situation is present in snakes (Halpern *et al.*, 1982). This constitutes another of the defining features of the medial EA of mammals (Tables 2 and 3).

2.14.5.3.3 Central extended amygdala of reptiles

The name 'central' amygdala was first applied to designate a cell group in the brain of the gecko (Smeets *et al.*, 1986a) that occupies a central location within the putative amygdaloid region. However, since this nucleus is the target for a projection from the AOB (Lohman and Smeets, 1993), it is not comparable to the central but to the medial amygdala of mammals (see above). The reptilian homologue of the mammalian Ce was identified a few years later. In research primarily devoted to the study of the efferent connections of the dorsal and ventral striatum, Russchen and Jonker (1988) realized that injection of tracers in the caudal basal ganglia, what they called striato-amygdaloid transition area (SAT), resulted in a distinct pattern of anterograde labeling. The SAT seemed to project to the lateral hypothalamus (lateral posterior hypothalamic nucleus, LHN; Lanuza *et al.*, 1997), to the midbrain tegmentum (like the rest of the striatopallidal system) and to more distant targets in the central gray, parabrachial area (Figure 15a) and medulla (nucleus of the solitary tract and dorsal motor vagal complex; Figure 15b). This is a pattern of connections identical to the one displayed by the mammalian central EA. It is important to note that the caudal SAT fuses with the BSTl, so that it is tempting to suggest that, together, both structures conform the central EA of reptiles.

Several lines of evidence are in agreement with this proposal of homology. Like the mammalian Ce and the dorsal and posterolateral BST, the SAT of lizards receives afferents from the parabrachial nucleus (Figure 15a), as well as from several nuclei in the posterior thalamus, including the nucleus rotundus (Guirado *et al.*, 2000) and the posteromedial and posterocentral nuclei (Lanuza *et al.*, 1998). In addition, it receives projections from the basolateral amygdala (PDVR and DLA; Novejarque *et al.*, 2004; Figure 15c) and from the rostral deep lateral pallium (dLC). Moreover, the SAT displays at least some of the remaining defining features of the mammalian central EA. Thus, in *Podarcis* the lateral aspect of the SAT and the region of the striatum adjacent to it, just medial to the NAOT, display a dense CGRPergic innervation (Martinez-Garcia *et al.*, 2002b; Figure 15d), which recalls the projection to the CeC and amygdalostriatal transition of the mammalian brain. The origin of these CGRPergic fibers is not clear but the most likely candidates are the two cell groups projecting to the amygdala that display CGRP-immunoreactive cells, namely the parabrachial nucleus and the ventral aspect of the posterior thalamic nuclei (Lanuza *et al.*, 1998). This is also a feature shared with the mammalian central EA (Schwaber *et al.*, 1988; Yasui *et al.*, 1991). Unfortunately, there are no detailed descriptions of the distribution of CRF and NT in the brain of reptiles. These data are needed to prove the hypothesis of homology between the mammalian central EA and the SAT of reptiles.

2.14.5.4 The Reptilian Amygdala: A Summary

All the data reviewed above convincingly demonstrate that the reptilian amygdala contains pallial and subpallial components comparable to those of the amygdala of mammals. The pallial amygdala of

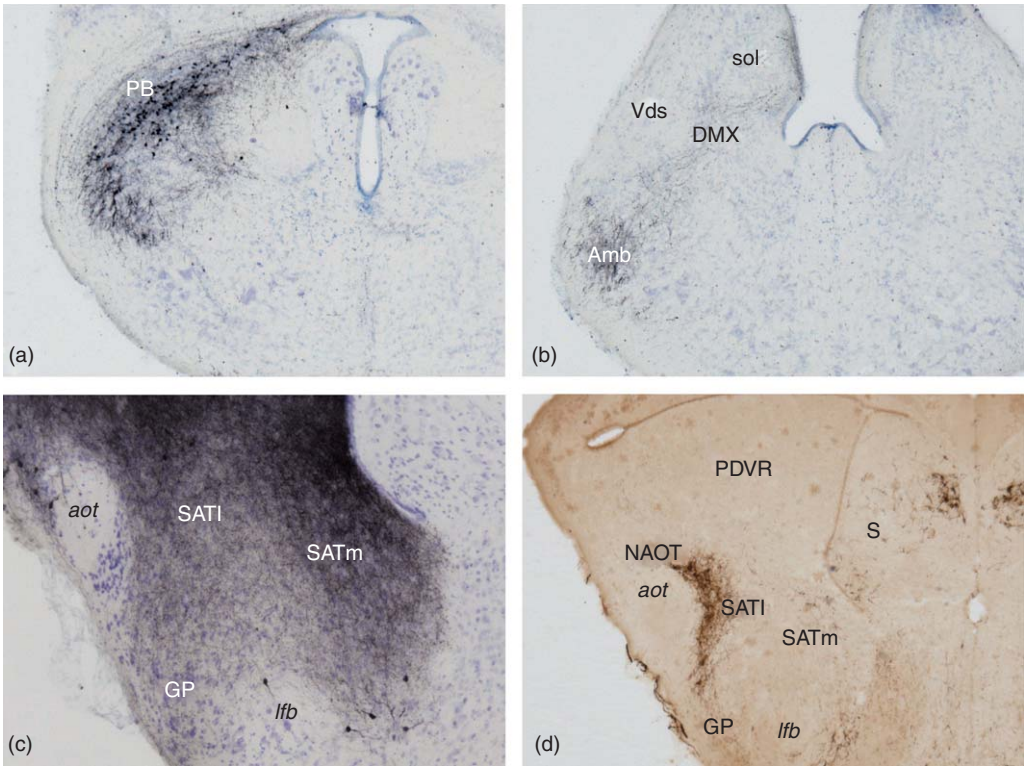

Figure 15 Connections and histochemical properties of the SAT. a and b, Tracer injections encompassing the SAT of *Podarcis* give rise to anterograde labeling in the lateral hypothalamus (Lanuza *et al.*, 1997), but also in the parabrachial area (a), where retrograde labeling is also visible, and in the medulla (b), including the nucleus of the solitary tract (sol), the dorsal motor vagal nucleus (DMX), and the ambiguus (Amb). c, Anterograde labeling in the SAT following a tracer injection in the PDVR and LA in *P. hispanica*. d, The lateral SAT (SATl) of the lizard *P. hispanica* shows a dense innervation by CGRP-immunoreactive fibers. The medial SAT (SATm) shows a much weaker innervation that is mainly composed of nests around the cell bodies.

reptiles is composed of cortical olfactory (VAA, portions of the LC) and vomeronasal centers (NS) plus deep, unlaminated regions (PDVR, LA, and DLA) that receive multimodal and unimodal afferents from different levels of the neuroaxis. An additional nuclear pallial nucleus that (in spite of its superficial location) is topologically deep to the vomeronasal cortex, the VPA, is part of a forebrain circuit closely related to the vomeronasal system which is rich in cells expressing receptors for sexual steroids.

The vomeronasal amygdala includes not only the NS (and VPA) but also a portion of the subpallium, the MA, and BSTm (medial EA), which is also very rich in receptors to sexual steroids. The pallial and subpallial centers of the vomeronasal amygdala are interconnected and project massively to centers in the limbic forebrain (septum, preoptic, and tuberal and premmamillary hypothalamus), probably involved in the control of reproductive and agonistic behaviors.

In contrast, the multimodal centers of the pallial amygdala (PDVR, LA, and DLA) project massively to the ventral striatum, including the Acb and the SAT. The latter is a part of the subpallial amygdala that shows long-distance projections reaching hypothalamic, tegmental, periaqueductal, pontine, and medullary targets. This pathway seems involved in the control of somatomotor, endocrine, and vegetative reactions. In addition, the deep lateropallial nucleus, the DLA, projects bilaterally to the dorsal striatum.

This picture is virtually identical to the one observed in mammals, thus suggesting that the amygdala as a whole has undergone quite a conservative evolution in the phylogeny of amniote vertebrates. As we will see, data from birds further support this view.

2.14.6 The Amygdala of Birds

Identifying the avian amygdala becomes a fundamental issue after the recent joint effort of an international forum of comparative neuroscientists to rename and reinterpret the telencephalon of

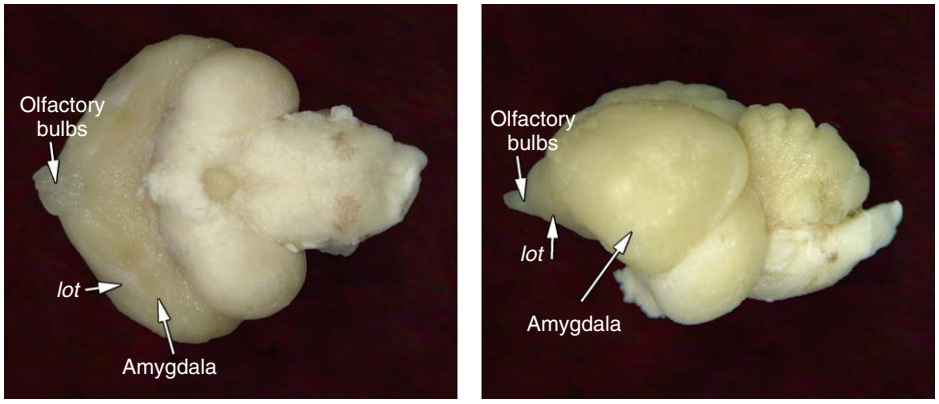

Figure 16 Ventral and lateral views of the brain of the quail (*Coturnix coturnix*). The regression of the olfactory system is evidenced by the small size of the olfactory bulbs (where accessory olfactory bulbs are absent) and the inconspicuous lateral olfactory tract (*lot*). An arrow points to the putative amygdala.

birds (Reiner *et al.*, 2004; Jarvis *et al.*, 2005). However, two features of the avian brain make this task especially difficult. First, as compared to mammals, birds possess very peculiar cerebral hemispheres, since they have a huge dorsal ventricular ridge (occupying an apparent subventricular position), but lack anything recalling the mammalian isocortex. This has resulted in important changes in the topographical relationships between the different areas of the pallium and subpallium of avian cerebral hemispheres, as compared to mammals. In addition, birds have undergone a regression of the olfactory and a virtual atrophy of the vomeronasal system, which, as we have seen, provide important inputs to the mammalian and reptilian amygdalae, where they constitute the defining features of their cortical and medial portions. This is clearly observed in a macroscopic view of the brain of birds (Figure 16), in which the olfactory bulbs are very small, the lateral olfactory tract is hardly visible, and no rhinal fissure is observed.

Nevertheless, once the reptilian amygdala and its components have been identified with a high degree of certainty, the similarities between the avian and reptilian brains (Ulinski, 1983) may be very helpful for our goal. In this section, we are using this strategy to identify the amygdala in the caudolateral telencephalon of birds and to delineate its main pallial and subpallial components by using the features that define them in both mammals and reptiles.

2.14.6.1 On the Nomenclature and Architecture of the Telencephalon of Birds

In this article, we will follow the revised nomenclature of the avian forebrain (Reiner *et al.*, 2004; Jarvis *et al.*, 2005). However some issues concerning this nomenclature need to be discussed prior to analyzing the identity of the amygdala of birds.

The caudal cerebral hemispheres of birds (Figure 17) includes several structures that conform a regular (superficial and layered) cortex, composed of several areas, from medial to lateral, the Hp, the APH, the area corticoidea dorsolateralis (CDL), and the CPi, that needed no renaming since their cortical nature was always recognized. Inner structures of the dorsal telencephalon include those pallial structures whose name contained the suffix striatum, which have been renamed to recognize their pallial nature. Thus, the deep pallium of the posterior telencephalon of birds is composed of the caudal edge of the mesopallium (M, formerly ventral hyperstriatum) and the caudal nidopallium (formerly caudal neostriatum), in which lateral and medial divisions are usually recognized (NCL and NCM respectively). The NCM includes the auditory field L. In addition, there is a region just deep to the CPi that extends dorsally up to the CDL, which is called the area temporoparieto-occipitalis (TPO).

More ventrally, the caudal poles of the lateral striatum (LSt; formerly paleostriatum augmentatum) and GP (formerly paleostriatum primitivum) occupy the medial aspect of the hemispheres. Just ventral to them (thus justifying the old name of ventral paleostriatum) there is a region now renamed as SpA, topologically superficial to the so-called lateral BST (occupying a juxtaventricular position).

Finally, ventral and lateral to the caudal subpallium there is a region traditionally called archistriatum and the nucleus teniae of the amygdala (TnA). The archistriatum was considered to be composed of several divisions that were named

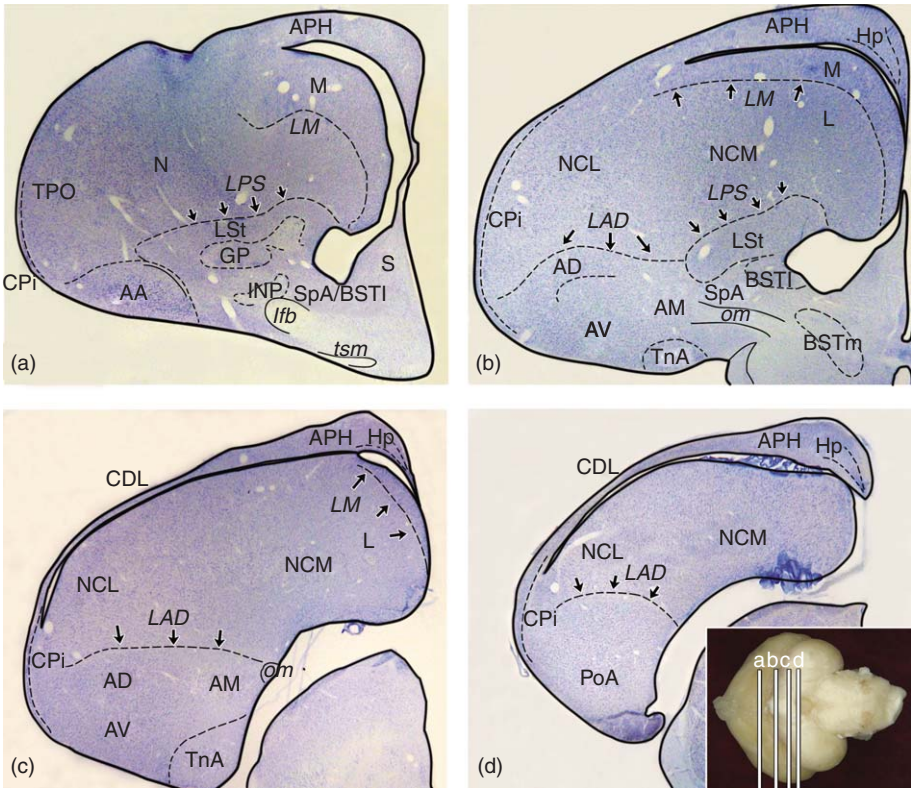

Figure 17 Cytoarchitecture of the caudal cerebral hemispheres of the chicken. Nissl-stained frontal sections through the left cerebral hemisphere of a newborn chicken at slightly precommissural (a); commissural (b); postcommissural (c); and caudal (d) levels of the telencephalon. The inset shows the approximate location of the sections on a ventral view of the avian brain. The different nuclei and cortical areas of the telencephalic structures are delineated using thin discontinuous lines, whereas thin solid lines indicate the main fiber tracts. Small arrows point to the laminae that separate the main divisions of the avian forebrain (also delineated in discontinuous lines), whose abbreviations are written in italics.

topographically as anterior, medial, intermediate (with dorsal and ventral subdivisions), and posterior. The new nomenclature reflects the putative pallial nature of the whole archistriatum by naming its parts as anterior arcopallium (AA), medial arcopallium (AM), dorsal arcopallium (AD; corresponding to the dorsal intermediate archistriatum), ventral arcopallium (AV; corresponding to the ventral intermediate archistriatum), and the posterior nucleus of the pallial amygdala (PoA, corresponding to the former posterior archistriatum).

Two problematic issues directly related to our purposes need to be discussed. First, the boundaries between the AA and the AD/AV (formerly intermediate archistriatum) have never been properly defined and the same happens with the border separating the AD/AV from the PoA. In this respect, the identity of the PoA is not clear and, consequently, different authors have labeled different structures as PoA (or posterior archistriatum), thus generating a confusing panorama (see, for instance, the extent of the PoA in Reiner and Karten, 1985, as compared to Kroner and Gunturkun, 1999). Most authors simply label as PoA (or posterior archistriatum) the caudal edge of the former archistriatum, thus including the caudal tip of the AD (like the latter, deep to the caudal-most CPi), plus the posterior aspect of the AV. Dealing with this, it is important to recall that the nowadays-accepted classification of the arcopallial-amygdaloid centers of birds is based on a topographical compartmentalization of this area introduced by Zeier and Karten (1971), who reported to have identified some four to eight cytoarchitecturally discrete nuclei in each of the major subdivisions of the former archistriatum. The poor understanding of the actual organization of the arcopallium/PoA is probably hindering our knowledge of its comparative and functional significance.

Second, the use of the term 'arcopallium' instead of the old name 'archistriatum' (Reiner *et al.*, 2004) is somewhat problematic since it excludes the possibility that a part of the former archistriatum may be subpallial. This is suggested by the distribution of

radial glia in developing chicken (Striedter and Beydler, 1997), indicating that the AM (formerly medial archistriatum) may be partially subpallial in nature and, therefore, part of the SpA. This issue requires a thorough analysis using modern techniques to identify the palliosubpallial boundaries in the avian brain.

2.14.6.2 Topological Identification of the Avian Pallial Amygdala

The mammalian and reptilian amygdalae contain lateropallial and ventropallial territories that, in turn, are composed of superficial laminar structures receiving subpial projections from the olfactory bulbs (the cortical amygdala) and deep nonlaminated centers (mainly multimodal), which constitute their basolateral amygdala. In this section, we are reviewing the literature available to identify and delineate these two pallial components of the avian amygdala (Figures 17 and 18). In addition, we will review the data on connections and histochemistry that may help to refine the comparison of the pallial amygdala of birds with that of reptiles and mammals.

2.14.6.2.1 Olfactory areas in the avian pallium: The avian cortical amygdala The small size of the olfactory bulbs of birds is probably the reason why their projections have been poorly investigated. Fortunately, Reiner and Karten (1985) traced the olfactory projections in pigeons by means of injections of tritiated amino acids. Their results demonstrate olfactory projections to the olfactory tubercle and the presence of abundant labeled fibers in the frontoarcopallialis (FA; frontoarchistriatal tract in the old nomenclature) through which they reach several parts of the pallium, including the whole CPi and more ventral regions in the caudal cerebral hemispheres. There, the olfactory projection reaches cortical areas

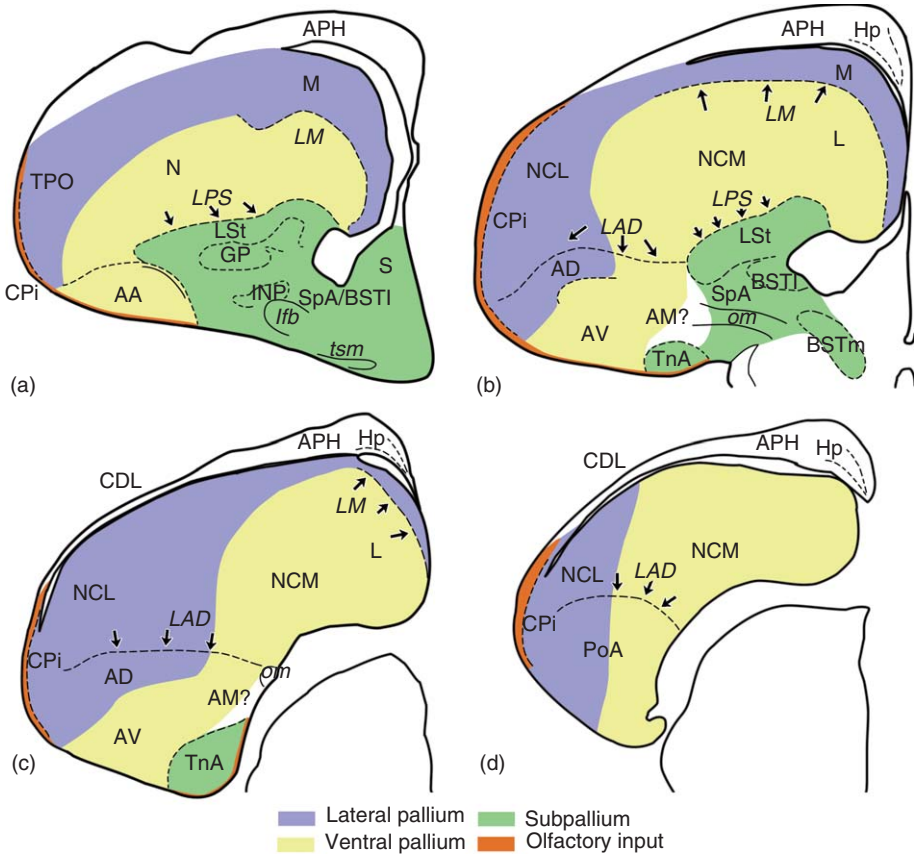

Figure 18 Pallial and subpallial territories of the avian amygdala. A schematic diagram of the amygdala of birds, based on the Nissl-stained sections of Figure 17, which shows the palliosubpallial boundary and the extent of the latero- and ventropallial territories within the pallial amygdala (based on Puelles *et al.*, 2000). The projection fields of the olfactory bulbs (based on the description by Reiner and Karten, 1985) are also indicated. The actual boundary of the ventral pallium with the subpallium is difficult to trace at the level of the AM (white region in the AM).

superficial to the AA, AD, and anterior part of the AV, up to the level of TnA. From there, olfactory fibers seem to enter the stria medullaris to reach the contralateral cerebral hemisphere through the habenular commissure. More caudally, a gap appears between the olfactory projection to the CPi and that to the TnA, so that apparently the structures superficial to the caudal portion of the AV are not olfacto-recipient. These results have been replicated in chick embryos using lipophilic tracers (Striedter *et al.*, 1998).

Reiner and Karten (1985) interpreted their results as demonstrative of the absence of olfactory projections to the amygdala in birds, with the exception of the olfactory pathway to the TnA. However, in the light of the results in reptiles and mammals, and of the interpretation of the nature and organization of the amygdala derived from them, the structures superficial to the AA and to the rostral AV, plus the caudal CPi, are to be interpreted as the cortical amygdala of birds. Reiner and Karten (1985) also discuss the lack of vomeronasal organ and of accessory olfactory bulbs and its possible consequences on the organization of the projections from the olfactory bulbs. In our view, this may have resulted in the disappearance of the projections to the superficial caudal aspect of the caudal AV that would have been the natural target for the projections from the AOB. This will be further discussed in relation to the existence or absence of a subpallial chemosensory amygdala (equivalent to mammalian Me).

2.14.6.2.2 The deep pallial amygdala in birds According to the general scheme of the amygdala of mammals and reptiles, those pallial structures deep to the olfacto-recipient cortical areas described above would constitute the pallial nuclear amygdala, e.g., the basolateral amygdala plus the AHA of birds. The distribution of radial glia in the cerebral hemispheres of chicken embryos (Kalman *et al.*, 1993; Striedter and Beydler, 1997) strongly suggests that the dorsolateral aspect of the arcopallium (pallial archistriatum, in the words of Striedter and Beydler, 1997) plus the caudal M, the NC, and TPO, are topologically deep to the olfacto-recipient cortical structures mentioned above (Figure 18). This was subsequently confirmed by combining an innovative method of tracing the fate of cells generated in specific zones of the telencephalic ventricles with labeling of the projections from the olfactory bulb in chicken embryos (Striedter *et al.*, 1998). In addition, as discussed above, the PoA contains the structures deep to the posterior edge of the CPi and fuses without clear boundaries with the caudal edge of the AD/AV. Consequently, its new name seems especially appropriate to designate a pallial amygdaloid structure.

These data indicate that the deep pallial amygdala of birds is conformed by, at least, the NC and the TPO plus the pallial arcopallium and the PoA.

2.14.6.2.3 Lateral and ventral pallial derivatives in the caudal avian cerebral hemispheres Although the olfactory projection is often taken as a marker of the lateral pallium (see, for instance, the title of the paper by Striedter *et al.*, 1998), data from the mammalian neuroanatomy reviewed above indicate that the olfacto-recipient cortical areas include lateropallial and ventropallial derivatives. This is fully supported by the pattern of expression of homeotic genes in the cerebral hemispheres of birds (Smith-Fernandez *et al.*, 1998), which indicates the lateropallial origin of the M and the ventropallial origin of the N. Using a similar approach and a detailed anatomical analysis, Puelles *et al.* (2000) mapped the ventropallial and lateropallial territories in the caudal cerebral hemispheres of chicken embryos. According to their results and their interpretation of the cytoarchitecture of the cerebral hemispheres of birds, the embryonic ventral pallial territories are composed of the rostral N, including the entopallium (E, formerly ectostriatum) and the basorostral pallial nucleus (Bas; formerly basal nucleus). In addition, the AV (classically called ventral intermediate archistriatum) displays a profile of gene expression typical of the ventral pallium that extends caudally into what most authors consider the PoA.

In turn, the lateral pallium includes the CPi, and the structures topologically deep to it, namely the whole M and the AD. Moreover, the lateral pallium seems to impinge into the PoA. As noted previously (Martinez-Garcia *et al.*, 2002a), topology and cytoarchitecture strongly suggest the existence of a lateropallial bridge connecting the rostral (M) with the caudal (AD) territories of the lateral pallium. This bridge is probably composed of the lateral aspect of the NC, including the TPO, part of which is indeed immediately deep to the CPi. This was fully confirmed by the pattern of expression of cadherins in the cerebral hemispheres of embryonic chicken (Redies *et al.*, 2001).

The position of the AA within this map requires further discussion. According to Puelles *et al.* (2000) and Redies *et al.* (2001), the AA (anterior archistriatum in the classical terminology) is part of the

subpallium. We consider, however, that the structure that they label as AA is a cytoarchitectonically distinct portion of the LSt of the embryonic chicken brain. In contrast, most of the avian neuroanatomists call AA to the anterior pole of what Puelles *et al.* (2000) and Redies *et al.* (2001) label as intermediate archistriatum, plus their nucleus of the LOT (see figures 3 and 4 in Redies *et al.*, 2001). If this interpretation is correct, the AA turns out to be a rostral ventropallial region (Figure 18) closely associated with the LOT (which in birds is named as frontal arcopallial tract, FA; Striedter *et al.*, 1998). As we will see, connectional data clearly support this view.

The palliosubpallial boundary is delineated by the lamina palliosubpallialis. However, at commissural and postcommissural levels, the AM is interposed between the SpA and the TnA, and crossed by the fibers of the occipitomesencephalic tract (OM) as they leave the cerebral hemispheres. Since the SpA and AM are subpallial, a transition between ventropallial and subpallial territories is expected at this level. Tracing this boundary becomes an unresolved and potentially important issue.

2.14.6.2.4 The avian pallial amygdala: A proposal of homologies with mammals The map of the pallial amygdala of birds depicted in Figure 18 leads to a proposal of homologies of the caudolateral cerebral hemispheres of birds with the mammalian pallial amygdala that is topologically consistent and shows congruence with the proposed map of the reptilian amygdala, with which the comparison is quite straightforward (Table 3). According to this proposal, within the lateral pallium, the CPi would contain the homologues of the mammalian lateropallial olfactory cortices, including the COApl and the transition areas with the piriform and entorhinal cortices (APi and TR, respectively). The structures of the avian brain deep to the CPi, including the lateral NC, TPO, AD, and the lateropallial PoA, would be the putative homologues for the mammalian deep lateropallial amygdala, the B nucleus.

The superficial ventropallial regions of the telencephalon of birds should be homologous to the olfactory and vomeronasal cortical areas of the mammalian amygdala. The AA is a rostral ventropallial region associated with the *lot* (FA), and consequently constitutes the most likely candidate for the avian homologue to the LOT of mammals. On the other hand, the superficial anterior part of the AV, which receives direct projections from the olfactory bulbs, occupies a position in the avian brain comparable that of the COAa in the mammalian amygdala. The deep anterior AV/AM seems topologically equivalent to the ABa of mammals, and therefore we conceive both structures as homologous.

In contrast, at the level of the caudal AV, a superficial olfacto-recipient cortex is lacking (this region is labeled as posterior archistriatum by Reiner and Karten, 1985). This occupies the topological position where the projections from the accessory olfactory bulbs should terminate (if they existed). Therefore, according to our view, birds have an only-deep caudal ventropallial region, where a vomeronasal cortex equivalent to the mammalian COApm is lacking, but the structures equivalent to the nuclei deep to it (ABp and AHA) are presumably located. In this respect, the location of the PoA in the caudal edge of the arcopallium (thus its name) suggests that its ventropallial portions are equivalent to the mammalian AHA (named posterior nucleus of the amygdala by Canteras *et al.*, 1992a) and the reptilian VPA. Finally, the NCM, a deeper ventropallial structure located in the dorsomedial PDVR of birds, is a good candidate for the mammalian L, thus equivalent to the reptilian PDVRdm (Table 3).

2.14.6.3 Connections and Histochemistry of the Avian Pallial Amygdala: Comparative Implications

There are few studies of the connections of the centers here proposed as the avian homologues of the pallial amygdala, and this is especially true for the putative cortical amygdala. Nevertheless, since the pioneer work by Zeier and Karten (1971) on the archistriatum of pigeons, all the published reports of projections of the arcopallium, PoA, and adjoining areas (Bingman *et al.*, 1994; Davies *et al.*, 1997; Dubbeldam *et al.*, 1997; Kroner and Gunturkun, 1999) coincide in indicating that the caudolateral pallial telencephalon originates a long descending projection known as occipitomesencephalic pathway (OM). This projection reaches not only parts of the hypothalamus (OM pars hypothalamii, OMH), as expected from a pallial amygdaloid structure, but also the posterior thalamus and deep midbrain (thus its name). This projection has been reported to reach also pontine regions, the reticular formation, and dorsal medulla (Zeier and Karten, 1971). No region of the caudal pallium of mammals or reptiles gives rise to such a projection. Thus, the OM seems exclusive of the avian brain. Consequently, we will not use this trait to discuss the identity of the avian pallial amygdala, but we devote a section to analyzing its possible origin, as well as its comparative and functional significance.

2.14.6.3.1 The connections of the cortical amygdala of birds The connections of the CPi have been studied in pigeons using large injections of anterograde and retrograde tracers (Bingman *et al.*, 1994). The results indicate that the CPi displays the characteristic projections of the piriform cortex, including associative and commissural projections and connections with other secondary olfactory centers, plus other connections that are difficult to interpret from a comparative viewpoint (e.g., connections with the dorsal mesopallium). In addition, the CPi is connected with the parahippocampal cortex and presumed deep pallial amygdaloid centers such as the AD, PoA, and TPO/N. It is also reciprocally connected with the bulbo-recipient subpallial amygdala (TnA). Moreover, it displays some descending projections to striatal territories, including the Acb and MSt (formerly lobus paraolfactorius), parts of the lateral septum, the BSTl, and the SpA. Finally, tracer injections in the CPi give rise to fiber labeling in the OM.

This pattern of intratelencephalic connections of the avian CPi is much more extensive than expected for a mere piriform cortex. Although the authors (Bingman *et al.*, 1994) accept that some of this labeling is seemingly due to the involvement of deep tissue in the injection site, these data can also be interpreted as suggestive of a field homology among the CPi of birds with the whole lateropallial olfactory cortex of reptiles and mammals. As discussed above, the olfacto-recipient lateral pallium seems to have undergone a differential compartmentalization in each group of amniotes.

Data on the ventropallial olfactory-related areas derive from the report by Davies *et al.* (1997) on the connections of the chicken archistriatum. Their results confirm the view put forward by Zeier and Karten (1971) that the AA is the region of the arcopallium giving rise to most projections through the anterior commissure. These contralateral projections mirror the intratelencephalic ipsilateral efferents of the AA, thus resulting in a pattern of bilateral intratelencephalic projections to extensive areas of the pallium (Hp, Wulst, M, and N). Bilateral efferents from the AA also target the olfactory bulbs and retrobulbar formation, the CPi, and portions of the AD and AV. Morever, the AA also shows bilateral projections to the subpallium, which specifically innervate the MSt (just lateral to the Acb) and olfactory tubercle. Finally, the AA projects massively to its contralateral counterpart.

This is demonstrative of the pallial nature of the AA (thus contradicting the view proposed by Puelles *et al.*, 2000), since only cortical areas are involved in commissural and ipsilateral projections to cortical areas (corticocortical pathways; see above). Moreover, this recalls the massive homotopic commissural projections of the mammalian LOT (Johnston, 1923) and its bilateral pathways to the olfactory system (olfactory bulbs, anterior olfactory nucleus, piriform and endopiriform cortex, olfactory tubercle), to atypical striatal structures (fundus striatum, IPAC), to parts of the pallial amygdala and to limbic and transitional cortical areas (Santiago and Shammah-Lagnado, 2004). Therefore, connectional data fully confirm our proposal of a homology of the avian AA with the LOT of mammals and reptiles (Table 3).

2.14.6.3.2 Connections of the avian basolateral amygdala The basolateral amygdala of mammals (B, L, and AB) receives sensory inputs from many different sources. In addition, its nuclei show differential projections to the dorsal and ventral striatum, to the hypothalamus, and to the cortical fields from which they receive their main inputs. As we describe below, most of these features are met by the proposed homologues to the basolateral amygdala in the brain of birds.

2.14.6.3.2.(i) Identity of the basal nucleus of the avian amygdala in the deep lateral pallium The B of the mammalian amygdala is the main source of projections to the dorsal and ventral striatum. The projections to both striatal compartments within the cerebral hemispheres have been thoroughly studied in pigeons twice by means of anterograde and retrograde tracing techniques (Veenman *et al.*, 1995; Kroner and Gunturkun, 1999). These studies reveal projections to dorsal striatal territories arising from some of the proposed pallial amygdaloid centers. Specifically, the deep lateropallial derivatives (deep CPi/TPO, NCL, and AD, plus maybe part of the PoA) project massively to the LSt. At least part of this projection, arising from rostral levels of the arcopallium, is bilateral. The deep lateropallial amygdala also projects to the ventral striatum, namely the Acb (formerly, the medial aspect of the lobus paraolfactorius). In many cases, this projection extends caudally to reach also the lateral BST and the SpA. Thus, tracer injections involving the posterior NCL and/or PoA (Veenman *et al.*, 1995; Kroner and Gunturkun, 1999) reveal a projection to the continuum Acb/BSTl-SpA. A similar pattern of anterograde labeling is found after large tracer injections into the CPi that also encompass deep structures (Bingman *et al.*, 1994; Veenman *et al.*, 1995).

This supports our hypothesis that the TPO/NCL, the AD, and its caudal continuation within the PoA

constitute the B nucleus of the amygdala of birds (Table 3). If so, these structures should display a convergent dopaminergic and cholinergic innervation, which constitutes the most remarkable defining histochemical feature of the mammalian B. In fact, birds show a dense dopaminergic innervation of the striatum plus a field of dopaminergic fibers in the caudolateral cerebral hemispheres that comprises the NCL, AD, and extends into the PoA (Figures 19a and 19b; Durstewitz *et al.*, 1999). These same pallial areas show a dense innervation by cholinergic fibers, as revealed by either ChAT immunostaining (Medina and Reiner, 1994) or AChase histochemistry (Figures 19c and 19d).

Therefore, the AD plus its neighboring areas within the deep caudal lateral pallium make up the most likely homologue of the mammalian B and the reptilian DLA. In fact, if one compares the images of the caudolateral cerebral hemispheres of birds (Figure 19) with similar images from the brains of

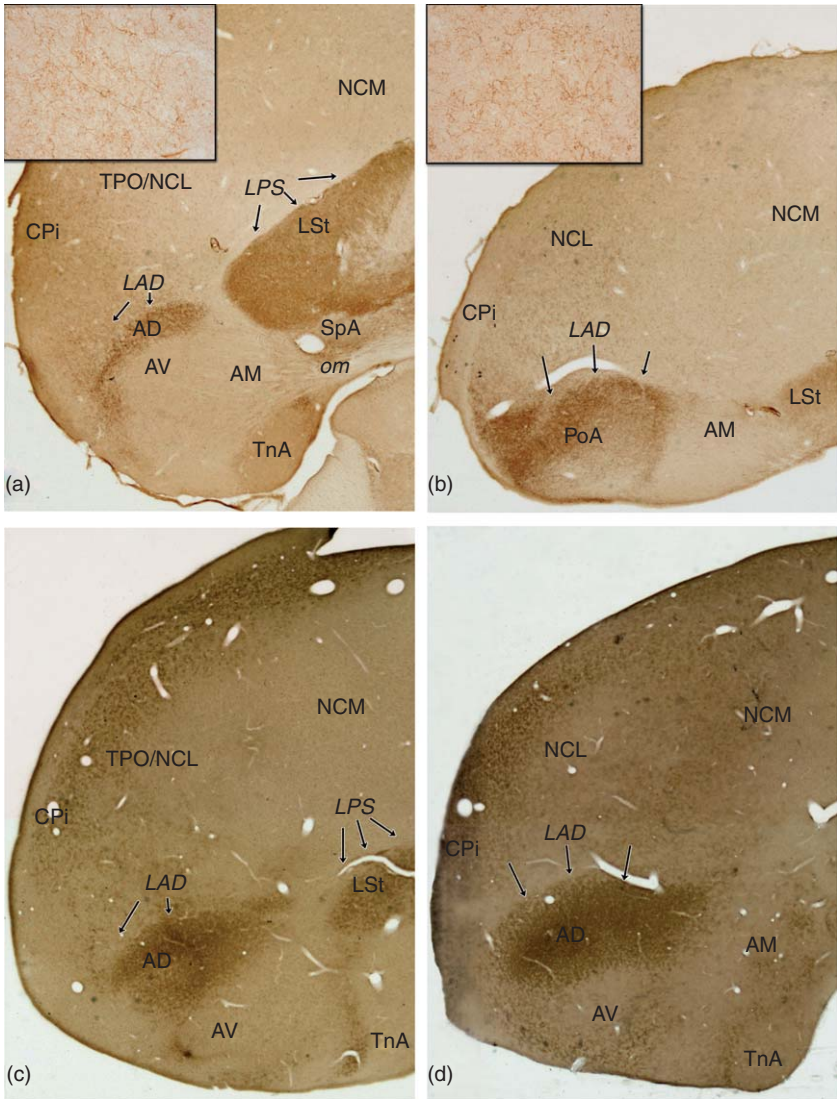

Figure 19 Histochemistry of the pallial amygdala of birds. a and b, The distribution of fibers immunoreactive for tyrosine hydroxylase (TH) in the caudal telencephalon of the quail (*Coturnix coturnix*) is shown in two frontal sections of the left hemisphere (slightly postcommissural (a) and caudal section (b)). Dopaminergic fibers innervate the striatum and parts of the amygdaloid complex. This includes a dense innervation of the AD and parts of the PoA and a scarcer innervation of the TPO and NCL (insets). In addition, the SpA also shows a remarkable TH-immunopositive innervation (a). c and d, Distribution of AChase histochemistry in two frontal sections of the left cerebral hemisphere of the chicken (*Gallus domesticus*) at slightly postcommissural (c) and caudal levels (d). The AD shows a remarkable AChase-positive innervation that extends into the TPO and NCL. Therefore, all three structures are targeted by convergent dopaminergic and cholinergic innervations, which supports their homology with the basal nucleus of the mammalian amygdala.

microsmatic lizards (such as *Anolis*; Box 1), the resemblance is astonishing. The lack or strong reduction in the vomeronasal system in birds and anole lizards has resulted in a similar displacement of the deep lateropallial amygdala. Their histochemical features, however, remain the same and clearly indicate that they are the sauropsidian homologues of the basal nucleus of the amygdala of mammals.

2.14.6.3.2.(ii) Deep ventropallial nuclei: The AV/AM and NCM The ventropallial basolateral amygdala of mammals contains a deep sensory interface, the L nucleus, and another more superficial nucleus projecting to the ventral striatum and hypothalamus, the AB. According to our hypothesis (Table 3), the avian AV/AM should display projections to the ventral striatum and hypothalamus (like the mammalian AB), whereas the deepest ventropallial amygdala, the NCM, would constitute the sensory interface of the pallial amygdala, like the mammalian L.

2.14.6.3.2.(ii).(a) The homology between the AV/AM and the mammalian AB The projections from the AV/AM to the ventral striatum have indeed been described. Tracer injections into the Acb of the pigeon (formerly medial lobus paraolfactorius; Veenman *et al.*, 1995; Kroner and Gunturkun, 1999) retrogradely label neurons in the lateropallial structures referred to above (NCL/TPO, AD, and, to a lesser degree, PoA) but also in the caudal AV (named archistriatum centrale by Veenman *et al.*, 1995). Anterograde tracing of this projection (Veenman *et al.*, 1995; Kroner and Gunturkun, 1999) indicates that it extends into the BSTl/SpA. The arcopallial projection to the Acb/BSTl/SpA also arises in part from the AM, as indicated by both retrograde (Veenman *et al.*, 1995; Kroner and Gunturkun, 1999) and anterograde tracing experiments (Davies *et al.*, 1997). This suggests that the AV/pallial AM are the avian counterpart of the mammalian AB, in terms of both topology and hodology.

Concerning the projections to the hypothalamus, our hypothesis predicts two projections arising from different areas of the AV/AM. Like the ABa, the rostral portions of the AV/AM should project to the lateral hypothalamus, whereas the caudal AV/AM, likely homologous to the mammalian ABp (and the reptilian PDVR and LA), would therefore project to the ventromedial tuberal hypothalamus. The existence of such a pathway in the brain of birds is evidenced by the presence of a zinc-rich terminal field in a nucleus of the caudal ventromedial hypothalamus of the chick (Faber *et al.*, 1989), clearly misidentified by the authors as the nucleus papillioformis, which seems to correspond to medial nucleus of the posterior hypothalamus (PMH). In addition, Montagnese *et al.* (1993) described a dense, zinc-containing terminal field in the PMH and an additional one in the nucleus tuberis of the premammillary/mammillary hypothalamus.

In this respect, the seminal paper by Zeier and Karten (1971) already described a tractus occipitomesencephalicus pars hypothalami (OMH) terminating mainly in the medial PMH (posterolateral and posteromedial nuclei) but also in the lateral hypothalamus and the so-called stratum cellulare internum and stratum cellulare externum. According to their description, this termination field extends into the infundibular hypothalamus at premammillary–mammillary levels. The site of origin of this zinc-rich arcopallial/amygdaloid projection (or projections) to the hypothamus is unclear. Zeier and Karten (1971) observed degeneration of the OMH after lesions of the posterior archistriatum and/or superficial parts of the caudal AV, plus the subpallial TnA (the latter projection is discussed below). However, they described the OMH as a single pathway, and did not contemplate the possibility of it being composed of several projections with different sites of origin in the archistriatum and termination fields in the hypothalamus, as apparently happens in mammals and reptiles.

Nevertheless, modern neuroanatomical techniques based on intra-axonic transport of tracers suggest that this is indeed the case. Thus, Davies *et al.* (1997) report anterograde labeling in the lateral hypothalamus after tracer injections centered in the anterior AM of the chicken. In contrast, injections in the caudal AM, just dorsal to the TnA in ring doves (Cheng *et al.*, 1999), specifically label fibers of the OMH directed to the preoptic anterior hypothalamus and to the PMH, which do not reach the mammillary–premammillary levels. This suggests that the AM includes a rostral portion displaying projections similar to those of the ABa and a caudal one, the hypothalamic projections of which recall those of the ABp. It is tempting to suggest that the anterior AM (projecting to the lateral hypothalamus) is topologically deep to the anterior olfacto-recipient archistriatum illustrated by Reiner and Karten (1985) in the pigeon, whereas the caudal AM, which projects to more medial areas of the preoptic to tuberal hypothalamus, is deep to the caudal, nonolfactory-recipient AV. However, studies on the histogenetic gradients and topological relationships of the arcopallium are lacking.

As a conclusion, like its putative mammalian homologue (the AB), the AV/AM of birds projects to the ventral but not the dorsal striatum. Apparently, part of this arcopallial region gives rise to a projection to the lateral hypothalamus. In addition, birds, like mammals and reptiles, display a zinc-rich projection directed to a cell group in the ventromedial posterior (tuberal) hypothalamus. This projection seems to arise, at least in part, from the caudal aspect of the AM, just dorsal to the TnA, which is therefore the most likely homologue of the mammalian ABp, in terms of both topology and connections. A systematic study of the projections to the hypothalamus from the caudal cerebral hemispheres using both retrograde and anterograde tracing techniques, and a deep analysis of the architecture of the arcopallium, PoA, and hypothalamus of birds, are urgently needed to clarify this issue further.

2.14.6.3.2.(ii).(b) Sensory afferents: The caudomedial nidopallium as the sensory interface of the avian amygdala In view of the organization of the reptilian amygdala we have described above, we expect to find a sensory interface of the pallial amygdala within the dorsomedial PDVR, which in birds is represented by the caudomedial nidopallium. In this respect, the NC is the target for important afferents from the different sensory centers of the cerebral hemispheres. This was reviewed in detail by Metzger *et al.* (1998) and Kroner and Gunturkun (1999), who realized that the NC is the target for thalamic and intratelencephalic sensory afferents. Thus, it receives projections from the secondary sensory areas of the neostriatum (fields L1 and L3, entopallial belt, frontal nidopallium, nucleus basalis, and intermediate nidopallium). The thalamic input to the NC arises mainly from the shell of nucleus ovoidalis and the dorsolateral posterior thalamic nucleus (DLP), which, according to Korzeniewska and Gunturkun (1990), constitute multimodal thalamic relays. Moreover, studies in chicken and quails strongly suggest that this thalamonidopallial pathway contains CGRP, as is the case for the projections to the L in mammals (see Lanuza *et al.*, 2000). In contrast to the NCL, the NCM seems mainly involved in intra-amygdaloid projections, since it does not display substantial projections to either the dorsal or the ventral striatum. Thus, NC projects to the AV and AD (Metzger *et al.*, 1998) in a topographic manner. These projections are reciprocated by arcopallial–nidopallial pathways (Kroner and Gunturkun, 1999).

These results indicate that the deep caudal cerebral hemispheres are organized very alike in birds and reptiles. In both groups, the caudal DVR receives afferents from different telencephalic and thalamic centers, and it is divided in two main areas with different embryological origin and connectional properties. The medial area (avian NCM and reptilian PDVRdm) is a ventropallial derivative mainly engaged in intra-amygdaloid projections within the ventral pallium (intra-amygdaloid) directed to the projecting areas of the pallial amygdala (AV and AD in birds, PDVRvm, LA, and DLA in reptiles). The lateral area is a lateropallial derivative (the avian NCL/TPO plus the AD; the reptilian DLA) that projects to the dorsal and ventral striatum. In this scheme, the NCM should be considered the sensory interface of the avian pallial amygdala, thus becoming functionally equivalent to its most likely homologue in the mammalian brain, the lateral nucleus of the amygdala. As in reptiles, in birds the possibility that the anterior sensory DVR (e.g., the sensory centers of the nidopallium, including the entopallial nucleus) constitutes an enlarged and highly specialized LA cannot be ruled out (Table 3). The presence of a primary auditory pallium immersed in the avian lateral amygdala, the field L, is a specific feature of the avian brain that probably had a strong influence in the organization of other parts of the amygdala of birds.

2.14.6.3.2.(ii).(c) Amygdalocortical projections Another attribute of the basolateral amygdala is the presence of projections to the cortical areas from which it receives projections. In mammals, this is represented by the projections arising in the basolateral amygdala (B and AB) directed to the prefrontal, insular, and perirhinal cortices. In reptiles, equivalent projections arise from the LA and terminate in the ADVR (Bruce and Butler, 1984; Martinez-Garcia *et al.*, 1993), thus constituting a corticocortical projection within the ventral pallium that reciprocates the ADVR–PDVR/LA projection. In contrast to mammals, in reptiles the amygdala does not project back to the dorsal pallial region (dorsal cortex; Hoogland and Vermeulen-Vanderzee, 1989; Lanuza *et al.*, 1998).

Birds apparently display a reptile-like organization of the amygdalocortical interconnections. Thus, the AV is interconnected with those portions of the anterior nidopallium that project to the pallial amygdala, namely the medial intermediate N, and the frontal N (Kroner and Gunturkun, 1999). This supports the proposed homology between the AV and the reptilian LA. However, pigeons also display projections from portions of the AV to dorsal pallial sensory areas such as the visual Wulst (part of the hyperpallium apicale), which provides a scarce

projection back to the AV (Shimizu *et al.*, 1995). This seemingly constitutes an apomorphic feature of the avian brain.

2.14.6.3.2.(iii) The posterior amygdala of birds and its homology with the mammalian AHA Several lines of evidence strongly suggest that the PoA constitutes the avian homologue for the AHA of the mammalian amygdala. From a topological point of view, the PoA and AHA occupy the caudal edge of the area bounding the ventral and lateral pallial territories. This is also consistent with the proposed scheme of the reptilian amygdala, since the position of the VPA (the reptilian homologue of the AHA; Table 3) in the reptilian cerebral hemispheres is clearly reminiscent of the avian PoA.

Moreover, most of the defining features of the mammalian AHA are accomplished by the PoA of birds. Thus, the PoA not only projects to the posterior medial hypothalamus, including the infundibular mammillary/premammillary levels (Zeier and Karten, 1971; Davies *et al.*, 1997; Kroner and Gunturkun, 1999), but also receives a projection from supramammillary–premammillary hypothalamus (Berk and Hawkin, 1985). Although there are no studies on the comparative significance of the mammillary region of the hypothalamus of birds, its interconnection of the PoA is reminiscent of the reciprocal connections displayed between the AHA and the PMv of mammals. This is supported by the fact that PoA, like the AHA, is the only structure of the pallial caudolateral cerebral hemispheres also projecting to the medial preoptic hypothalamus (Absil *et al.*, 2002).

The PoA is also connected to the septohippocampal system. Thus, reciprocal connections of the APH with the caudal PoA were reported in the pigeon by Casini *et al.* (1986), and recently confirmed by Atoji and collaborators (Atoji *et al.*, 2002; Atoji and Wild, 2004). In addition, the PoA projects unidirectionally to the portion of the ventral lateral septum that receives projection from the APH (Atoji and Wild, 2004). Therefore, the ventral lateral septum receives a projection from two pallial centers, one in the hippocampal formation (APH) and the other in the amygdala (the PoA), which are interconnected. This circuit recalls the one established by the mammalian AHA with the CA1-subiculum and the ventral lateral septum, thus giving strong support to our hypothesis of homologies (Table 3).

It is important to stress the similarity in the pattern of connections between the caudal AV/PoA and the whole medial EA, including the TnA (see below), concerning its connections with the hypothalamus and septohippocampal system. Thus, like the mammalian AHA, the caudal PoA is functionally related to the medial EA.

In this respect, the only feature expected for the homologue of the mammalian AHA (Tables 1 and 3) that seems not accomplished by the PoA, is the expression of receptors to sexual steroids (Simerly *et al.*, 1990). Data on this issue are, however, somewhat contradictory. Thus, whereas Watson and Adkins-Regan (1989) reported the presence in the quail of a few cells accumulating steroids (mainly estrogens) "in the basal archistriatum dorsal and lateral to the borders of the large-celled nucleus teniae," these results were not replicated using immunohistochemical detection of receptors (Balthazart *et al.*, 1989, 1992). On the other hand Metzdorf *et al.* (1999) described the presence of scattered cells expressing aromatase throughout the archistriatum (arcopallium plus PoA), not only in songbirds but also in nonsongbird species. It is interesting to note that songbirds display cells expressing receptors for sexual steroids in all the vocal centers of the forebrain, including the high vocal center in the mesopallium–NC interface and the nucleus robustus arcopallialis (Gahr *et al.*, 1993; Metzdorf *et al.*, 1999; Gahr, 2000). In addition, areas of the posterior AD, AV, and PoA next to the robustus also express androgen receptors in canaries (Balthazart *et al.*, 1992; Gahr and Wild, 1997) and zebra finches (Gahr and Wild, 1997; not detected by Balthazart *et al.*, 1992). The presence of cells expressing aromatase in the arcopallium/PoA of some nonsongbirds and of cells expressing receptors to steroids in arcopallial/PoA nonvocal centers in songbirds is suggestive of a minor expression of steroid receptors of this particular region.

As a conclusion, on topological and hodological grounds the PoA seems to be the avian homologue of the AHA of the mammalian amygdala. The lack or very low expression of steroid receptors by its cells seems, however, an apomorphic feature of birds, the significance of which is not yet understood.

2.14.6.3.2.(iv) The occipitomesencephalic tract, the somatomotor arcopallium, and the significance of birdsong According to our proposal, the amygdala of birds includes the whole arcopallium, the TnA, and the PoA (plus the posterior DVR: NCM, NCL, and TPO). This contrasts with the view, put forward by Zeier and Karten (1971), that the former archistriatum is composed of a limbic (amygdaloid) part that projects to the hypothalamus (TnA, AM, and PoA), and a

somatomotor one (AA, AD, and the dorsal part of the AV, sometimes called jointly central archistriatum) that is the origin of the OM. The rationale for this view comes from the fact that the OM, as traced in pigeon by Zeier and Karten (1971), innervates parts of the thalamus (mainly the dorsal posterior thalamus), tectal and tegmental structures in the midbrain (thus its name), the lateral reticular formation, lateral pontine nuclei, sensory nuclei in the dorsal medulla, and even the cervical spinal cord. The resemblance of the course of the OM, as defined by Zeier and Karten (1971) in pigeons, with a fascicle of the pyramidal tract of goats (Bagley's bundle), led the authors to propose that the central archistriatal regions originating in the OM should be considered equivalent to the sensorimotor cortex of mammals. Since then, this view has been further refined. In brief, the projections of parts of the arcopallium to the dorsal striatum have also been argued in favor of the comparative meaning of the arcopallium (or part of it) as a cortical sensorimotor area comparable to the infragranular layers of the mammalian isocortex (Veenman *et al.*, 1995). In line with this view, Veenman *et al.* (1995) propose that the thalamo-recipient parts of the nidopallium would be equivalent to isocortical layer 4 and the rest of the nidopallium would represent the supragranular layers of the avian isocortex. In this framework, the arcopallium is interpreted as the main motor output region of the cerebral hemispheres (Figure 20a).

However, several anatomical and functional observations seriously challenge this view. First, as discussed above, the OM has no counterpart in the reptilian brain, so that it probably represents an apomorphic character of the avian forebrain. In addition, the Wulst is a much more likely candidate for the avian homologue of the sensorimotor cortex (Medina and Reiner, 2000), a view that is topologically consistent since, like the mammalian isocortex, the Wulst is a dorsopallial derivative (but see Martinez-Garcia, 2003). Second, lesions of the AM encompassing the OM make animals apparently tame (thus recalling the effects of amygdaloid lesions; see Weiskrantz, 1956) but do not impair their movement (Phillips, 1964). In contrast, temporary inactivation of the LSt of pigeons results in a complete halt of movements so that animals appear paralyzed and unresponsive when handled or moved (Kalenscher *et al.*, 2003). These data suggest that telencephalic motor control in birds is executed by efferent projections from the LSt and, maybe, the somatomotor Wulst, instead of the OM.

Therefore, it is time to analyze which is the function of the OM on the control of behavior. The results of intra-axonic transport of tracers in pigeons (Wild *et al.*, 1993; Kroner and Gunturkun, 1999) and chicken (Davies *et al.*, 1997) have not revealed any target of the OM caudal to the nucleus of the lateral lemniscus. The caudal terminal fields reported by Zeier and Karten (1971) to show degeneration after transections of the OM are likely due to involvement of the BSTl/SpA in the lesion (Berk, 1987). In their study of the auditory forebrain of the pigeon, Wild *et al.* (1993) realized that the OM is a link within the auditory circuitry of the avian forebrain. Thus, the OM originates in the portion of the arcopallium that receives projections from the auditory N and terminates mainly in auditory thalamic (ovoidalis shell, nucleus semilunaris paraovoidalis, nucleus paramedianus internus) and midbrain/pontine centers such as the dorsomedial part of nucleus intercollicularis (ICo/DM) and the nucleus of the lateral lemniscus. On the other hand, songbirds display a very developed OM (see The Evolution of Vocal Learning Systems in Birds), which arises from a distinct arcopallial cell group called nucleus robustus arcopallialis (RA; Wild, 1993; Wild *et al.*, 1993). The RA directly innervates the tracheosyringeal hypoglossal motoneurons that control the syringe and premotor neurons for the respiratory system in the nucleus retroambigualis (Wild, 1997), thus having quite a direct control of the motor patterns leading to song generation.

Although this has led to the suggestion that the RA constitutes a portion of the motor cortex (Bottjer *et al.*, 2000) devoted to song control, in this kind of analysis the behavioral/physiological meaning of birdsong has largely been ignored (Cheng and Durand, 2004). Darwin already recognized vocalization as a form of emotional expression. Nonsongbirds, including pigeons, doves, chicken, and quails (which have often been used in anatomical studies), emit a repertoire of vocalizations for all kind of social interactions, including agonistic–territorial, parental, and reproductive ones. It is generally recognized that alarm calls or peeps emitted by newborn chicken when separated from the hen (distress peeps) are emotional behaviors. This has led Cheng (2003) to propose that song and vocalizations are amygdala-dependent behaviors. The pathway she suggests to mediate vocalization includes projections from the TnA to the PMH and then to midbrain song centers, namely the ICo/DM, whose electrical stimulation elicits singing. However, kainic acid lesions of the archistriatum of young chicken result in a decrease of distress calls (loud peeps; Phillips and Youngren,

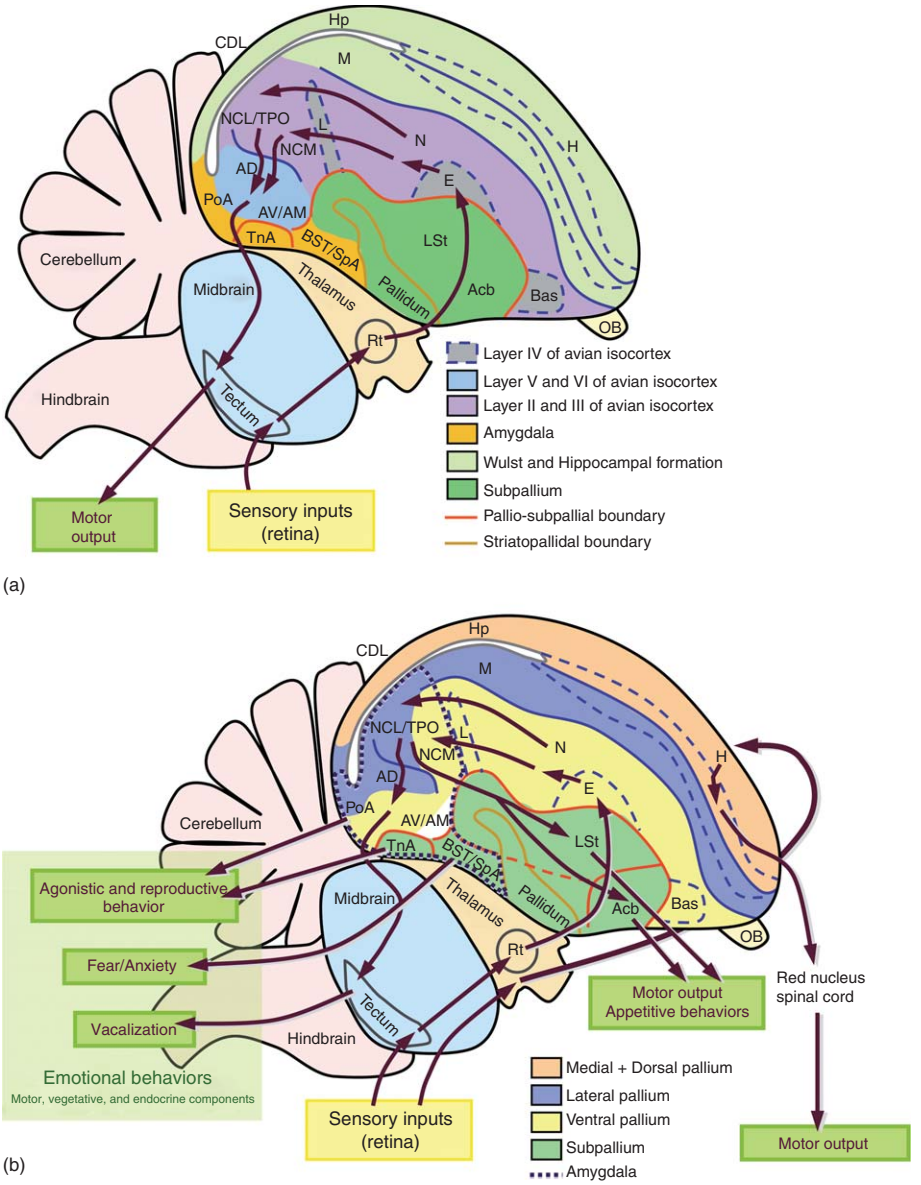

Figure 20 Alternative views on the comparative and functional neuroanatomy of the avian cerebral hemispheres. a, Diagram summarizing the classical view of the comparative neuroanatomy of the subventricular cerebral hemispheres of birds. According to this view, they include pallial regions equivalent to the granular (sensory nidopallium, including field L, basorostral and entopallial nuclei), supragranular (rest of the nidopallium, including the NCL and NCM), and infragranular layers of the mammalian isocortex (arcopallium). The arcopallium thus constitutes the main motor output region of the whole system, whereas the OM appears comparable to the mammalian pyramidal tract. In this view, the pallial amygdala of birds is restricted to the PoA. Arrows indicate the flow of sensory information (exemplified in the visual system) and the main intratelencephalic pathways for sensorimotor integration. b, Schematic diagram of the view proposed in this article. The pallial territories are labeled and the boundaries of the avian amygdala are delineated by a discontinuous line. Three main motor outputs, used for coordinating different kinds of response, arise from different output regions of the cerebral hemispheres. A motor cortex, engaged in coordinating pure motor actions through direct projections to the spinal cord and red nucleus, is present in the Wulst (hyperpallium). In addition, the lateral and ventral pallia are involved in processing sensory information (again, the visual system is drawn as an example) to elaborate different kinds of emotional response. Appetitive ones (involving delayed reward acquisition) are executed through palliostriatal pathways, whereas direct amygdaloid projections to the hypothalamus, midbrain, and brainstem are used to coordinate the motor, vegetative, and endocrine components of innate and learned emotional responses such as agonistic and reproductive behaviors, fear and anxiety, or vocalizations (song emission) used in both contexts. a, Modified from Jarvis, E. D., Gunturkun, O., Bruce, L., *et al.* 2005. Avian brains and a new understanding of vertebrate brain evolution. *Nat. Rev. Neurosci.* 6, 151–159.

1986), but no other visible motor impairments. Involvement of the OM in the control or generation of vocalizations is also suggested by the fact that in both songbirds (Wild, 1993) and nonsongbirds (Wild *et al.*, 1993) the OM reaches directly the midbrain song centers (ICo/DM). In this context, the OM can be envisaged as a projection of the avian amygdala accomplishing a specialized emotional behavior that includes generating or modulating the motor patterns leading to vocalization but also, very likely, the accompanying endocrine and vegetative responses (Figure 20b). The courtship and/or territorial singing of songbirds constitutes a sophisticated behavior with a strong emotional component that, in many species, includes a process of motor learning, for which intra-amygdaloid circuits are especially appropriate (Cheng and Durand, 2004).

2.14.6.4 The Subpallial Amygdala of Birds

The subcortical components of the avian cerebral hemispheres extend caudally into the amygdaloid complex. There, a subpallial striatopallidal amygdala is expected, which probably contains homologues of both the medial and central EA. As we discuss in detail below, the lack of a vomeronasal system in birds has not been accompanied by a disappearance of the medial EA.

2.14.6.4.1 The medial extended amygdala of birds The TnA has traditionally been considered the avian counterpart of the medial amygdala of mammals, based on three lines of evidence. First, it is one of the targets for the projections from the olfactory bulbs (Reiner and Karten, 1985). In birds, where the accessory olfactory bulb does not exist, the counterpart of the mammalian Me may still receive a projection from the main olfactory bulb, as occurs at least in the MeA of mammals and the region superficial to the MA associated with the stria medullaris in reptiles (Figures 10b and 10c). Second, the TnA also accomplishes another of the defining features of the medial EA of mammals (Table 2), since its cells express receptors to sexual steroids, especially to estradiol, in both nonsongbirds (Balthazart *et al.*, 1989, 1992; Watson and Adkins-Regan, 1989; Aste *et al.*, 1998; Foidart *et al.*, 1999) and songbirds (Metzdorf *et al.*, 1999). Third, the TnA contributes to the OMH (Zeier and Karten, 1971; Cheng *et al.*, 1999), through which it projects specifically to the medial preoptic region, the lateral hypothalamus, and medial nucleus of the posterior hypothalamus (which, as discussed above, seems to be the avian counterpart of the VMH). This projection continues further caudally to reach regions of the premammillary hypothalamus (Cheng *et al.*, 1999).

The remaining connections of the TnA, as described by Cheng *et al.* (1999) in doves and starlings, are remarkably similar to those described for the different portions of the Me of mammals (Canteras *et al.*, 1995). Thus, the TnA projects to a ventral striatal territory comprising the caudal Acb and the BSTl/SpA, which mimics the projections from the MeA to the Acb and central EA of mammals (the identity of the avian central amygdala is discussed below). In addition, the TnA projects to parts of the intermediate to caudal arcopallium, which would represent projections from the medial to the basolateral amygdala similar to those described in mammals. In addition the TnA is connected to the septohippocampal system consisting of projections to the ventral lateral septum and reciprocal connections with the parahippocampal cortex (AHP; Cheng *et al.*, 1999), for which comparable projections are found in the connections of the Me of mammals with fields CA1 and subiculum of the hippocampal formation.

The OMH, which at least partially arises from the TnA (Cheng *et al.*, 1999), courses through a region located just caudal and mostly ventral to the anterior commissure that links the cerebral hemispheres with the preoptic region. Along its course through this area, the OMH fibers show varicosities. This is suggestive of this area being the avian counterpart of the posteromedial BST of mammals. Aste *et al.* (1998) studied in detail the cytoarchitecture and the distribution of vasotocinergic and aromatase-expressing cells in this area and reported a sexual dimorphism in the vasotocinergic cell population (Panzica *et al.*, 2001). Therefore, they concluded that this area constitutes the medial BST of birds (BSTm) and named it accordingly. In fact, like the mammalian posteromedial BST, the avian BSTm is also rich in receptors to sexual steroids (see references cited above) and displays a similar set of connections with the preoptic hypothalamus (Absil *et al.*, 2002), the premammillary hypothalamus (Berk and Hawkin, 1985), and the PoA (Zeier and Karten, 1971; Kroner and Gunturkun, 1999).

Therefore, birds seem to possess a medial EA composed of, at least, the TnA (plus maybe parts of the AM just dorsal to it; Cheng *et al.*, 1999) and the BSTm. Unlike its mammalian and reptilian counterparts, the medial EA of birds does not receive vomeronasal inputs but instead does receive a scarce olfactory one. However, it shares

with the medial EA of the remaining amniotes the main defining features of this part of the subpallial amygdala. It is rich in receptors to sexual steroids and shows sexual dimorphism, including sexually dimorphic projections containing vasotocin. It is connected to the preoptic, lateral, ventromedial (medial posterior), and premammillary divisions of the hypothalamus, and receives ascending projections at least from the latter. This strong resemblance suggests a similar role of the medial EA in the control of behavior in all amniotes.

2.14.6.4.2 The central extended amygdala The identity of the central amygdala of birds is an unresolved issue. However, our understanding of the organization of the central EA of mammals and reptiles helps us to delineate its counterpart in the avian forebrain using appropriate histochemical and connectional data.

As in reptiles, in birds the first clues to the identity of the central EA came from studies on descending projections of the cerebral hemispheres. Thus, Berk (1987) demonstrated a projection from the basal telencephalon to the lateral hypothalamus, SN, parabrachial, pericoerulear, and subcoerulear areas and to the dorsal vagal complex and nucleus of the solitary tract in the medulla. When traced retrogradely, this projection was seen to arise from the former BST, now renamed BSTl, and the former ventral paleostriatum, now called SpA. In mammals, the only area of the cerebral hemispheres giving rise to a similar set of projection is the central EA.

This proposal of homology fits the remaining available data on the connections and neurochemistry of the avian brain. Thus, the projection of the BSTl/SpA to the parabrachial region was further analyzed by Wild *et al.* (1990), who also reported an ascending projection of the parabrachial region back to the BSTl/SpA. Therefore, like the central EA of mammals, the BSTl/SpA of birds is reciprocally connected to the parabrachial region. As in mammals, the ascending projection of the parabrachial area reaches not only the subpallial amygdala (BSTl/SpA) but continues to the pallial one to reach at least parts of the arcopallium, PoA, and TPO/NCL, thus giving additional support to our proposal of homologies for the pallial amygdala (see Box 2).

The intratelencephalic connections of the BSTl/SpA also agree with their homology with the mammalian EA. Thus, as reviewed above, the NCL/TPO and AD as well as most of the AV (Veenman *et al.*, 1997; Kroner and Gunturkun, 1999), display substantial projections to the BSTl and/or SpA. This recalls the important projections from the basolateral amygdala to the central EA in the mammalian brain. Additional data on the connections of the BSTl/SpA, which also fit our proposal of homologies, are described in the literature. Thus, the BSTl and SpA receive an important projection from the thalamus, including the periovoidal region (Durand *et al.*, 1992) and the adjoining dorsolateral and dorsointermediate cell groups of the posterior thalamus (Wild, 1987a, 1987b). These cell groups apparently constitute the posterior intralaminar thalamus of birds on the basis of its location, afferents, multimodal physiological response (Gamlin and Cohen, 1986; Wild, 1987a; Korzeniewska and Gunturkun, 1990; Durand *et al.*, 1992), and the presence of abundant CGRP immunoreactive neurons (Lanuza *et al.*, 2000; Durand *et al.*, 2001). Therefore, the BSTl/SpA, like the mammalian central EA, receives a dense afferent projection from the posterior intralaminar thalamus. These thalamic cell groups are the same ones that project sparsely to the NC (Metzger *et al.*, 1998; Kroner and Gunturkun, 1999), to the TnA, and to parts of the arcopallium (Durand *et al.*, 1992; Cheng *et al.*, 1999). This can be interpreted as the presence in birds of a massive projection of the posterior intralaminar thalamus to the central EA (and medial EA) and a sparse one to the basolateral amygdala. This mimics the situation in mammals and, therefore, supports our proposal on the identity of the avian amygdala. Therefore, the available data on the anatomical relationships of the BSTl/SpA support its homology with the central EA of mammals, but a detailed analysis of the connections of the central EA is still needed to improve our understanding of the organization of the avian forebrain.

Finally, neurochemical data fully support the view of the BSTl/SpA as the avian central EA. Thus, like the central EA of mammals, the BSTl/SpA displays populations of CRFergic (Richard *et al.*, 2004) and NTergic cells (Atoji *et al.*, 1996; see figure 5f in Reiner *et al.*, 2004) and a dense innervation by CGRP-immunoreactive fibers (Lanuza *et al.*, 2000). Comparison of the location of CGRP fibers and CRF-immunoreactive cells in the BSTl/SpA reveals a differential distribution. Cells expressing CRF (Figure 21a), like those expressing NT, are mainly located in the BSTl and extend into the SpA, whereas the CGRPergic innervation is very dense in the anterior SpA (Figure 21b) and much scarcer in the BSTl. This also recalls the situation in mammals (Figures 6e and 6f), where CRFergic cells are mainly distributed in the CeM

Box 2 On the identity of the prefrontal cortex of birds

The efforts of comparative neuroanatomists to understand the evolution of the brain have led to a continuous search for counterparts of areas of the mammalian brain with important and/or well-defined functions, in the brain of nonmammals. Although the prefrontal cortex (PFC) may not be present in all mammals (see Do All Mammals Have a Prefrontal Cortex?), it is nevertheless a paradigmatic example of these attempts. Thus, Mogensen and Divac found that the TPO/NCL of the avian cerebral hemispheres displays two of the defining features of the PFC. First, the TPO/NCL shows a high content of dopamine (Divac and Mogensen, 1985; Divac *et al.*, 1985), due to a dense meshwork of dopaminergic fibers (see, for instance, Waldmann and Gunturkun, 1993; Wynne and Gunturkun, 1995), arising from the ventral tegmental cell groups (mainly A10) (Metzger *et al.*, 1996), which recalls the dopaminergic innervation of the PFC of mammals. The presence of a dense dopaminergic innervation arising from the A9–A10 tegmental cell groups, however, does not constitute by itself a defining feature of the prefrontal cortex since in the mammalian brain the B nucleus of the amygdala is also targeted by a similar dopaminergic pathway (see main text).

In addition, Mogensen and Divac (1982, 1993) reported that lesions of the avian TPO/NCL resulted in deficits in behavioral tasks of delayed alternation. This seems indicative that the NCL/TPO is involved in working memory, a kind of memory that in mammals is dependent on prefrontal cortex function (Goldman-Rakic, 1996). Since then, the homology of the TPO/NCL with the mammalian PFC has gained a wide acceptance (Reiner, 1986). This view and the idea that the N and M are part of the isocortex of birds were mutually reinforcing.

Functional experiments demonstrate that the amygdala and the orbitofrontal cortex are part of the neural machinery for the control of those forms of associative learning (both Pavlovian and instrumental) that involve delayed reinforcement and reward expectancy (Cardinal *et al.*, 2002; Holland and Gallagher, 2004). In other words, in the face of a given stimulus (cue) the animal learns to behave (action) so that it gets a rewarding item (usually food, sucrose, or water). During learning the stimulus becomes a reward-predicting cue. Execution of those behaviors demands that reward expectancy during the delay phase (between the detection of the reward-predicting cue and reward consumption) is encoded by the activity of cells responding to the cue during the delay phase, usually just prior to the action leading to reward acquisition. In a go/no-go task that uses an odor as the reward-predicting cue and sucrose (vs. quinine) consumption as the reward, these working memory cells have been observed in mammals in both the basolateral amygdala and orbitofrontal PFC (Schoenbaum *et al.*, 1999).

Similar working memory cells, including reward expectancy-encoding cells, are found in the NCL of pigeons (Kalt *et al.*, 1999; Diekamp *et al.*, 2002), executing comparable (but more instrumental) go/no-go tasks, in which the cue was auditory or visual. These results led the authors to conclude that the NCL contains the analogue of the mammalian PFC. As they further discuss, differences in the topological position of both structures (revealed by the different patterns of expression of homeotic genes during development) make their homology very unlikely.

As reviewed above, the mammalian B and parts of the orbitary and medial and insular PFC are extensively and massively interconnected (McDonald, 1998). Consequently, in their functional classification of the mammalian amgydaloid nuclei, Swanson and Petrovich (1998) considered the B nucleus as part of the frontotemporal cortical system. Therefore, it is not surprising that lesions of the avian counterpart of the B nucleus lead to behavioral impairments reminiscent of those resulting from lesions of the PFC in mammals. On the other hand, in a forebrain lacking a true isocortex, like the avian one, it is likely that the amygdaloid portion of the frontotemporal system has assumed the functions of the whole system. In other words, it is sensible that the TPO/NCL and other deep lateropallial derivatives of the avian cerebral hemispheres (such as the AD and lateropallial PoA) are not just the homologues of the B nucleus of the mammalian amygdala but also the analogues of the PFC, including its dorsolateral portion.

and CeL, whereas the CGRPergic innervation is concentrated in the lateral-most capsular division of the central amygdala (CeC).

The name BSTl is suggestive of a homology of this juxtaventricular cell group with parts of the BST of mammals and, as a consequence, of the SpA with the mammalian Ce. The histochemical data discussed above clearly indicate that this is not the case. In mammals, the central EA is composed of two centers separated by the internal capsule, namely the Ce and anterior posterolateral BST. However, in the forebrain of birds (as in

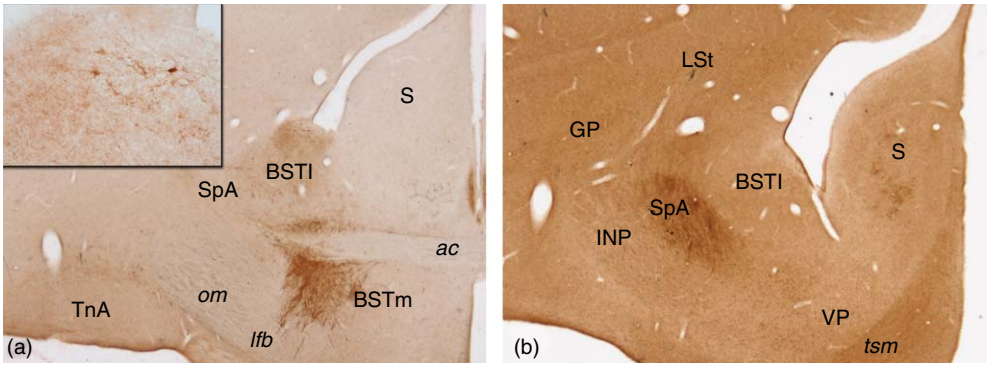

Figure 21 Histochemical characterization of the avian central extended amygdala. a, CRF-immunoreactivity in the BSTl of the left cerebral hemisphere of a quail (the inset shows a detail of the labeling). This is the main CRFergic cell group in the telencephalon of birds that supports the homology of the BSTl with parts of the central EA of mammals. CRF-immunoreactive fibers (probably arising from the reactive cells in the BSTl; Richard *et al.*, 2004) are observed in the lateral aspect of the BSTm. b, Frontal section of the left telencephalic hemisphere of a chicken through the anterior aspect of the BSTl, which has been processed for the immunohistochemical detection of CGRP. A dense CGRPergic innervation is observed in the former ventral paleostriatum, now renamed SpA. Taken together, these data (and additional data reported in other species) suggest that the central EA of birds is composed of the BSTl and SpA.

reptiles), an internal capsule is lacking and both groups are fused together: the SpA is adjacent and topologically superficial to the BSTl. We envisage both structures as the avian counterpart of the entire central EA of mammals in a case of field homology.

2.14.6.5 The Extent of the Avian Amygdala

The renewed avian neuroanatomical terminology (Reiner *et al.*, 2004) recognizes the homology of parts of the avian cerebral hemispheres with the mammalian amygdala. These include the TnA and PoA, as well as the so-called SpA. Our analysis confirms that these cell groups display histochemical, hodological, and topological features comparable to parts of the mammalian amygdala. As indicated in Table 3, the PoA seems comparable to the AHA of mammals, the TnA to part of the medial EA, and the SpA to part of the central EA.

Thus, the new nomenclature of the avian forebrain leaves it without a recognized homologue for the basolateral amygdala of mammals (L, B, and AB; Figure 20a). The search for this homologue has been hindered by the lack, in turn, of a clear idea of the identity of the central EA of birds. Now, when a wide consensus has been reached (in the Nomenclature Forum) to name SpA and BSTl to the structures composing the central EA of birds, it is time to look seriously for the avian basolateral amygdala. Any candidate for this should be ventro- and lateropallial, and provide a strong input to a continuum in the ventral striatopallidal telencephalon linking the central EA (SpA and BSTl) with the Acb. In addition, it should be adjacent to the known components of the avian amygdala (TnA, SpA, and PoA).

This simple reasoning, the quite straightforward comparison of the avian telencephalon with the cerebral hemispheres of reptiles, together with a cladistic analysis, leads us to reinterpret the comparative meaning of the arcopallium and the NC (Table 3; Figure 20b). They are the pallial derivatives of the avian brain that are best positioned using topological, embryological, and hodological data, to constitute the avian basolateral amygdala. In addition, their histochemical properties are comparable to those of the mammalian and reptilian components of the basolateral amygdala.

This solid hypothesis requires serious consideration. This would not only contribute to our better understanding of the organization and evolution of the brain of amniotes, but would give an extraordinary impulse to avian neurobiology. If we are right, birds are the amniote vertebrates that display a bigger and more accessible basolateral amygdala. Moreover, birds display a complex behavior that includes remarkable learning capacities such as auditory and visual imprinting, passive avoidance learning, song and instrumental conditioning (Jarvis *et al.*, 2005). Therefore, birds might constitute the ideal animal model for the study of the amygdaloid function (Box 2).

2.14.7 The Evolutionary Origins of the Amniote Amygdala

The common pattern of organization of the amygdala in reptiles, birds, and mammals described

above suggests that the amygdaloid complex of the ancestral amniote already possessed a pallial amygdala with lateropallial and ventropallial components and a subpallial EA with medial and central components. In this section, we discuss whether this pattern of organization of the amygdala was already present in anamniotes. To do so we review the available data on the structure and function of the telencephalon of amphibians, and some functional data available in fishes.

2.14.7.1 Functional Data in Teleostean Fishes

The telencephalon of ray-finned (actinopterygian) fishes is everted (Nieuwenhuys, 1963; see Evolution of the Nervous System in Fishes), instead of evaginated like the telencephalon of tetrapod vertebrates. Therefore, the structures located medially in the pallium of ray-finned fishes correspond to those with a lateral location in the telencephalon of tetrapods, and vice versa. This has led to the hypothesis that the amygdala should be located medially in the telencephalon of fishes, whereas the Hp should be positioned laterally. Anatomical and functional data strongly support this view as it concerns the hippocampal pallium (Northcutt and Braford, 1980; Nieuwenhuys and Meek, 1990; Braford, 1995; Northcutt, 1995; Butler, 2000; Rodriguez *et al.*, 2002; Salas *et al.*, 2003). In contrast, very few anatomical data are available either to support or discard the putative homology between the medial pallium of fishes and the amygdaloid complex of tetrapod vertebrates (but see Braford, 1995; Butler, 2000). There are, however, some functional studies (Portavella *et al.*, 2002, 2004) showing that the medial pallium of teleost fishes is involved in avoidance conditioning (a case of emotional learning). Therefore, topological and functional data suggest that ray-finned fishes and land vertebrates probably share an ancestor that already possessed an amygdala (medially located in everted brains, laterally located in evaginated brains) involved in fear/aversion acquisition and expression. Whether this structure includes pallial and/or subpallial derivatives is, at present, unclear.

2.14.7.2 Anatomical Data in Amphibians

The existence of an amygdala in the telencephalon of amphibians was already suggested in early anatomical studies by Herrick (1921). After that, the amphibian amygdala was defined as an area receiving direct projections from the accessory olfactory bulb (Scalia, 1972; Northcutt and Royce, 1975). The classical understanding on the organization of the amphibian amygdala divides it, on topographical grounds, into a lateral and a medial part (pars lateralis and pars medialis; Northcutt and Kicliter, 1980; Neary, 1990). However, recent data on neurochemistry (Marin *et al.*, 1998), hodology (Bruce and Neary, 1995c; Marin *et al.*, 1997a; Moreno and Gonzalez, 2003, 2004; Roth *et al.*, 2004; Laberge and Roth, 2005), and developmental gene expression (Brox *et al.*, 2003, 2004; Moreno *et al.*, 2004) have led to a complete redefinition of the amphibian amygdaloid complex (Figure 22). Although, at present, there are some discrepancies on the nomenclature of the amphibian amygdaloid complex, the terminology by Marin *et al.* (1998) and Moreno and Gonzalez (2003, 2004) will be used in the present section, since it is the most useful one for comparative purposes.

2.14.7.2.1 Vomeronasal and olfactory projections to the amygdala in amphibians The telencephalic target of the accessory olfactory bulb projections corresponds to the classical amygdala pars lateralis (Northcutt and Kicliter, 1980; Neary, 1990) but, given its comparative meaning, it was recently renamed as medial amygdala by Marin *et al.* (1998) (see also Moreno and Gonzalez, 2003). However, other authors keep using a purely topographical nomenclature, and name the structure receiving the bulk of the projection from the accessory olfactory bulb as lateral amygdala in anurans (Roth *et al.*, 2004) and caudal amygdala in urodeles (Laberge and Roth, 2005). Therefore, the same structure is named medial or lateral amygdala by different authors studying different species. However, from a comparative point of view, there is a certain consensus in considering the vomeronasal target within the caudal ventrolateral telencephalon homologous to the medial amygdala of mammals (the possible existence of a vomeronasal pallial amygdala in amphibians is discussed below).

Regarding the olfactory projections, the main structure receiving direct input from the main olfactory bulb is the lateral pallium (Northcutt and Kicliter, 1980; Neary, 1990). Within the lateral pallium, a dorsal and a ventral division have classically been recognized (Neary, 1990). Using neurochemical data, Marin *et al.* (1998) suggested that the ventral division of the lateral pallium was an amygdaloid structure, which in turn has been divided into an anterior amygdala (Aa, previously called striatopallial transition area by the same authors; Marin *et al.*, 1997a, 1997b) and a lateral amygdala (La, Figure 22) (Marin *et al.*, 1998; Moreno and Gonzalez, 2004).

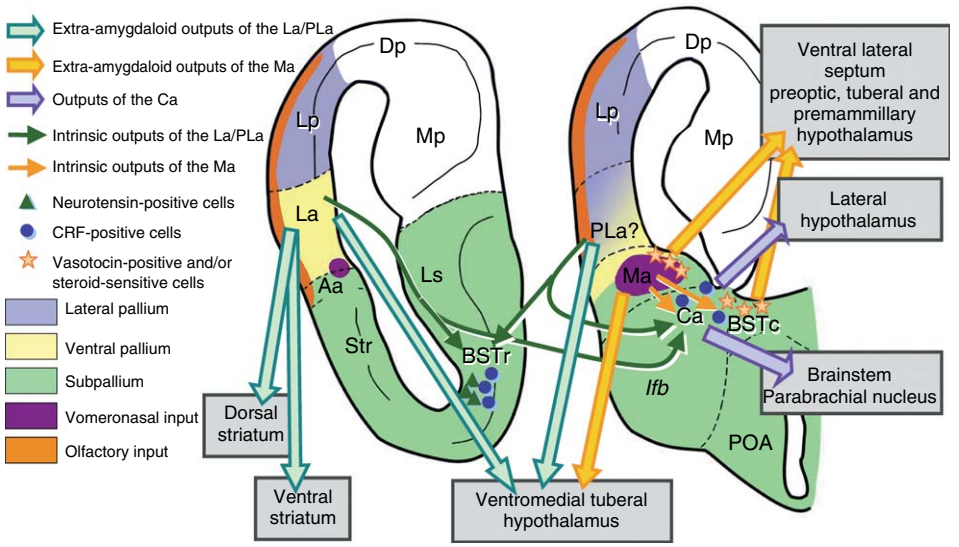

Figure 22 The amphibian amygdala. Schematic view of the amygdala of amphibians, based on two frontal sections (rostral (a); caudal (b)) of the left cerebral hemisphere of an anuran. The map of pallial territories (based on Brox *et al.*, 2003, 2004 and Moreno *et al.*, 2004), the bulbar afferents, the connections with the hypothalamus and striatum, and the intrinsic connections are represented. The PLa is depicted in a color mixture to represent that its ventropallial or lateropallial nature is uncertain. The Ma is depicted as composed of ventropallial (dorsal) and subpallial (ventral) regions. In addition, the location of cells expressing receptors for sexual steroids and/or immunoreactive for vasotocin, as well as the distribution of cells immunopositive for corticotropin-releasing factor and neurotensin (see section 2.14.7.2.5 of the text for the appropriate references), are depicted. These data suggest the existence of a medial and central EA, as well as a pallial amygdala in which cortical and deep nuclei are not separated (due to the low degree of cell migration).

2.14.7.2.2 New data on the divisions of the amphibian pallium: The pallial amygdala As discussed above, the pattern of expression of developmental genes in vertebrate embryos has revealed the existence of a pallial region interposed between the lateral pallium and the striatum, the ventral pallium. Similar studies in amphibians (Smith-Fernandez *et al.*, 1998; Bachy *et al.*, 2001, 2002; Brox *et al.*, 2003, 2004; Moreno *et al.*, 2004) indicate that a ventral pallium is also present in ampibians. In anurans, this is mainly represented by the La (formerly ventral division of the lateral pallium), which should therefore be homologous to the ventropallial derivatives of the amygdala of amniotes.

In addition, these studies indicate that, although the medial amygdala (as defined by Moreno and Gonzalez, 2004) (Ma, see Figure 22) is mainly subpallial (Brox *et al.*, 2003; Moreno and Gonzalez, 2004), a part of it (very likely its dorsal aspect adjacent to the La) might be ventropallial (Brox *et al.*, 2004). This suggests that the amphibian brain displays homologues for both the medial amygdala of amniotes (subpallial Ma; see below) and the ventropallial vomeronasal cortex (COApm of mammals, NS of reptiles), as previously suggested (Northcutt and Kicliter, 1980; Scalia *et al.*, 1991). The homology and boundaries of this putative ventropallial portion of the Ma with the vomeronasal cortex of amniotes should be further explored by analyzing in detail the connections and neurochemistry of the amphibian Ma.

The anterior amygdala is a relatively small structure located in a rostral position close to the accessory olfactory tract (Marin *et al.*, 1998). Available data on developmental gene expression and the presence of GABAergic cells suggest that it is a subpallial structure (Gonzalez *et al.*, 2002b; Brox *et al.*, 2003; Moreno and Gonzalez, 2004), but its amniote homologue is unknown.

One of the main conclusions of the study of the developmental gene expression patterns in the amphibian telencephalon is that there appears to be no amygdaloid division originated in the embryonic lateral pallium, in contrast to the situation in mammals (B and COApl), reptiles (DLA and LCc), and birds (NCL/TPO, AD, and lateropallial PoA). Therefore, the lateropallial amygdala would be an evolutionary acquisition of the amniote lineage. The opposite interpretation derives from recent data on the expression of the homeobox gene *xEmx1* (Brox *et al.*, 2004), which suggest that the posterior La (PLa) of anurans is a lateropallial derivative. However, in contrast to the lateropallial derivatives of the amniote amygdala (see above), the PLa of amphibians projects to the ventromedial hypothalamus (Bruce and Neary, 1995b; Moreno and

Gonzalez, 2004; see below). Thus, the possibility that the La of amphibians contains a lateropallial portion equivalent to the B and COApl of mammals requires further analysis (Figure 22).

2.14.7.2.3 Projections to the hypothalamus: The pallial stria terminalis of amphibians Besides its afferents from the olfactory bulbs, the other widely accepted defining feature of the amygdala of amniotes is the presence of important projections to the hypothalamus, mainly to its medial tuberal division. The telencephalic projections to the hypothalamus have been studied in anurans (Neary, 1995; Moreno and Gonzalez, 2004, 2005) and the results indicate that the amphibian La gives rise to important projections to the core of the ventromedial hypothalamus. Therefore, the La of amphibians is the ventropallial, olfacto-recipient structure that originates a major projection to the ventromedial hypothalamus through the stria terminalis. These features suggest that the LA includes the amphibian homologues for the mammalian AB and of the overlying COAa, as well as the PDVR and ventropallial olfactory amygdala of sauropsids.

The pallial component of the stria terminalis of mammals also includes fibers arising from the AHA that reach mainly the preoptic and tubero-premammillary hypothalamus (VMH shell and PMv). A homologue for the AHA of mammals, equivalent to the VPA of reptiles and the avian ventropallial PoA, is still to be found in amphibians. A useful clue to explore this possibility would be the study of the expression of receptors for sexual steroids in the La of amphibians, since they are present in the AHA of mammals and its reptilian homologue (VPA), although this trait has apparently been lost in birds (Table 3). Dealing with this, the few available data (Davis and Moore, 1996; Perez *et al.*, 1996) suggest that steroid-sensitive cells are lacking in the La of amphibians, but detailed studies are needed to clarify this issue.

2.14.7.2.4 The amygdalostriatal pathways and the lateropallial amygdala of amphibians The telencephalon of amniotes displays a set of amygdalostriatal projections arising from the pallial amygdala. First, the deep lateropallial amygdala (B in mammals, DLA in reptiles, AD and TPO/NCL) projects massively to the ipsilateral (and also to the contralateral) dorsal and ventral striatum, including its caudal extension, the central EA. In addition, parts of the ventropallial basolateral amygdala (mammals, AB; reptiles, PDVR and LA; birds, AV and AM) project to the ipsilateral Acb and to the central EA.

Anatomical studies in anurans and urodeles indicate the existence of similar projections in amphibians. Thus, the rostral La projects to the ipsilateral Acb (Marin *et al.*, 1997a, 1998; Moreno and Gonzalez, 2004), as well as to more caudal striatopallidal territories, including the central amygdala, the bed nucleus of the stria terminalis, the VP, and the nucleus of the diagonal band. This projection system recalls the connections of the pallial amygdala to the central EA (see next section), and further supports the homology of the La of amphibians with the basolateral division of the amygdala of amniotes.

Both anurans and urodeles display a bilateral projection to the dorsal striatum that arises from the La (Marin *et al.*, 1997a). However, since the amphibian La seems a ventropallial derivative, it is not clear whether this projection is equivalent to the amygdaloid pathway to the dorsal striatum of amniotes, which arises from the deep lateropallial nuclei (mammals, B; reptiles, DLA; birds, TPO/NCL, AD, and lateropallial PoA). In fact, the portion of the La for which a lateropallial nature has been suggested, the PLa (Brox *et al.*, 2004), apparently does not project to the dorsal striatum (Moreno and Gonzalez, 2004). In addition, no portion of the amphibian La shows remarkable immunoreactivity for either choline acetyltransferase (Marin *et al.*, 1997c) or for tyrosine hydroxylase (Marin *et al.*, 1998), which constitute the most outstanding histochemical features of the deep lateropallial amygdala of amniotes. The absence of a cholinergic innervation of the pallial amygdala of amphibians could be explained in view of the scarce population of ChAT-positive cells in the basal telencephalon of anurans and urodeles (Marin *et al.*, 1997c; Gonzalez *et al.*, 2002a). In contrast, the lack of dopaminergic input to the lateropallial amygdala is surprising, given that other dopaminergic inputs to the telencephalon are notably conserved (Marin *et al.*, 1998).

As a conclusion, at present it is unclear whether a homologue for the lateropallial amygdala of amniotes is present in amphibians. The lateropallial nature of the PLa (Brox *et al.*, 2004) is not consistent with its massive projections to the hypothalamus and the lack of projections to the dorsal striatum. Detailed analysis of the chemoarchitecture, development, and connections of the LA of amphibians is needed to clarify this issue.

2.14.7.2.5 The subpallial amygdala of amphibians The available data on the connections and neurochemistry of the telencephalon of amphibians strongly suggest that the caudal subpallium

includes structures comparable to the EA of amniotes. Moreover, there is evidence supporting the view that amphibians possess a central and a medial EA comparable to their amniote homonyms.

2.14.7.2.5.(i) The medial extended amygdala of amphibians As we have already discussed, the caudal cerebral hemispheres of amphibians include a subpallial structure that is targeted by the projections from the accessory olfactory bulb (Northcutt and Kicliter, 1980; Neary, 1990), which is now named medial amygdala (Ma) to suggest its homology with its mammalian homonym (Moreno and Gonzalez, 2003). In fact, like the medial amygdala of amniotes, the amphibian Ma gives rise to important projections to the hypothalamus (Neary, 1995; Moreno and Gonzalez, 2003, 2005; Roth *et al.*, 2004), including preoptic and tuberoinfundibular levels (ventromedial hypothalamus). In addition, the amphibian Ma projects to the central amygdala (see below), the BST, the Acb, and ventral lateral septum and nucleus of the diagonal band (Moreno and Gonzalez, 2003). In turn, the hypothalamic targets of the Ma (preoptic, and tuberoinfundibular hypothalamus) project back to it. This set of connections clearly recalls those of the medial amygdala of amniotes.

As we have discussed for reptiles and birds, in amphibians a portion of the BST (mainly its caudal aspect, BSTc; Moreno and Gonzalez, 2003) is interconnected with the Ma. This suggests that amphibians, like amniotes, also possess a medial EA composed of the Ma and the BSTc. Histochemical studies reveal further similarities between the medial EA of amphibians and amniotes. Thus, the medial EA of anurans and urodeles (and even of Gymnophiona; Gonzalez and Smeets, 1997; Hilscher-Conklin *et al.*, 1998) displays a population of cells immunoreactive for arginine vasotocin (Smeets and Gonzalez, 2001) that extends into the preoptic hypothalamus, thus recalling the medial EA of amniotes. This vasotocinergic neuronal population is sensitive to steroid hormones (Boyd, 1994), so that this cell group is sexually dimorphic at least in certain seasons (Boyd *et al.*, 1992). Accordingly, the medial EA of anurans (Morrell *et al.*, 1975) and urodeles (Davis and Moore, 1996), like its counterpart in amniotes, is rich in receptors to sexual steroids, especially estrogens. In addition, vasotocin-immunoreactive fibers innervate some forebrain centers (ventral lateral septum, ventromedial infundibular hypothalamus; Smeets and Gonzalez, 2001) that are reached by projections of the Ma (Moreno and Gonzalez, 2003) and are also sensitive to sexual steroids (Kelley *et al.*, 1975; Morrell *et al.*, 1975; Davis and Moore, 1996). Therefore, like amniotes, amphibians display a sexually dimorphic forebrain circuit in which the medial EA apparently gives rise to vasotocinergic projections. This strongly supports the homology of the medial EA of amphibians and amniotes. In this respect, the absence of a vasotocinergic innervation of a pallial amygdaloid center (specifically in the La), which would be part of this circuit in the amphibian forebrain, again suggests that amphibians lack a cell group equivalent to the AHA of mammals, the VPA of reptiles, and the ventropallial PoA of birds. However, detailed studies of the neurochemistry and connections of the amphibian pallium are needed to determine whether a pallial, vomeronasal-related, and steroid-sensitive pallial amygdaloid center is already present in the brain of amphibians.

2.14.7.2.5.(ii) The central extended amygdala of amphibians The caudal part of the striatum was renamed central amygdala (Ca) by Marin *et al.* (1998) on the basis of its chemoarchitecture and connections (Marin *et al.*, 1997a, 1997b). The amphibian Ca is interconnected with the parabrachial region, the lateral reticular zone, and the nucleus of the solitary tract (Marin *et al.*, 1997a, 1997b). Moreover, at least some of the projections to the tuberal hypothalamus originally attributed to the striatum are likely to originate in the Ca (Neary, 1995; Marin *et al.*, 1997b). In addition, the Ca receives important afferents from the other two amygdaloid divisions, the Ma (Moreno and Gonzalez, 2003) and La (Moreno and Gonzalez, 2004). Therefore, the afferent and efferent connections of the amphibian Ca resemble those of the mammalian central nucleus of the amygdala.

In amphibians, the projections of the La to the Ca continue rostrally to innervate a continuum of structures that include the rostral BST (BSTr) and the Acb. This recalls the projection from the basolateral to the central EA in amniotes, thus suggesting that the amphibian brain possesses a central EA composed of the Ca plus the BSTr. Available neurochemical data are consistent with this hypothesis. Thus, in *Xenopus* the Acb shows large neurons expressing CRF (Yao *et al.*, 2004), whereas the Ca, Ma, and BSTc display smaller and scattered CRF-immunopositive cells. In addition, the only study of the distribution of NT in the amphibian brain (Bello *et al.*, 1994) reports a few NT-immunoreactive cells in the BSTr.

2.14.7.3 What's New in the Amniote Amygdala?

The data reviewed above show that amphibians possess an amygdaloid formation with pallial and subpallial components. Within the pallium, the La and maybe the dorsal aspect of the Ma are ventropallial derivatives (Brox *et al.*, 2004; Moreno and Gonzalez, 2004), although the posterior La may be lateropallial (Brox *et al.*, 2004). Both the La and Ma project to the hypothalamus (Moreno and Gonzalez, 2003, 2004). In addition, the La also projects to the dorsal and ventral striatum (Marin *et al.*, 1997a). The subpallial amygdala of amphibians consists of most of the Ma, the Ca, and the BST (Marin *et al.*, 1998; Brox *et al.*, 2003, 2004; Moreno and Gonzalez, 2003, 2004). The latter two structures apparently display long descending projections directed to parts of the hypothalamus (Neary, 1995; Moreno and Gonzalez, 2003), tegmentum, and brainstem, including the parabrachial region (from which they receive in turn an important input) and the dorsal medulla (Marin *et al.*, 1997a, 1997b). In addition, the Ca and BSTr are reached by projections from the presumed amphibian homologue for the basolateral amygdala (Moreno and Gonzalez, 2004).

Therefore, the amygdala of amphibians already shows the pattern of organization present in amniotes. In spite of the relative lack of studies on the amphibian brain, the available data suggest that the subpallial amygdaloid centers (medial and central EA) were already present in the anamniote forebrain and underwent a conservative evolution during the anamniote–amniote transition.

However, the pallial amygdalae of amniotes and anamniotes display relevant differences that deserve being considered. First, it is still unclear whether the existence of a lateropallial amygdala is an acquisition of amniotes or whether anamniotes already possess it (see above). Moreover, the pallium of amphibians shows a low degree of radial migration (as compared with amniote pallium) and this applies for the presumed pallial amygdala. Consequently, in the amphibian pallial amygdala there is no differentiation between cortical (superficial, olfactorecipient) and deep pallial nuclei, but the cells of the pallial amygdala projecting to the EA, striatum, and hypothalamus (which in amniotes are mainly deep) are directly reached by olfactory inputs (Moreno and Gonzalez, 2004, 2005). Although the La of anurans also receives afferents from multimodal dorsal thalamic nuclei (anterior and central nuclei; Moreno and Gonzalez, 2004), this suggests that amygdala of anamniotes is mostly influenced by olfactory and vomeronasal information. In this respect, it is important to note that the cerebral hemispheres of amphibians lack true pallial visual, auditory, and somatosensory centers (thalamic sensory inputs reach only the striatum; see Evolution of the Amphibian Nervous System). In contrast, amniotes display pallial regions (reptiles, ADVR; birds, nidopallial sensory regions; mammals, isocortical sensory areas) that receive sensory thalamic inputs, and project directly and/or indirectly to the deep pallial amygdala (Figures 23 and 24).

2.14.8 The Amygdala and the Evolution of the Vertebrate Forebrain

2.14.8.1 The Amygdala: Physiology and Behavior

Considering together all the data reviewed above, it is clear that the amygdala has a long history and that it has undergone a conservative evolution during the phylogeny of tetrapods. This comparative perspective allows us to pose again one of the central questions of the research on the amygdala: whether the amygdala is a functional system (Swanson and Petrovich, 1998; Figure 23a). As we have seen, reptiles, birds, and mammals display a similar set of pallial and subpallial centers in the caudal cerebral hemispheres that can be grouped into two main circuits, structured around the central and medial EA respectively (Figure 23b). The roles of these two circuits in physiology and behavior are discussed below.

2.14.8.1.1 The roles of the central/basolateral amygdala The central EA is one of the output centers for the basolateral amygdala. Thus, the L, B, and AB of mammals, and their homologues in the remaining amniotes, project massively to the central EA. This projection, however, also extends to other striatal territories, including the whole Acb and the dorsal striatum (the latter projection arising exclusively from the B and its avian and reptilian homologues). This system is clearly multimodal as it receives inputs from cortical, thalamic, and brainstem centers, as well as diverse modulatory (mostly aminergic and cholinergic) afferents. In addition, it receives chemosensory inputs thanks to the presence of important superficial-to-deep projections within the amygdala, which convey the olfactory and vomeronasal stimuli received by the cortical (superficial) amygdala to the basolateral and central amygdala.

The role of this circuit has been extensively studied in mammals using different experimental approaches. The results of these studies indicate that the outputs through the central EA mediate fear/anxiety reactions to incoming stimuli, whereas

the amygdalostriatal pathways are part of the reward system of the brain.

2.14.8.1.1.(i) Expression and acquisition of fear/aversion In primates and nonprimate mammalian species (Roozendaal *et al.*, 1990; Davis and Shi, 1999; Choi and Brown, 2003; Kalin *et al.*, 2004; Rosen, 2004), lesioning or inactivating the central amygdala and/or anterior and posterolateral BST diminishes the expression of fear and anxiety

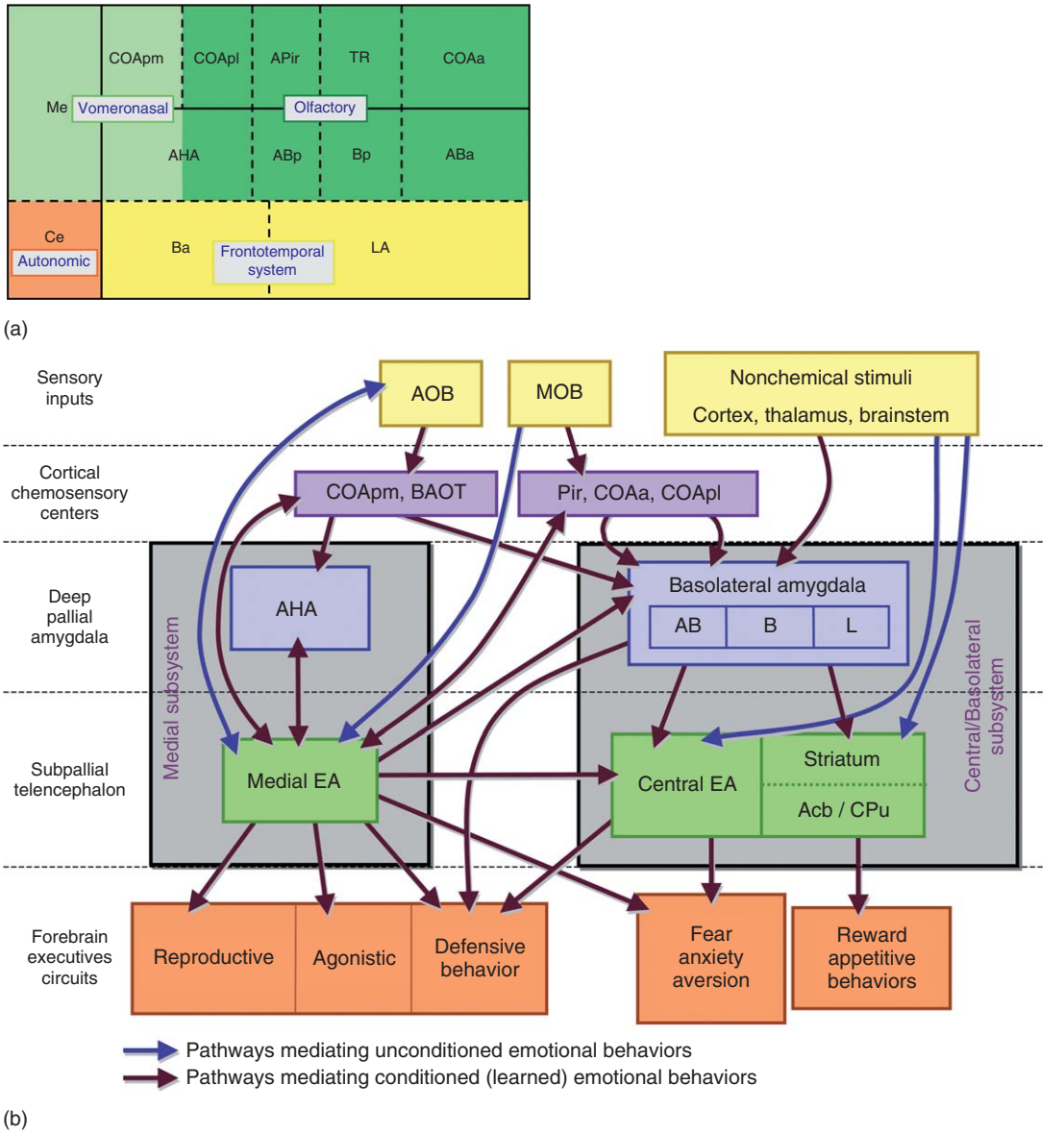

Figure 23 Is the amygdala a functional system? According to the view put forward by Swanson and Petrovich (1998) (a) the amygdala is not a functional system but is composed of structures belonging to the olfactory/vomeronasal, autonomic, and frontotemporal cortical systems. However, the available data on the anatomy and function of the amygdala suggest an alternative scheme on the functional anatomy of the amygdala (b). According to it, the different nuclei of the amygdala are interconnected to conform two functional subsystems, namely the central/basolateral subsystem and the medial subsystem. The former subsystem coordinates innate and learned reactions of fear/anxiety/aversion (through the descending projections of the central EA) or of attraction/reward-directed behaviors (through its projections to the striatum) to virtually any stimulus. The medial subsystem is primarily involved in the coordination of responses to chemosensory stimuli (olfactory and vomeronasal) that constitute species-specific emotional behaviors, such as reproductive/agonistic behaviors to conspecifics (responses to pheromones), and defensive behaviors to conspecifics (as a component of agonistic behaviors) or to predator vomeronasal organ-detected signals. Both subsystems are interconnected, in such a way that olfactory and vomeronasal stimuli can elicit fear/aversion or appetitive behaviors (medial to basolateral/central). On the other hand, nonchemosensory stimuli can modulate the response to pheromones (basolateral/central to medial), although this influence is mediated by direct projections from the basolateral amygdala to the defensive forebrain circuit, rather than through intra-amygdaloid connections.

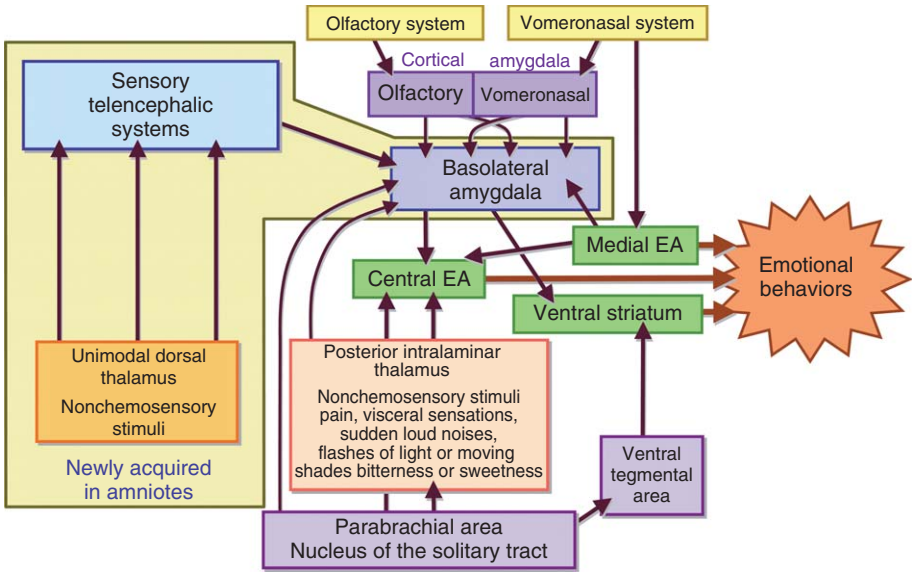

Figure 24 Evolution of the amygdala in tetrapods. The comparative study of the amygdala suggests that all tetrapods have a subpallial amygdala (composed of medial and central EA), which together with the striatum mediates unconditioned emotional behavioral responses to different stimuli (including chemical and nonchemical ones). Moreover, the ancestral amygdala of tetrapods also possesses a cortical amygdala that receives chemosensory information (mainly olfactory), and might contribute to these responses. Two new acquisitions characterize the amygdala of amniotes: (1) the existence of a deep pallial (nuclear) amygdala, namely the basolateral amygdala; and (2) the presence of unimodal nonchemosensory cortical areas in the pallium (dorsal and/or ventral depending on the class). Both have become interconnected, thus allowing processing of stimuli (recognition and identification of their spatial and/or temporal configuration) prior to their emotional evaluation. This has a great adaptive value that has potentiated these traits in all the evolutionary lines of extant amniotes.

against several fear-eliciting stimuli. Comparable functional studies are scarce in nonmammals. However, it has been shown that tonic immobility, a form of prolonged stillness and decreased responsivity induced by threatening stimulation (e.g., physical restraint), which constitutes one of the fear-related ultimate defensive behavioral resources, is reduced by lesions of the former archistriatum of birds (Maser *et al.*, 1973), or the SAT (central EA homologue) in lizards (Davies *et al.*, 2002). Tonic immobility is also part of the behavioral repertoire of some mammals, such as guinea pigs (and maybe humans, where it has been related to catatonia; Moskowitz, 2004). In guinea pigs, tonic immobility seems controlled by the central and basolateral amygdala (Ramos *et al.*, 1999; Leite-Panissi and Menescal-de-Oliveira, 2002). Some of its components, such us the profound analgesia associated with tonic immobility (Leite-Panissi *et al.*, 2001), are dependent on the integrity of the central amygdala and its projection to the periaqueductal gray (Leite-Panissi *et al.*, 2001, 2003).

The role of the descending pathways of the central amygdala in fear expression is further supported by experiments of electrical stimulation. One of the most common behaviors related to stress, discomfort, and anxiety is vocalization. For instance, rats display several kinds of ultrasonic and sonic vocalizations that convey information on their emotional state, some of which are induced by stress and anxiety and are mediated by descending projections arising from the periaqueductal gray to the vocal controlling motor nuclei (Sanchez, 2003). In this respect, electrodes implanted into the amygdala and along the trajectory of the stria terminalis in monkeys elicit different kinds of vocalization (Jurgens, 1982), including purring and chattering calls (which express a self-confident, challenging attitude) and alarm peep and groaning calls indicative of flight motivation and resentment and associated to social stress. Like in rats, the anatomical pathways responsible for these vocalizations in monkeys include periaqueductal regions that are targets for amygdaloid projections (Dujardin and Jurgens, 2005). Moreover, in guinea pigs, the central amygdala is involved in the expression of pain-related vocalization, so that vocalizations induced by noxious stimuli can be decreased by intra-amygdaloid infusions of cholinergic- and opioid-related drugs (Leite-Panissi *et al.*, 2004).

It has also been shown that electrical stimulation of the central amygdala (Rosen and Davis, 1990; Koch and Ebert, 1993) enhances acoustic startle response, one of the well-studied models of

conditioned fear in rats. In fact, it is now well established that conditioned fear to a previously neutral stimulus (conditioned stimulus) is mediated by a process of Pavlovian association between unconditioned (e.g., footshock) and conditioned stimuli (a tone, a light) that apparently takes place in the basolateral amygdala thanks to *N*-methyl-D-aspartate (NMDA)-mediated synaptic plasticity (Davis, 1994; LeDoux, 2000; Lee *et al.*, 2001; Rosen, 2004). In this respect, it has been suggested that the central EA is involved in conditioned, but not unconditioned fear responses (Choi and Brown, 2003; Rosen, 2004). However, the central EA of mammals (more specifically, its capsular and lateral divisions) receives direct nociceptive inputs from the parabrachial area and posterior intralaminar thalamus (Gauriau and Bernard, 2002), which very likely constitute powerful unconditioned fear-eliciting stimuli. These nociceptive-related afferents of the central EA are rich in the peptide CGRP (see above). In this respect, Borszcz (1993, 1995) reported that in order for a painful stimulation (tailshock) to support fear conditioning, it must generate vocalization afterdischarges (vocalizations that extend beyond the termination of the unconditioned stimulus). It is important to note that lesions of the central amygdala abolish the unconditioned vocalization afterdischarges elicited by tailshock (Borszcz and Leaton, 2003). Therefore, the central amygdala (and very likely the whole central EA) is involved in the generation of fear/anxiety or aversion responses to conditioned and, at least, to some unconditioned fear-eliciting stimuli, such as pain.

The presence of a dense CGRPergic innervation of portions of the central EA of reptiles (Martinez-Garcia *et al.*, 2002b) and birds (Lanuza *et al.*, 2000) suggests that this is a general role for the central EA of, at least, amniotes. This is strongly supported by experiments of electrical stimulation of the amygdala in crocodiles (Keating *et al.*, 1970) and iguanas (Distel, 1978), which elicit fear-related behaviors such as fleeing accompanied by vocalization, pupillary dilation, and hyperventilation. In birds, there is evidence that the descending projections arising from the former archistriatal region mediate escape responses (Phillips, 1964). In addition, Phillips and Youngren (1986) demonstrated that kainic acid lesions of the archistriatum of young domestic chicks reduced fear, as expressed by distress calls (peeps). More recently, it has been shown that the archistriatum is indeed involved in the expression of unlearned fear- or anxiety-related behaviors, such as avoidance of the center of an open field (Lowndes and Davies, 1995). It is still unknown whether these reactions are mediated by the archistriatal projections to the BSTl/SpA or by direct descending projections (like the OM). As a conclusion, despite the fact that more functional studies in nonmammals are needed to refine this view, there is ample evidence suggesting that the circuit composed of the basolateral and central amygdaloid divisions is involved in the expression of unlearned fear and anxiety elicited by unconditioned stimuli (at least pain), and in the acquisition and expression of conditioned fear and distress.

2.14.8.1.1.(ii) Amygdalostriatal pathways: The amygdala and reward In contrast to the data reviewed above, it has been reported that lesions of the basolateral amygdala or of its ventral striatal targets (Acb) in different mammalian species selectively impair learning instrumental responses that result in a delayed reinforcement, which results in impulsive choice (Baxter and Murray, 2002; Cardinal *et al.*, 2004). This indicates that the mammalian amygdalostriatal pathways, together with the prefrontal cortex and the tegmental dopaminergic cell groups, constitute the reward system of the mammalian brain (Baxter and Murray, 2002; Holland and Gallagher, 2004; Schultz, 2004). Therefore, the amygdala is involved not only in the expression of negative emotions (fear, anxiety, aversion) and related learning (conditioned fear and anxiety), but also in positive emotions (reward, attraction, and appetitive behaviors; Kelley, 2004) and related learning (goal-directed behavior through stimulus–reward associations).

In agreement with the current view of homologies between the mammalian and avian brains, lesions of the Acb in birds give rise to a mismanagement of effort economy leading to impulsive behavior when a short delay separates the instrumental response from reward acquisition (Izawa *et al.*, 2001, 2003), or to a complete inability to work for a reward when a long delay is imposed (Kalenscher *et al.*, 2003). To our knowledge there are no data on that issue in any other nonmammalian vertebrate, but the available data in birds suggest that the ventral striatal output of the amygdala of nonmammals, like its mammalian counterpart, is involved in reward expectation and in the generation of behavior using this reward as a goal. This involves not just detecting the reward but also a learning to respond to cues predicting the reward.

2.14.8.1.2 The roles of the medial extended amygdala The second system of the mammalian amygdala is mainly composed of the secondary vomeronasal centers, since it includes the vomeronasal cortex (COApm) and the medial EA, plus a

deep pallial nucleus, the AHA. Their pattern of connections with the hypothalamus and the septohippocampal system, as well as the presence of receptors to sexual steroids in most of the centers of this circuit, suggests that this system is involved in the control of reproductive and agonistic behaviors elicited by conspecific chemical signals (odorants and/or pheromones). In addition, there is compelling evidence of a role for the medial amygdala of mammals in defensive reactions to some predators. The neural basis of both functions is discussed below.

2.14.8.1.2.(i) The medial amygdala and reproductive function The medial EA of mammals, together with the COApm and AHA, are surely activated by vomeronasally detected chemical signals from conspecifics of the same or the other gender. Studies carried out in rodents reveal that these pheromones elicit neuroendocrine changes in conspecifics (e.g., Whitten, Vanderbergh, Bruce, and Lee-Boot effects: Halpern, 1987). In addition, vomeronasal organ-detected pheromones elicit behavioral responses that include agonistic/territorial ones (intermale aggression, territorial countermarking), attraction as well as facilitation of courtship and sexual behaviors, including paracopulatory (e.g., vocalizations) and mounting/lordosis (Halpern and Martinez-Marcos, 2003).

The neural mechanisms of the neuroendocrine responses to pheromones (Bronson and Whitten, 1968) or mating (mating-induced ovulation in females; Bakker *et al.*, 2001), probably involve interactions of the medial EA with gonadotropin-releasing hormone-expressing cells of the rostral medial preoptic region (Swanson, 1987). Nevertheless, the whole system of hypothalamic projections of the medial EA seems to be necessary, since lesions of the ventral premammillary nucleus block pheromone-induced ovulation (Beltramino and Taleisnik, 1985). Thus, in many mammalian species exposure of females to male pheromones induces a luteinizing hormone surge mediated by the vomeronasal organ (Beltramino and Taleisnik, 1983) that induces ovulation. A similar effect is found in males in response to female pheromones (Coquelin *et al.*, 1984; Fernandez-Fewell and Meredith, 1998), accompanied by *c-fos* activation in medial preoptic cells (Fewell and Meredith, 2002). In addition, electrical stimulation of the vomeronasal organ (probably involved in pheromone detection (Halem *et al.*, 1999; see The Evolution of the Vomeronasal System) in hamsters leads to activation of the luteinizing hormone-releasing hormone population of the preoptic area (Meredith and Fewell, 2001). In other species, such as ferrets, ovulation is not spontaneous but it is induced by mating (Carroll *et al.*, 1985). In agreement with this, in most studied mammals mating leads to *c-fos* activation in the luteinizing hormone-releasing hormone-expressing preoptic cells (Fernandez-Fewell and Meredith, 1994; Wersinger and Baum, 1996; Pfaus and Heeb, 1997; Bakker *et al.*, 2001; Meredith and Fewell, 2001), but also of the medial EA. A similar pathway may account for the acceleration or delay of puberty in prepubertal females by conspecific chemosignals. In this respect, it has been shown that vasopressinergic inputs to the gonadotropin-releasing hormone cells, most of which might arise from the medial EA (see above), are important for the modulation of luteinizing hormone surges (Dobson *et al.*, 2003).

As noted above, the medial EA is interconnected with a series of forebrain centers that are involved in reproductive behavior. These include several nuclei of the medial hypothalamus, such as the medial preoptic nucleus, the tuberal nucleus, the ventrolateral aspect of the VMH, and the PMv (Canteras, 2002). Within the cerebral hemispheres, the remaining vomeronasal amygdaloid nuclei (COApm and AHA), together with parts of the septohippocampal system, including the ventral aspect of the lateral septum, are also part of this circuit. The principal outputs of this circuit are the medial preoptic nucleus and VMH, which are involved in the control of sexual behavior of males (Hull *et al.*, 2002) and females (Blaustein and Erskine, 2002) respectively. In agreement with this, most of the nuclei conforming this circuit are sexually dimorphic and express receptors to sexual steroids that very likely mediate the modulatory effects of steroid hormones on copulation, territorial aggression, and other forms of reproductive and agonistic behavior. Therefore, the medial EA, COApm, and AHA are in a good position to facilitate innate agonistic or reproductive behavioral responses (Kollack-Walker and Newman, 1995) to pheromones and other chemical cues from conspecifics.

Functional studies of the counterparts for the medial amygdala and/or AHA of nonmammalian vertebrates are restricted to birds. Thus, lesions of the TnA in male quails (Thompson *et al.*, 1998) result in an impairment of copulatory and paracopulatory behaviors, including courtship vocalizations, thus reinforcing the view that it is homologous to parts of the medial amygdala of mammals. Similar lesions in other avian species, such as ring doves and starlings (Cheng *et al.*, 1999), lead to changes in social behavior such as increased cooing in female doves, interpreted by the

authors as an indifference to concurrent male attacks, and social detachment and lack of social inhibitions in starlings.

The connections from the medial EA to the lateral septum seem to be fundamental to modulate agonistic behavior. Using different bird species, Goodson and collaborators (Goodson, 1998a, 1998b; Goodson and Adkins-Regan, 1999; Goodson *et al.*, 2004) have shown that vasotocinergic (and vasoactive intestinal peptidergic) innervation of the lateral septum, presumably arising from the medial EA (see above), modulates mate competition, aggression, and territorial down song but not courtship. A role for vasotocin in modulating agonistic behavior has also been shown in mammals, amphibians, and fish, thus suggesting that this circuit has a long evolutionary history with a well-conserved role (Goodson and Bass, 2001).

2.14.8.1.2.(ii) The medial amygdala: Defensive behavior and predator-elicited fear Besides a role in modulating conspecific-related behaviors, the medial amygdala of mammals seems to mediate innate fear to chemical cues derived from common predators, such as cats or foxes (Dielenberg and McGregor, 2001). Confrontation of a rat to cat fur or to a chemical derived from fox feces (2,5-dihydro-2,4,5-trimethylthiazoline, TMT) innately provokes endocrine (increased corticosterone and adrenocorticotropic hormone levels), vegetative (increased arterial pressure) and behavioral components of fear (freezing in some conditions, and escape or hiding if this is allowed; Rosen, 2004).

Although the vomeronasal organ is presumed to be involved in the detection of pheromones (by definition, secreted by conspecifics), there is experimental evidence suggesting that some of the predator-related substances that elicit innate fear are also detected by the vomeronasal organ. Thus, rats do not display fear (increased hiding) if a worn cat collar is present but a mesh wire avoids direct contact with it (Dielenberg and McGregor, 1999), thus indicating that the cat-derived fear-eliciting substance is not volatile, as shown for some vomeronasal organ-detected conspecific pheromones (Moncho-Bogani *et al.*, 2002, 2005; Luo *et al.*, 2003). In agreement with this, cat-derived chemicals induce *c-fos* (Dielenberg and McGregor, 2001) in the medial EA, including the MeP (especially the MePV) and parts of the BST. In addition, the forebrain defensive circuit (Canteras, 2002) seems strongly activated: the ventral lateral septum, the anterior hypothalamus, the dorsomedial aspect of the VMH, and the dorsal premammillary nucleus. Surprisingly, despite the clear signs of fear displayed by the rats studied by Dielenberg and McGregor (2001) when confronted with cat-derived chemicals, neither their central nor their basolateral amygdala was activated by these stimuli. This led the authors to suggest that the medial amygdala is responsible for unconditioned fear, whereas the central/basolateral amygdala is just involved in the expression of conditioned fear (as discussed above; Rosen, 2004). This is partially confirmed by the effects of lesions of the medial or central amygdala on fear elicited by cat odors in rats (Li *et al.*, 2004). The results indicate that the medial but not the central amygdala is involved in generating fear of cat-derived chemicals in rats. A role of the medial amygdala in fear and stress induced by other stimuli (such as acute restraint or footshock) is also possible (Pezzone *et al.*, 1992; Rosen *et al.*, 1998; Dayas *et al.*, 1999; Kubo *et al.*, 2004), although this might be a strain-specific trait (Ma and Morilak, 2004) and the circuitry involved is unknown.

The expression of *c-fos* induced by TMT has recently been studied (Day *et al.*, 2004) and the pattern of cerebral activation differs from that of cat-derived chemicals. Thus, although in both cases the defensive forebrain circuitry is activated, TMT only activates the MeA, but not the MeP. In addition, TMT elicits a strong activation of the central amygdala. These differences can be attributed to the sensory organ used to detect TMT and the cat-related fear-eliciting chemical. Whereas the cat-derived chemical seems a vomeronasal organ-detected stimulus (Dielenberg and McGregor, 2001), TMT is a volatile chemical that displays a strong odor to the human nose, thus it is very likely detected by the main olfactory system. In agreement with this, it strongly activates the granular layer of the main olfactory bulb and those portions of the medial amygdala that show direct inputs from the main olfactory bulb, namely the MeA (see above). Since the MeA projects to the central amygdala, this depicts a circuit for odor-induced unconditioned fear that includes the main olfactory bulb, its direct (and maybe indirect) projections to the MeA and its projection to the CeA (Myers and Rinaman, 2005).

Therefore, the medial amygdala of mammals seems not only involved in coordinating agonistic and reproductive behaviors in response to conspecific pheromones, but also fear/defensive reactions to odors and vomeronasal organ-detected substances secreted by common predators. In other words, the medial amygdala seems the key center to orchestrate innate responses to biologically significant chemicals (pheromones and odorants) derived from conspecifics and predators. Similar functional studies carried out in reptiles suggest that they can also

use chemical stimuli to detect predators and generate anticipatory defensive reactions. Thus, many crotaline snakes display defensive reactions to one of its predators, the kingsnake (*Lampropeltis getula*) which are mediated by the vomeronasal organ (Miller and Gutzke, 1999). Similar functional studies are not available in birds, but since the sign stimuli for detecting predators are not chemical but visual or auditory, the neural circuitry mediating predator-elicited defensive reactions is unlikely to use the medial EA.

2.14.8.1.2.(iii) The amygdala as a functional system In their insightful review of the structure and function of the amygdala, Swanson and Petrovich (1998) proposed that the term 'amygdala' should be abandoned since it is neither a structural nor a functional unit. From a functional viewpoint, they considered the amygdala as composed of portions of the autonomic (central amygdala), chemosensory (cortical, medial, AHA, ABa, Abp, and Bp) and frontotemporal (the rest of the basolateral amygdala, L and Ba) systems of the brain (Figure 23a).

Our review of the available data in different vertebrates reveals, however, that the amygdala is composed of two functional subsystems that, together, control several aspects of behavior and physiology (Figure 23b). The subsystem that has its output through the central EA also includes portions of the basolateral amygdala that Swanson and Petrovich (1998) consider as part of the olfactory amygdala (ABa, ABp, Bp) and a frontotemporal nucleus (L). Therefore, the central/basolateral subsystem of the amniote amygdala encompasses structures belonging to the three functional divisions of the amygdala proposed by Swanson and Petrovich (1998), thus suggesting that a great part of the amygdala does act as a functional system. Concerning the medial subsystem of the amygdala, it is exclusively composed of nuclei belonging to Swanson and Petrovich's olfactory compartment of the amygdala. However, there is evidence that the medial amygdala is involved in the generation of autonomic and/or endocrine stress responses (Dayas *et al.*, 1999; Kubo *et al.*, 2004), so that it could equally be considered as part of the autonomic amygdala together with the central amygdala.

The central/basolateral and medial subsystems of the amygdala, as proposed here, are also interconnected and connected with similar forebrain centers through which they can manage, jointly, important behaviors. Thus, as we have discussed above, the MeA receives not only vomeronasal but also olfactory inputs, and projects to parts of the central EA. This connection provides a vomeronasal and/or olfactory input to the central EA that could mediate fear/anxiety reactions to this kind of stimuli (Day *et al.*, 2004; Myers and Rinaman, 2005). This does not rule out the possibility that parts of the medial EA mediate some fear/anxiety reactions, without involvement of the central EA, to either chemosensory stimuli (Dielenberg and McGregor, 2001; Li *et al.*, 2004) or nonchemosensory stressors (Dayas *et al.*, 1999; Kubo *et al.*, 2004).

In addition to the role described in mediating reproductive, agonistic, and defensive behaviors in response to chemosensory cues, the medial subsystem of the amygdala is surely involved in the appetitive behaviors due to the reinforcing value of sexual pheromones (Moncho-Bogani *et al.*, 2002, 2005). The rewarding value of these pheromones is likely to be mediated by indirect projections from the medial subsystem of the amygdala to the ventral striatum through the central/basolateral subsystem (Moncho-Bogani *et al.*, 2005) and, therefore, these behaviors are dependent on the interconnection between the two amygdaloid subsystems.

Finally, parts of the central/basolateral subsystem of the amygdala, namely the posterior AB, give rise to a strong projection to the defensive system of the hypothalamus (a portion of the pallial stria terminalis), e.g., the anterior hypothalamus and the dorsomedial (core) VMH. Canteras (2002) interprets this as a pathway mediating defensive reactions to nonchemosensory stimuli, namely visual, somatosensory, and auditory ones.

As a conclusion, the functional division proposed by Swanson and Petrovich (1998) does not seem tenable in the light of the evidence reviewed. In contrast, the amygdala is composed of two functional subsystems, named here as central/basolateral and medial, which, together with other forebrain centers, govern emotional behavioral responses to different kinds of stimuli. These include goal-directed behaviors that relay on delayed reward and involve learning (output to the striatum), fear/anxiety/aversion (central EA), and reproductive/agonistic and defensive behaviors. Interactions of both subsystems are needed to accomplish these functions.

2.14.8.2 Evolution of the Emotional Brain: The Amygdala and the Evaluation of Incoming Stimuli

Emotional behaviors can be expressed either in response to intrinsically attractive and reinforcing stimuli, such as sexual pheromones, sweet and

salty taste, or in response to intrinsically aversive/fear-eliciting ones, such as pain or disgusting visceral sensations, chemicals from predators, startling loud noises or lights (Figures 23b and 24). The presence of some direct sensory inputs to the EA and to the striatopallidal telencephalon allows quick, automatic responses to these stimuli. Thus, nociceptive stimuli reach directly parts of the central EA through the CGRP-enriched projection from the parabrachial area and intralaminar thalamus (Gauriau and Bernard, 2002), and this pathway seems to mediate unconditioned fear reactions (Borszcz and Leaton, 2003). In addition, fear/defensive reactions to predator-related chemical stimuli (cats) seem mediated by the vomeronasal/olfactory inputs to the medial amygdala (Li *et al.*, 2004). On the other hand, direct access of rewarding stimuli to the ventral striatum mediates most appetitive behaviors related to natural reinforcers, such as food and water intake, salt and sweet appetite, and sexual behavior (Pecina *et al.*, 2003; Kelley, 2004).

As we have seen, the basolateral amygdala of amniote vertebrates receives convergent afferents from all the sensory systems, but it is evident that not every stimulus reaching the amygdala elicits fear or reward. However, when a neutral stimulus coincides with an attractive or aversive stimulus (US), Pavlovian conditioning occurs and the previously neutral stimulus results in a conditioned response similar to the one provoked by the US with which it is associated. In other words, the animals acquire fear or attraction to previously neutral stimuli that become, in that way, emotionally labeled. The synaptic plasticity in the basolateral amygdala might mediate this kind of Pavlovian conditioning, and the conditioned response is mediated by the palliosubpallial projections within the amygdaloid circuit. Our review indicates that this circuit for emotional behavior and emotional learning is present in all the amniotes studied and, therefore, constitutes one of the defining features of the forebrain.

Although most of the models of emotional conditioning use somatosensory or gustatory stimuli as US and auditory or visual ones as conditioned stimuli, vomeronasal stimuli can play an important role as US. Thus, vomeronasal organ-detected chemicals apparently elicit innate fear/defensive reactions (rat defensive behavior to cat fur; Dielenberg and McGregor, 1999). Studies in several rodents reveal that the innate attraction to possible mates (Moncho-Bogani *et al.*, 2002, 2005) as well as different aspects of copulatory (Beauchamp *et al.*, 1982, 1985; Del Punta *et al.*, 2002; Leypold *et al.*, 2002; Stowers *et al.*, 2002) and paracopulatory behaviors (ultrasonic vocalizations; Wysocki *et al.*, 1982) to mates are mediated by nonvolatile pheromones apparently detected by the vomeronasal organ. In addition, similar stimuli seem to elicit agonistic behaviors such as competitive signaling (Hurst and Beynon, 2004) or intermale aggression (Clancy *et al.*, 1984; Del Punta *et al.*, 2002; Leypold *et al.*, 2002; Stowers *et al.*, 2002).

Dealing with this, it is important to stress that there is a significant olfactory–vomeronasal convergence within the basolateral amygdala of amphibians, reptiles, and mammals (see above), which can mediate olfactory–vomeronasal conditioned learning, thus providing olfactory stimuli with an attractive or aversive significance. (Indeed, neurons in the basolateral amygdala respond to a particular odor depending on whether it was previously paired with a pleasant or unpleasant taste (Schoenbaum *et al.*, 1999). A similar neural mechanism would mediate olfactory–vomeronasal associations.) This kind of associations would confer a predictive value to odors that can be detected from a distance due to their volatility. This allows the animal to anticipate its reactions to pheromones or vomeronasal organ-detected predator signals (which are usually nonvolatile; Wysocki *et al.*, 1980; Dielenberg and McGregor, 1999; Moncho-Bogani *et al.*, 2002, 2005; Luo *et al.*, 2003), thus being able to trail the source of attractive pheromones, or to avoid or flee from repulsive or fear-eliciting ones. This has a huge adaptive value and confers advantage to animals showing this ability.

In our view this kind of association might have constituted the primary role of the pallial amygdala in guiding behavior and was already present, at least, in the ancestral tetrapods. Thus, even in amphibians the medial amygdala (which receives the bulk of the projection from the accessory olfactory bulb) and the LA (which receives an important input from the main olfactory bulb) are interconnected (Moreno and Gonzalez, 2003, 2004). In addition, direct thalamic afferents to the amygdala are scarce but present in amphibians, so that they probably occurred very early in the vertebrate evolutionary history. These pathways might primarily convey information on simple but innately significant stimuli (e.g., pain, visceral sensations, sudden loud noises or lights) that would contribute to the emotional tagging of odors. In this way, the vertebrate amygdala became a neural center involved in the emotional labeling of odors by association with either attractive or fear-eliciting chemosensory (mainly vomeronasal) and nonchemosensory stimuli.

2.14.8.3 The Amygdala and the Evolution of the Pallium in Vertebrates

In all the amniotes studied, the pallial amygdala also receives important afferents from the sensory pallial areas of the telencephalon. There, specialized unimodal regions of the dorsal thalamus convey nonchemosensory stimuli to specific areas of the cortex, where these stimuli are processed by means of complex circuits that include palliopallial excitatory projections and local interneurons mediating feedback and feedforward inhibitory processes. In contrast, amphibians do not possess unimodal thalamocortical sensory pathways (Martinez-Garcia, 2003), but their dorsal thalamic sensory nuclei project to the striatum (Endepols *et al*., 2004; see Evolution of the Amphibian Nervous System). Therefore, sensory cortical areas first appeared in amniotes, where they are represented by the anterior DVR of reptiles and birds (entopallium, nucleus basorostralis of the pallium, field L of the nidopallium), the visual and somatomotor Wulst of the avian hyperpallium, and the sensory isocortex of mammals (Figure 24). The appearance of these cortical sensory areas in amniotes was accompanied by a differentiation within the pallial amygdala of superficial cortical areas receiving direct olfactory inputs, and deep nuclear territories engaged in interconnections with the nonolfactory sensory cortex, e.g., a true basolateral amygdala.

Processing information about the environment prior to conveying it to the amygdala would have been strongly selected during the evolutionary history of amniote vertebrates, since it allowed the animals to react differently to stimuli with a similar configuration but a different emotional value. This may have resulted in an increase in size and complexity of the sensory telencephalon in all the evolutionary lines of amniotes, a phenomenon that seems to have occurred independently in each line. In therapsid reptiles, leading to mammals, sensory processing mainly took place in the mediodorsal pallium, which developed into a complex isocortex with primary sensory and complex associative regions that provide highly processed sensory information to the amygdala. In sauropsids, this sensory processing occurred mainly in the ventral and lateral pallial territories, thus resulting in the development of the DVR, which, at least in birds, includes associative areas that project to the amygdala.

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2.15 The Evolution of Vertebrate Eyes

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Glossary

archaea	Also called archaeobacteria, these are genetically and metabolically different from all other known bacteria. They appear to be living fossils, survivors of an ancient group of organisms that bridged the gap in evolution between bacteria and eukaryotes, the multicellular organisms.
Cambrian	The first period of the Paleozoic era in geology, characterized by desert land areas, warm seas, and rapid early diversification of marine life resulting in the rise of almost all modern animal phyla.
cryptochrome	Photosensory receptors mediating light regulation of growth and development in plants; recently found in animals.
eye	An organ that can produce an image by comparing the light intensities/wavelengths coming from different directions.
metazoan	Multicellular organism.
phenotype	The observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences.
phylogeny	The evolutionary development and history of a species or higher taxonomic grouping of organs or organisms.
rhodopsin	The pigment sensitive to light in vertebrate retinal rods of the eyes, consisting of the seven transmembrane domain protein, opsin, and retinal.

2.15.1 Introduction

Light from the sun carries energy essential for all life on earth and has been a profound selective force, driving the evolution of cellular and molecular processes that harvest the sun's energy. Light is also the premier source of information for many species, and this selective pressure led to the evolution of light-sensing organs, including eyes, that harvest information carried by light. Basically, from the beginning of biological evolution on our planet over 5 billion years ago, sunlight has both fueled and informed life. Light, and the light/dark cycle from our rotating planet are arguably second only to sex as the most important selective forces ever to act on biological organisms. Energy and information are also essential inside cells in the form of DNA and mitochondria and, similar to light, they have an evolutionary history essential to life. One of the most remarkable consequences of light on earth has been the evolution of mechanisms that convert photons not only into energy but also into signals useful to organisms. The evolution of eyes and other structures that collect and use light to represent information from incoming photons has left a remarkable evolutionary trail. And in understanding the genetic, biochemical, and structural remnants of eye evolution, one must follow Ernst Mayr's dictum: "evolution is an affair of phenotypes." Nowhere is this more evident than in the varieties of eyes and the diversity of mechanisms to convert photons into energy useful to the owners of those eyes.

How did eyes evolve? Darwin, the great English naturalist who first brought the systematic

explanatory power of evolution to bear on the bewildering biological complexity of our planet, felt that eyes offered a special challenge to evolutionary thinking because they are such "... organs of extreme perfection and complication ..." (1859). He was quite explicit on this point, saying "... that the eye ... could have been formed by natural selection seems, I freely confess, absurd in the highest possible degree." Although this is most often cited in relation to Darwin's thinking on eyes, he also wrote:

> Reason tells me, that if numerous gradations from a simple and imperfect eye to one complex and perfect can be shown to exist, each grade being useful to its possessor, as is certainly the case; if further, the eye ever varies and the variations be inherited, as is likewise certainly the case; and if such variations should be useful to any animal under changing conditions of life, then the difficulty of believing that a perfect and complex eye could be formed by natural selection, though insuperable by our imagination, should not be considered as subversive of the theory.

Indeed, there are features of eye evolution that challenge the imagination, but we are coming closer to a fuller understanding of how eyes evolved.

More than a century later, new discoveries and new insights that reach from molecular to macroscopic levels of analysis reinforce Darwin's prescient writing. Although we still have much to learn from the evolution of eyes, both about the existing eyes as well as the processes of evolution that produced them, several new findings have guided our understanding about the origins of eyes (see The Role of Vision in the Origin and Evolution of Primates).

Excitement about eye evolution comes from discoveries across the spectrum of biological investigation. Molecular biologists who seek fundamental similarities among organisms have found some clusters of genes implicated in eye development that are conserved in eyes across large phylogenetic divides. We also now know that vertebrate genomes contain nearly twice as many genes encoding light-transducing opsin proteins as were once thought to be present. Moreover, physiologists have identified two fundamentally different kinds of eye phenotypes in single organisms. In fact, within the eye of at least one vertebrate, there are now known to be two fundamentally different kinds of phototransduction, each apparently serving separate but overlapping functions. Evolutionary biologists interested in understanding why organisms and their parts are so different have found new types of eyes, both in the fossil record and in living animals. What do these different approaches to the evolution of eyes tell us? Together they offer complementary views of eye evolution and possibly the beginnings of a clearer story about how and how often eyes arose during evolution.

2.15.2 Eye Variation: Structural and Functional Adaptations

2.15.2.1 Adaptations to General Constraints

In his monumental book, Walls (1942) provided remarkable insights into all aspects of the vertebrate eye. Moreover, this classic has numerous illustrations, many drawn by Walls himself, with details about the range and variety of vertebrate eye phenotypes. Indeed, the variety of eyes is astonishing, reflecting the range of adaptations produced by selective pressures for vision in different habitats. There are many features common to all eyes, however, which are a consequence of fundamental physical constraints on their construction. Since eyes collect and focus light, their structure ultimately depends on the physical properties of light, which set limits on the optical features of eyes. For example, eyes have evolved to be sensitive within a narrow range of wavelengths, relative to the broad spectrum of energy produced by sunlight (see Figure 1). This is most likely due to the fact that early evolution occurred in water, which strongly filters light (Fernald, 1988). Selection for biochemical mechanisms sensitive to this limited range of wavelengths predisposed the sensitivity that emerged during subsequent evolution. Even though many species long since moved onto land where they

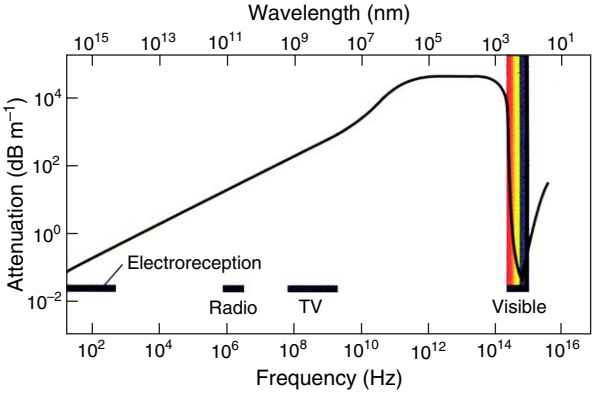

Figure 1 The attenuation (decibels/meter) of electromagnetic (EM) radiation in water as a function of wavelength (nm) and frequency (Hz). This illustrates that attenuation of EM radiation by water is generally quite high except for two ranges: under 10^3 Hz and from 10^{14} to 10^{15} Hz. This accounts for the usefulness of low-frequency signaling and electroreception to weakly electric fish and to the range of frequencies we now term visible light. The band of EM radiation we now consider visible light is transmitted through water with an attenuation six orders of magnitude lower than that of adjacent wavelengths. Redrawn from Fernald, R. D. 1988. Aquatic adaptations in fish eyes. In: Sensory Biology of Aquatic Animals (*eds.* J. Atema, R. R. Fay, A. N. Popper, and W. N. Tavolga), pp. 185–208. Springer.

are exposed to the broader spectrum of electromagnetic radiation from the sun, most animal eyes remain limited to seeing within the narrow band. However, insects and some species of fish and birds later evolved additional receptor types for ultraviolet (UV) light (e.g., Viltala *et al.*, 1995). Thus, the narrow range of wavelength sensitivity is a residual reflection of our aquatic origins and illustrates how early evolutionary solutions persist in the evolved organs.

Of the approximately 33 animal phyla, about one-third have no specialized organ for detecting light, one-third have light-sensitive organs, and the remaining third are animals with what we would consider eyes (Land and Nilsson, 2002). Image-forming eyes appeared in 6 of the 33 extant metazoan phyla (Cnidaria, Mollusca, Annelida, Onychophora, Arthropoda, and Chordata), and these 6 contribute about 96% of the known species alive today (Land and Fernald, 1992), suggesting that eyes are, indeed, useful. Existing eyes have many shapes and sizes, reflecting the diverse solutions to the problem of obtaining an image. Eyes can range in size from a fraction of a millimeter to tens of centimeters in diameter. The range of eye types, sizes, and locations suggests that they can evolve relatively easily (see below).

2.15.2.2 Optical Systems of Eyes

Eye optical systems fall into three classes based on their image-forming mechanisms: images formed via shadows, images formed via refraction (e.g., lens and/or cornea), and images formed via reflection. These different optical types were first systematically analyzed by Land (1981), who has contributed significantly to our understanding of eyes and particularly their optical function. The physical laws governing the behavior of light are well known and these fundamentally limit how an eye can be formed, whether it produces an image, records the direction of incident light, or simply the presence of light. For this reason, similar structures have arisen in distinctly unrelated animals such as fishes and cephalopods. The chambered or camera eyes in these two lineages are similar in a large number of details, even though their owners are phylogenetically distant (Packard, 1972). Both evolved spherical lenses to achieve sufficient refractive power for focusing light underwater, but the inverted retinal layers of fishes (and all vertebrates) are distinctly different from the noninverted, somewhat simpler retinas of cephalopods. Macroscopically, these eye types and the animals bearing them are not homologous, even though there are striking similarities and even homologies at the molecular and developmental levels, which are at the heart of understanding eye evolution.

The major optical types of eyes (Figure 2) consist of systems that detect shadows, refraction, and reflection. This range of eye types reveals that a limited number of optical solutions actually have persisted in organisms.

The greatest variety of eyes exists among invertebrates. These animals have both camera eyes (e.g., cephalopods) and compound eyes (e.g., *Drosophila*). Moreover, invertebrates also have the greatest variety of eyes as regards number and location on given species. Whereas vertebrates settled on paired, chambered eyes with lenses on the head, invertebrate species may have multiple, nonpaired eyes and eyes in remarkable locations. For example, certain butterflies have light detecting organs located such that darkness signals successful copulation (Arikawa *et al.*, 1996a, 1996b). In addition, Nilsson and colleagues (Nordström *et al.*, 2003) recently described a visual system in the planula of a box jellyfish *Tripedalia cystophora*, with eyecups directly connected to motor cilium. In this case, there is no nervous system to process visual information because the eyes are a complete sensorimotor system unto themselves.

While primitive eyes provide information about intensity and possibly the direction of a light source, more advanced eyes also inform their owners about wavelength and contrast and can provide high-resolution images of an illuminated scene via the concentration of cone photoreceptors in one area such as the fovea in many vertebrates.

There is great variation in the capacities of eyes depending on development and ultimately their structure. For example, resolution of an image, as measured in subtended degrees, differs by approximately 13-fold among vertebrates and even more between vertebrates and invertebrates. Eagles have the greatest acuity that is around 10 000-fold greater than that found in planaria (Land and Nilsson, 2002). Similarly, a comparison of relative sensitivities among vertebrates reveals a range of 4×10^5 between highly sensitive deep-sea animal vision and human foveal vision (Land and Nilsson, 2002).

Another remarkable adaptation is differential wavelength sensitivity of photoreceptor types resulting in the ability to distinguish colors (see The Comparative Biology of Photopigments and Color Vision in Primates, Evolution of Color Vision and Visual Pigments in Invertebrates). The selective pressures for evolution of such wavelength discrimination appear to have been quite pervasive. Very likely the added value of better

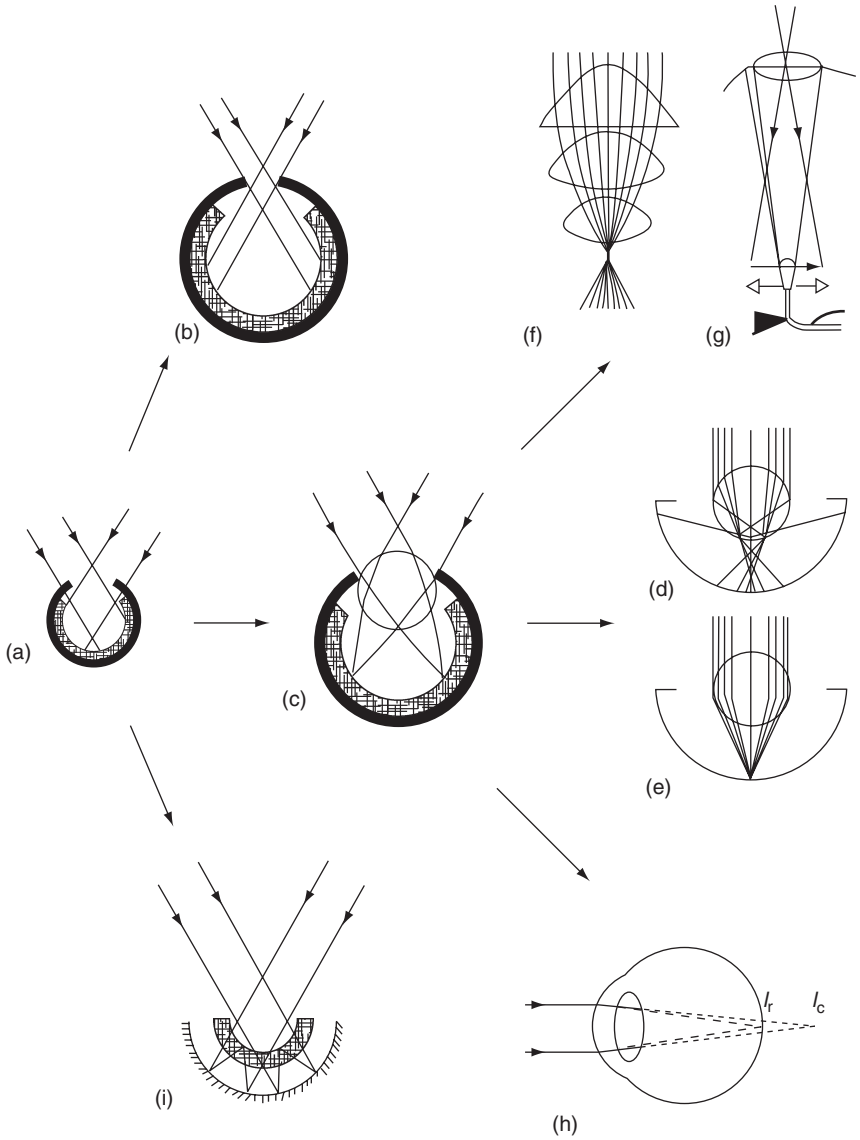

Figure 2 Likely evolutionary sequence of single-chambered eyes. Arrows indicate functional developments, not specific evolutionary pathways. a, Pit eye, common throughout the lower phyla. b, Pinhole of *Haliotis* or *Nautilus*. c, Eye with a lens. d, Eye with homogeneous lens, showing failure to focus. e, Eye with lens having a gradient of refractive index. f, Multiple-lens eye of male *Pontella*. g, Two-lens eye of *Copilia*. Solid arrow shows image position and open arrow the movement of the second lens. h, Terrestrial eye of *Homo sapiens* with cornea and lens; I_c, image formed by cornea alone; I_r, final image on the retina. i, Mirror eye of the scallop *Pecten*. Redrawn from Fernald, R. D. 2000. Evolution of eyes. *Curr. Opin. Neurobiol.* 10, 444–450.

contrast discrimination, which increases the likelihood of identifying food, mates, and predators, would have been enhanced with chromatic information (e.g., Nagle and Osorio, 1993; Osorio and Vorobyev, 1996). Indeed, recent work comparing eight primate taxa suggests that trichromatic vision evolved where leaf consumption was critical (Lucas *et al.*, 2003). In support of this idea, many species of diurnal reptiles and birds have colored retinal filters, composed of oil droplets, which appear to have evolved to increase the number of colors that can be discriminated, suggesting selective pressure for improved color vision (Vorobyev, 2003).

2.15.2.3 Lenses: Multiple Protein Types and Gene Sharing

Eyes collect light through an aperture and focus it with a lens onto photoreceptor cells specialized to convert photons into neural signals. Some eyes exist without pupils and even without lenses (*Nautilus*), but eyes that evolved to give their owners a clear view of the environment on a short timescale do

have lenses. Lenses are constructed of tightly packed proteins, so could the composition of lenses yield insight into how eyes evolved?

In vertebrates, lenses are formed from modified epithelial cells and contain high concentrations of soluble proteins, known as crystallins because of their organized packing into arrays. In contrast, in most invertebrates, the lens proteins are secreted by specialized cells of the eye. Recently, lenses of mitochondrial origin have been found in the two pairs of eyes of the parasite *Neoheterocotyle rhinobatidis* (Rohde *et al.*, 1999). Despite their distinct cellular origins, for a lens to function optically, its constituent proteins must be distributed to produce a radial gradient of refractive index that is low at the edge of the lens and high in the center (see Kroeger *et al.*, 1999; Land, 2000). An exact gradient of refractive index is essential for vision in animals living in water but is also found in terrestrial vertebrates and invertebrates. Perhaps most remarkably, cephalopods assemble their spherical lens from two distinct embryological sources, yet manage to produce the required gradient of refractive index (Jagger and Sands, 1999).

Until quite recently, the 10 or so crystalline proteins found in lenses were thought to be unique to lens tissue and were also thought to have evolved for this function. Of the large number of crystallins, alpha and beta-gamma crystallins are indeed specialized lens proteins in vertebrates, related to heat shock protein and schistosome egg antigen, respectively. However, the remaining vertebrate lens proteins are not conserved, but rather comprise a diverse group, many of which are used as enzymes elsewhere in the body. Surprisingly, most of these taxon-specific lens proteins are actually products of the same genes as the enzymes; this double use has been termed gene sharing by Wistow (1993a, 1993b). For example, a crystalline protein in the duck lens was shown to be similar to a metabolic enzyme, argininosuccinate lyase, and the lens protein and metabolic enzyme are encoded by the same gene, not from duplicated genes. Such sharing might possibly have been a prelude to gene duplication. This molecular opportunism is so effective that it has also occurred both in cephalopods (Tomarev and Zinovieva, 1988) and in *Drosophila* (Janssens and Gehring, 1999). One possibility is that since lenses need the production of a relatively large amount of protein, genes that have been upregulated in other tissues might be selected as appropriate.

Perhaps the most remarkable example of a lens from an unusual source is found in the brittlestar (*Ophiocoma wendtii*). These animals form crystal lenses as a part of their skeletal armor from calcite crystals. The crystals, oriented to bring light onto the photoreceptive surfaces in the body, focus the light much as corrective lenses might and effectively concentrate the light by approximately 50 times (Aizenberg *et al.*, 2001).

The common cellular strategy of assembling lenses from diverse proteins seems to be a convergent evolutionary solution that has occurred in many vertebrates independently. The exquisite gradient of the refractive index that evolved in vertebrates and invertebrates alike resulted because it is the only way known for making an optically useful lens. What remains unknown is how such diverse protein species are assembled through folding and organization that preserves key properties of transparency and suitable refractive index gradient along the axis of the lens. The challenge for understanding lens development is to identify the mechanisms responsible for organizing diverse proteins into a functioning lens. This knowledge could provide useful insights into eye evolution from the perspective of lens assembly.

2.15.2.4 Capturing Light: The Opsin/Retinal Solution

Evolution has left its mark in the DNA sequences of the main light-capturing molecule, opsin, in the biochemistry of transduction, and in the association between the active proteins and other molecules essential for phototransduction. Vertebrate visual pigments (opsins) appeared before eyes (Land and Fernald, 1992) and evolved along at least seven lines, diverging from an ancestral type, before teleost fish diverged from other vertebrates (e.g., Hisatomi *et al.*, 1994) and indeed before deuterostomes split from the protostomes (Terakita, 2005), suggesting that a common ancestor had multiple opsin genes. This surmise has been confirmed with recent evidence (see below). That visual pigments evolved along parallel lines following an ancient divergence is widely accepted, though there are some differences in exact interpretation (Okano *et al.*, 1992).

Opsins are seven transmembrane proteins (30–50 kDa) that associate with a nonprotein moiety, the chromophore retinal. Among the approximately 1000 opsin forms that have been described to date, the phylogenetic differences among the seven major groups correspond to specific functional classifications (Figure 3). These classes differ in several ways, including their transduction via different G-proteins. For example, vertebrate and invertebrate photosensitive opsins are heterotrimeric guanine nucleotide-binding protein (G-protein) -coupled receptors that use 11-*cis*-retinal or a close variant as their chromophore. Vertebrate rod and cone opsins signal through

photoreceptor-specific G-proteins called transducins, whereas invertebrate opsins signal through the Gq family of G-proteins. Photo responses are terminated by a combination of phosphorylation of the excited opsin, the binding of arrestin proteins, which is then followed by regeneration of the active chromophore form needed for photosensitivity.

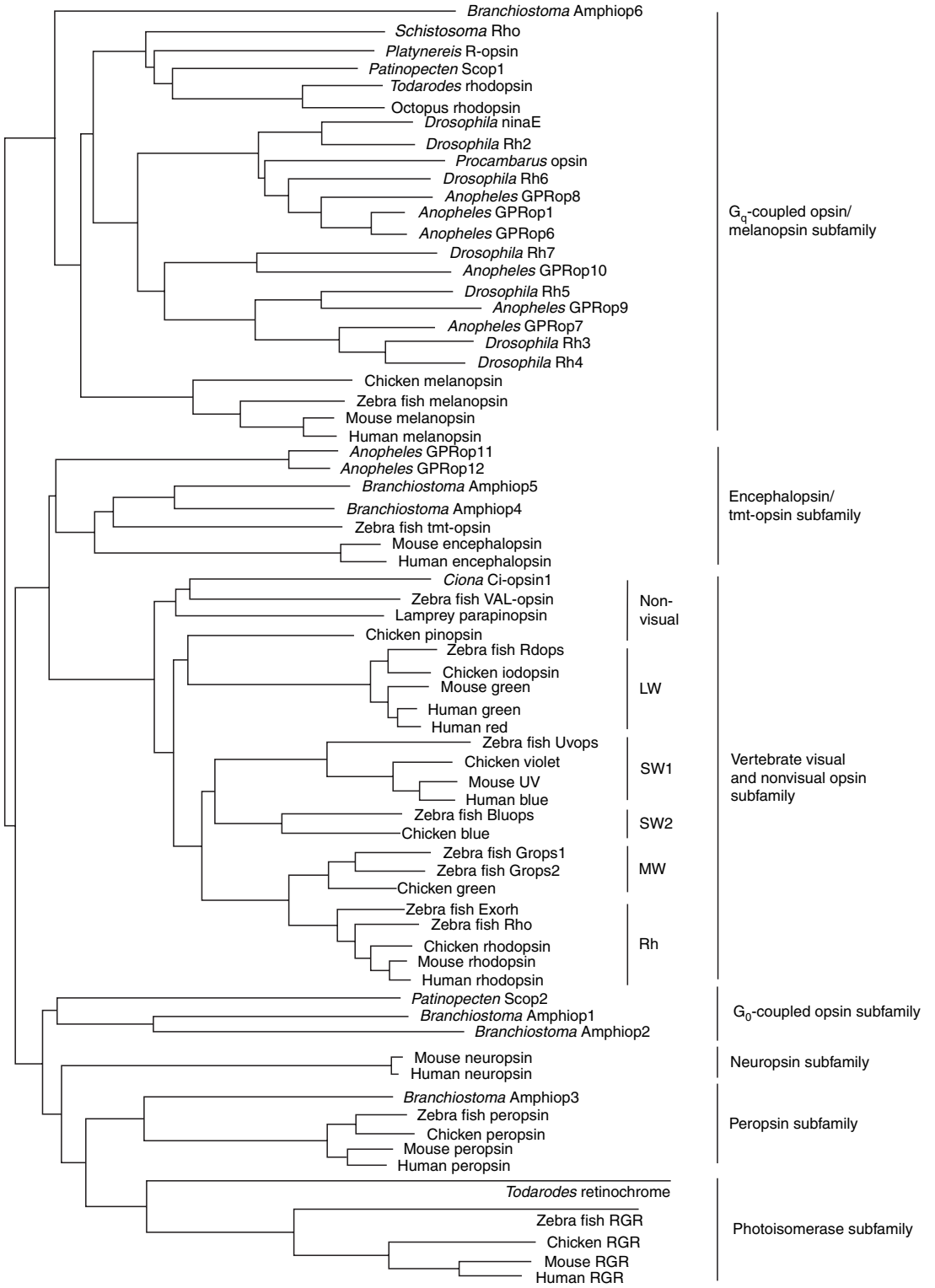

Figure 3 Phylogenetic tree of known opsins. These naturally segregate into seven families. Reprinted from Terakita, A. 2005. The opsins. *Genome Biol.* 6, 213.

A great deal is known about the detailed evolutionary relationships among rhodopsin molecules; some of this based on understanding the interaction between retinal and opsin (Marsh and Griffiths, 2005). Indeed, rhodopsin function is very well understood (e.g., Menon *et al.*, 2001) and the adaptive radiation of pigment types due to natural selection for particular wavelength responses has been described for some special cases (e.g., East African cichlids, Sugawara *et al.*, 2002; squirrelfish, Yokohama and Takenaka, 2004). However, there has been considerable variance in spectral sensitivities that likely resulted from specific selective advantages for one solution over another. Detailed comparisons between terrestrial vertebrates and insects, for example, reveal that there are not unique solutions to encoding both spatial and spectral information. Mammals and bees use long wavelength receptors for luminance and color vision, whereas flies and birds have evolved separate sets of photoreceptors for the two purposes (Orsio and Vorobyev, 2005).

Primate photopigments also offer examples of recent evolutionary change in these important molecules. For example, Old World monkeys, apes, and humans have trichromatic vision, while New World monkeys are polymorphic, having dichromatic or trichromatic color vision (Jacobs, 1996). In this context, *Homo sapiens* may be unique in the polymorphism found in our color vision system (e.g., Neitz *et al.*, 1996). This variance in the number and kinds of photopigments in the human retina might reflect the reduced selective pressure on color vision. The subtlety of selective pressures on chromatic detection can be found in many species. It is particularly evident in the variation within a single species of bluefin killifish, where the relative abundance of cone types depends on whether the animals live in springs or swamps (Fuller *et al.*, 2003). The novel differential spectral sensitivity in these populations is produced through differential expression of cone classes in the retina, rather than via modification of the spectral tuning of opsin molecules, showing that there are different ways to achieve different kinds of chromatic sensitivity.

Another mechanism for temporal modulation of wavelength sensitivity in cone photoreceptors has been described in Pacific salmon (*Oncorhynchus gorbuscha*). As salmon move from being planctivores living in surface waters where UV light is abundant to fish-eating predators in deeper waters where blue-green light prevails, they remodel their UV-sensitive cones with insertion of an opsin that is tuned to blue wavelengths (Cheng and Flamarique, 2004). A similar mechanism has been previously reported in winter flounder (*Pseudopleuronectes americanus*) in which a single opsin type in juveniles, located in hexagonally arranged single cones, is replaced by three different opsin types in photoreceptors arranged in a square array in the metamorphosed adult (Evans and Fernald, 1993; Evans *et al.*, 1993).

These examples show that animals have evolved eyes with resolution, sensitivity, and wavelength detection to match their needs, even as those needs change during their life history. The best understood aspects of visual transduction is that which is used for the main visual input in both vertebrates and invertebrates. The role of the other opsin families is beginning to be understood, although a great deal remains mysterious.

2.15.3 Evolutionary Issues

2.15.3.1 Origins of Eyes

Logically, eyes might be monophyletic, having evolved from a single progenitor, or polyphyletic, having arisen more than once during evolution. Salvini-Plawen and Mayr (1977) compared overall structure, photoreceptor types, developmental origins of eye tissue, position of receptor axons, and other anatomical markers among eyes using current fauna. Based on this analysis, they came to the conclusion that eyes evolved not once but at least 40 different times, and possibly many more (reviewed in Land and Fernald, 1992). This multiple-origins hypothesis, based on morphological evidence, has been challenged more recently by results from molecular experiments. Specifically, Gehring and Ikeo (1999) proposed that because a single, well-conserved master gene, *Pax6*, can initiate eye construction in diverse species, eyes must have arisen from a single ancestor. Did eyes appear many times in the course of evolution making them polyphyletic, as claimed by Salvini-Plawen and Mayr based on phenotype, or have all eyes descended directly from a common, primitive form, making them monophyletic, as claimed by Gehring and Ikeo (1999) based on genes controlling development? Since this original debate erupted, there have been several salient discoveries that suggest eyes arose more than once and we carry the evidence within our own eyes!

By the Cambrian period (570–500 Mya), eyes were present in the form of very simple eyecups, useful for detecting light but not for processing directional information. Although the causes are unknown, explosive speciation, or the big bang of animal evolution, happened during the Cambrian (Conway-Morris, 1998). Existing eye types improved radically, coincident with the appearance of carnivory and predation. The evolution of ocular structures

has proceeded in two stages (Figure 2; Land and Fernald, 1992). First was the production of simple eyespots, which are found in nearly all the major animal groups and contain a small number of receptors in an open cup of screening pigment (Land and Fernald, 1992). This kind of detector cannot play a role in recognizing patterns but rather in distinguishing light from dark. The second stage in eye evolution is the addition of an optical system that can produce an image. Image-forming eyes occur in 96% of known species distributed among six phyla. Among the known eye types are at least 11 distinct optical methods of producing images, the most recently described is a telephoto lens, identified in the chameleon in 1995. Indeed, six of the optical mechanisms have only been discovered in the past 25 years.

Since camera-type eyes are demonstrably superior in several respects (Nilsson, 1989), why do all animals not have them? Certainly, camera-type eyes require big heads and bodies to hold them and this likely restricted the number of animals that have followed this evolutionary path. Also, it is probable that, having evolved one eye type, conversion to another type requires intermediate stages that are much worse or useless compared with the existing design. This would make a switch essentially lethal to animals that depend on sight. Although this argument makes sense intuitively, some existing cases of novel optical combinations suggest this is probably not the whole story.

Textbooks tend to group animal eyes into two groups, the camera-type or simple eyes and the compound eyes, which may be didactically useful since such a dichotomy reflects a real and fundamental difference in optical mechanisms, but it conceals a remarkable diversity of optical systems subsumed under each heading.

For example, Nilsson and Modlin (1994) described a mysid shrimp (*Dioptromysis paucispinous*) that has a combined simple and compound eye: partly compound with multiple facets exactly like the eye of an insect, and partly simple with a single lens focusing an image on a sheet of receptors like that of a human. These shrimp are about 5 mm long with nearly spherical eyes at the ends of stalks. In addition to the facets (approximately 800–900), there is a single giant facet facing the shrimp's tail, which the shrimp frequently rotates forward, probably to get a better look at something since that facet has roughly five times the acuity (but much lower sensitivity) than the rest of the eye. It is as if the shrimp is carrying a pair of binoculars for the occasional detailed look at something ahead of it. The discovery that simple and compound eye types can be found in a single animal raises the question of how a developmental program could produce this outcome.

2.15.3.2 Developmental Evidence of Eye Evolution

Classical experimentation on ocular development focused on vertebrate eyes, a specialized extension of the brain. Experimental models were primarily limited to mice and chicks due to their extensive prior exploitation as model organisms. The beautiful images available today make the often subtle but distinctive morphological changes during eye development seem much more obvious than they were when first observed. With scanning electron microscopy and sophisticated methods of timing the state of tissue development, it is possible to watch unfolding of the production of an eye (e.g., see University of North Carolina website, 'Relevant Website').

Eyes develop from the prospective forebrain, beginning in the eyefields, which are made up of cells of the anterior neural plate. As the prosencephalon grows, this region moves forward until the optic groove forms, and the neuroectoderm of the groove locally contacts the surface ectoderm, inducing the lens placode. As the placode invaginates to form the lens vesicle, the optic vesicle forms the bilayered optic cup, which ultimately becomes the eye. The interaction between the optic vesicle and the lens placode was identified as the organizer of the lens by Spemann (1924). The presumptive lens arises from the lens placode, a thickening of the ectoderm in contact with the optic vesicle. Coincident with this change is the onset of expression of proteins that will form the lens. Other structures of the eye are formed by large- and small-scale tissue movements, caused and accompanied by the expression of tissue-specific genes at that site. The cornea arises from the surface ectoderm over the lens and from migrating mesenchyme derived from the neural crest. Many of the original observations about the role of specific tissue bits in these processes resulted from exquisite embryonic manipulations related to transplantation experiments. For example, Nieuwkoop (1963) identified, among other things, the source tissue essential for the induction of eye production.

With well-described macroscopic change in hand, the next challenge is to synthesize the phenomenological, macroscopic morphological observations with molecular explanations of eye development and understand what this tells us about evolution.

The morphological process of eye development has been viewed as a set of steps toward a final tissue arrangement. Underlying this apparently straightforward sequence of large-scale events, however, are distributions of gene expression with substantial overlap in both time and space. Gene expression is

closely regulated, and specific gene products are used repeatedly, which makes the causal relationships difficult to conceptualize. Nonetheless, progress in characterizing the genes responsible for particular steps in eye development has been reasonably rapid, as shown in several recent reviews (Harland, 2000; Chow and Lang, 2001; Graw, 2003). Functions for at least 15 transcription factors and several signaling molecules have been described in human and mice eyes, based on developmental disorders and/or molecular manipulations (e.g., Graw, 2003). As with other molecular actors, both the transcription factors and signaling molecules are expressed during ocular development and also in a wide range of other tissues. This suggests that the particular combination of expression patterns is important for the proper functioning of these genes in eye development.

As is now well known, the paired box gene 6 (*PAX6*), a member of the family of genes that encode transcription factors with a homeodomain and a paired domain, appears to be important in eye formation across many species. The remarkable demonstration that *PAX6* can induce eyes where they should not be (ectopic) in *Drosophila* (Halder *et al.*, 1995), and similar subsequent demonstration in vertebrates (Chow *et al.*, 1999), led to the suggestion that there might be master control genes responsible for development and differentiation of ocular tissue in many species. Subsequent work has suggested that the term 'master control gene' is a misnomer, however, since a suite of genes is required, collectively, to initiate eye development, and transcription factors are a necessary part of the initiation process. Moreover, as noted above, the genes in question actually have dynamic spatial and temporal expression during many stages of eye development, in addition to expression for essential purposes in other tissues. Nonetheless, it is remarkable that some of the same genes appear in the context of eye development, despite great evolutionary distance among the owners of the eyes. How this might have occurred is discussed below.

For *Drosophila* eyes, it is now known that a collection of seven genes, encoding transcription factors and two signaling molecules collaborate to make eyes (reviewed in Kumar, 2001). These nuclear factors (*eyeless* (*ey*), *twin of eyeless* (*toy*) – both of which are *PAX6* homologues – *sine oculus* (*so*), *eyes absent* (*eya*), *dachshund* (*dac*), *eye gone* (*eyg*), and *optix*), and signaling systems, including the Notch and receptor tyrosine kinase pathways, act via a complex regulatory network that is reasonably well understood (see Kumar, 2001, figure 1). The master gene hypothesis is not supported, because deletion of any of these genes causes loss or radical reduction in the *Drosophila* compound eye and, surprisingly, any gene except *sine oculus*, in collaboration with certain signaling molecules, can cause ectopic expression of an eye in a limited set of imaginal disks. This means that the whole troupe is needed to produce a reasonable eye. Why this might be so is suggested by recent work showing that the *eya* gene products are phosphatases, the first case in which a transcription factor can itself dephosphorylate other proteins to fine-tune gene expression (Li *et al.*, 2003). This elegant work demonstrated the details of interactions among *Six1*, *Dach*, and *Eya* in the formation of the kidney, muscle, and inner ear, as well as eyes, suggesting that this suite of genetically interacting proteins has been recruited repeatedly during evolution for organogenesis of different structures.

It is difficult to abandon the heuristic of hierarchical regulatory processes in development originally proposed by Lewis to characterize homeotic properties of bithorax and antennapedia genes, but molecular analysis of eye development shows that this concept may not be useful in this case. Instead, eye development appears to need new ways of thinking about how complex tissues are made and how such organs arose in evolution. The widespread and redundant activities of specific genes during ocular development (e.g., Chauhan *et al.*, 2002; Baumer *et al.*, 2003) suggest that hierarchies, if they exist, are unknown and the more likely scenario is the orchestrated activity of a suite of molecular actors.

As described above, the diversity of eyes confirms their dynamic evolutionary past. Explosive speciation, or the big bang of animal evolution, occurred during the Cambrian (Conway-Morris, 1998), when existing eye types appear to have improved radically, coincident with the onset of carnivory and predation. Many selective forces were likely at work (Fernald, 2000), including perhaps the first instances where light enabled behavioral signals (Parker, 1998), so no predominant selective force can be claimed. The rapidity of eye evolution has always been a question, but, using a simulation, Nilsson and Pelger (1994) suggested that about 2000 sequential changes could produce a typical image-forming eye from a light-sensitive patch. With reasonable estimates, this suggests that an eye could evolve in less than half a million years, making the virtual explosion of eyes during the Cambrian seem reasonable (Land and Nilsson, 2002). After the Cambrian, three phyla emerged: arthropods, mollusks, and chordates. Although these groups all use the opsin molecule to capture

light, details of the structure and function of their eyes differ considerably.

One of the most interesting developmental differences among extant eyes is the embryonic origin of the different structures in vertebrate and cephalopod eyes (summarized in Nilsson, 1996). Cephalopod eyes form from an epidermal placode through successive infoldings, whereas vertebrate eyes emerge from the neural plate and induce the overlying epidermis to form the lens as described above. It is also noteworthy that the cephalopod eyes lack a cornea, which is present in all vertebrates whether aquatic or not.

In addition to the differences in embryonic origin, photoreceptor cells divide into either ciliary or microvillar structures to provide the membrane surface for the opsin molecule (Salvini-Plawen and Mayr, 1977). Microvilli predominate in invertebrates, whereas vertebrate photoreceptors are ciliary. Physiological responses are also quite different, with the microvillous receptors of arthropods and mollusks depolarizing to light, and the ciliary receptors of vertebrates hyperpolarizing to light. In phototransduction, vertebrate photoreceptors exploit cyclic guanosine 5′-monophosphate (GMP) as a second messenger system, while invertebrates use inositol trisphosphate (Fernald, 2000). And, even though opsin is the key molecule for detecting light, mechanisms for regeneration (e.g., reisomerization) of the chromophore/opsin system are dramatically different among phyla (Gonzalez-Fernandez, 2003).

2.15.3.3 Functional Evidence about Eye Evolution

Until recently, the photodetection systems we understood well were localized primarily to eyes and pineal glands and a few other sites in the body such as the skin. For each of these, a canonical opsin and related transduction cascade were known. Specifically, ciliary structures associated with specific G-proteins are known from vertebrate eyes and microvilli associated with inositol phosphate signaling cascades are known from invertebrate eyes (see above). Then, in several laboratories, each of these phototransduction cascades was found in unexpected organisms. Arendt *et al.* (2004) found that the polychete ragworm (*Platynereis dumerilii*), in addition to the rhadomeric photoreceptors in its eyes, had ciliary photoreceptors in the brain. They also showed that the typical types of opsins associated with each photoreceptor type were both expressed in the ragworm and localized only with that type (e.g., vertebrate c-opsin in the brain and invertebrate r-opsin in the eye). This means that the two main types of eyes exist in a worm.

The idea that two kinds of photoreceptors might exist in a single organism was first suggested by the pioneering work of Gorman, who with colleagues showed physiological and morphological data suggesting that both types of photoreceptors exist in a scallop, *Pecten irradians* (Gorman and McReynolds, 1969, 1971). These investigators found depolarizing and hyperpolarizing responses to light stimuli from cells located in different layers of the scallop retina, with depolarizing potentials arising from the proximal layer and hyperpolarizing potentials form the distal layer. The investigators interpreted their data solely with respect to the various kinds of selective advantages each response type might have but did not consider the evolutionary implications, though their data support the existence of the two canonical receptor types in one organism.

Meanwhile, in vertebrates, a parallel set of results has been appearing. A small population of intrinsically photosensitive retinal ganglion cells have been discovered that play key roles in the regulation of nonvisual photic responses. These rely on melanopsin (see Figure 3), an opsin first identified in vertebrate melanophores, brain, and eyes by Provencio *et al.* (1998). The melanopsin in the retina was soon shown to be in the form of photosensitive ganglion cells (Berson *et al.*, 2002), required for normal light-induced circadian phase shifting (Panda *et al.*, 2002), and yet could not function without normal rods and cones (Ruby *et al.*, 2002), meaning that its signals are combined with those from rods and cones somewhere in the visual system. Photosensitive ganglion cells comprise a non-image-forming system that can detect the presence or absence of light but not much more. Subsequent functional analyses showed that retinal melanopsin functions via a phototransduction cascade that resembles invertebrate opsins and, in another similarity to invertebrates, has intrinsic photoisomerase activity (Panda *et al.*, 2005; Qiu *et al.*, 2005). Adding to the remarkable set of discoveries, melanopsin-expressing ganglion cells in the primate retina have been shown to signal color and radiance levels to the lateral geniculate nucleus (Dacey *et al.*, 2005). So, not only do vertebrates carry a version of the invertebrate visual transduction system with them, but it is used in a variety of ways, including to provide information to the image-forming visual system.

Taken together, these findings show that at least two kinds of photoreception existed in the urbilateria, before the split into three Bilateria branches at the Cambrian (Figure 4), and, importantly, each of these branches still carry versions of

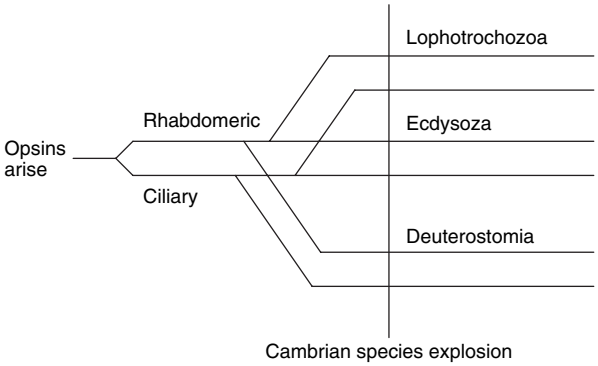

Figure 4 Schematic phylogeny of the Bilateria showing that the distinct rhabdomeric and ciliary organization of opsins preceded the splitting of the urbilateria. Based on Nilsson, D. E. 2005. Photoreceptor evolution: Ancient siblings serve different tasks. *Curr. Biol.* 15, R94–R96.

these two systems. In addition, cryptochromes, also discovered very recently (Cashmore *et al.*, 1999), are another photoreceptive system that is not based on opsin, has no molecular amplification, and is found in both plants and animals. To date, cryptochromes have been shown to play a role in circadian rhythms (Green, 2004) and control of the iris muscle in birds (Tu *et al.*, 2004) as well as many functions in plants. Considering that seven families of opsin have been described in humans (see Figure 3), we can expect more surprises in the detection of light. The additional opsins discovered recently have not yet been functionally characterized, but the evidence suggests that there are no more opsins to be discovered (Kumbalasiri and Provencio, 2005). Even so, figuring out how all the existing opsins work together is a daunting challenge.

2.15.3.4 Parallel Evolutionary Universe?

One of the persistent issues in the evolution of eyes, as noted above, is whether eyes evolved once or many times. Though it seems quite clear that there were at least two kinds of phototransduction (e.g., ciliary and rhabdomeric) before the urbilateria split into three families (see Figure 2), energy and information are harvested in archaea and eukaryotic microbes using a system that clearly arose independently, via convergent evolution. Microbial, or type 1 rhodopsins, named to distinguish them from the visual pigments or type 2 rhodopsins, function to harvest light for energy, to guide phototaxis, and probably many yet undiscovered functions (Spudich *et al.*, 2000). While the number of known type 2 (visual) rhodopsins has increased dramatically over the past several years (see above), the number of known type 1 rhodopsins has rapidly increased with the harvesting and genetic sequencing of ocean samples from a handful to over 800 (Spudich and Jung, 2005). These type 1 rhodopsins are widely dispersed on the planet, found in organisms living in both freshwater and seawater, salt flats, and glacial seas, among others.

There are several fundamental differences between types 1 and 2 rhodopsins. First, there is no evident phylogenetic relationship between the genetic sequences of type 1 and type 2 rhodopsins. As more type 1 opsins are discovered, a connection may become apparent, but given the current state of knowledge, this seems unlikely. Second, the type 1 rhodopsins reveal convergent solutions to the mechanisms for converting photon energy. Both rhodopsin types consist of seven transmembrane domain proteins and, in each, retinal is attached in a Schiff base linkage via a lysine residue in the seventh helix (Spudich *et al.*, 2000). However, type 1 rhodopsin (25–30 kDa) has a different organization of its intramembrane domains from type 2 rhodopsin (35 kDa), which reflects the fundamental difference in their signaling cascades. Whereas type 1 rhodopsins function within the membrane to pump ions or signal to other integral membrane proteins, type 2 rhodopsins signal via G-proteins, receptor kinases via the cytoplasmic loops (see above and Spudich *et al.*, 2000). Retinal is used in association with both apoproteins, but these are photoisomerized quite differently. In the familiar, type 2 rhodopsins, 11-*cis* retinal is transformed to all *trans* upon absorbing light, whereas in type 1 rhodopsins, all *trans* retinal is transformed to 13-*cis* when absorbing light.

Taken together, the remarkable convergence of type 1 and 2 rhodopsins suggests that in the course of evolution, an opsin apoprotein associated with retinal has been discovered and exploited twice. Clearly, when the seven transmembrane protein is appropriately solvated with retinal, it is useful for transforming the energy of photons into more useful forms. This also suggests that progenitors of the type 1 opsins may have existed in earliest evolution before the divergence of archaea, eubacteria, and eukaryotes. This means that the light-driven ion transport mechanism for deriving energy used in association with retinal 1 preceded the evolution of photosynthesis as a means for using the sun's energy (Spudich and Jung, 2005). We can now wonder whether a proto eye-like structure using rhodopsin 1 remains to be found that would allow a comparison of an additional independent solution to extracting information from light.

2.15.4 How Did Eyes Evolve?

Eyes exist in a variety of shapes, sizes, optical designs, and locations on the body, but they all

provide similar information about wavelength and intensity of light to their owners. Different tissues have been recruited to build lenses and retinas across the phyla. In contrast, all eyes share the same mechanism of absorbing photons, i.e., the opsin–chromophore combination has been conserved across phylogeny. Despite new findings yielded by powerful molecular techniques, all evidence still suggests that eyes have a polyphyletic origin, particularly since the discovery that two photodetection systems had evolved prior to the split of the urbilateria into three families. Clearly, eyes as we know them contain homologous molecules responsible for many structural, functional, and even developmental features. Given a growing list of homologous gene sequences among molecules in the eye across vast phylogenetic distances, the challenge is now to discover what makes the eyes of *Drosophila*, squid, and mouse so different. Understanding what makes eyes different may be a bigger challenge than finding what they have in common.

It seems increasingly evident that as eyes evolved, different functional mechanisms have been generated by recruiting existing gene programs. From genome sequencing, we know that there are far fewer genes in organisms than previously thought, so the use and reuse of genes and their products in combinatorial assemblies as reported for known genomes make sense. In the development of eyes, this seems to be the rule not the exception. Specifically, in the evolution of eyes, it seems likely that light sensitivity evolved early in the Cambrian in the form of a proto-opsin molecule in association with the chromophore, retinal. This molecular combination, sensitive to light, became associated with the genes *pax6* (Sheng *et al.*, 1997), and possibly *eya* (based on its phosphatase activity (Li *et al.*, 2003)). One can imagine that this combination was recruited and worked well in early evolved eyespots and other light-sensing organs. It would not be surprising, for example, to find these genetic players in the recently described eye without a nervous system (Nordström *et al.*, 2003). As different eye types evolved over time, there was probably repeated recruitment of particular gene groups, not unlike improvisational groups of actors, interacting to produce candidates for selection. The evolutionary fiddling through which various combinations or routines were tried could have led to numerous parallel evolutionary paths for eyes as we now envisage.

From this, two different mechanisms for transmitting the photic information to surrounding cells were selected for, one in ciliary and one in rhabdomeric photoreceptors. These two systems are likely present in all organisms, as described above for worms and mice. The big surprise is that both of these transduction systems persisted, with each selected as the primary visual system for a major branch of animals. So the answer to the question of whether eyes evolved from a single prototypical eye (monophyletic), or whether they evolved repeatedly (polyphyletic), appears to be that quite evidently eyes arose at least twice and probably many times. And, as described above, given the vast number of organisms using rhodopsin 1, we should not be surprised if additional eyes appear in the biological world in the future.

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2.16 The Evolution of Ultraviolet Vision in Vertebrates

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Glossary

in vitro assay	Visual pigments are constructed by expressing opsins in cultured COS 1 cells, regenerated with 11-*cis*-retinal, and purified by immunoaffinity chromatography. Ultraviolet–visible spectrophotography was used to determine the wavelength of maximum absorption of the resulting visual pigments.
λ_{max}	Wavelength of maximal absorption of a visual pigment.
SWS1 pigments	Short wavelength-sensitive type 1 pigment group; one of the five evolutionarily distinct groups of visual pigments found in the retina that contain UV and violet pigments.
UV pigments	Visual pigments with λ_{max} values of ~360 nm.
violet pigments	Visual pigments with λ_{max} values of ~390–440 nm.
visual pigments	Photo-sensitive molecules, each of which consists of a transmembrane protein, opsin, and the chromophore, either 11-*cis*-retinal or 11-*cis*-3,4-dehydroretinal.

2.16.1 UV Vision and Violet Vision

We receive a broad spectral irradiance from the sun. Most organisms have developed vision systems to utilize energies in the narrow spectral range between 400 and 700 nm (Yokoyama, 2000). By the early 1930s, it was already well recognized that the behavior of a variety of insects was strongly influenced by their ultraviolet (UV) vision, detecting the wavelengths at ~360 nm (Jacobs, 1992). In contrast, a serious survey of UV vision in vertebrates did not start until the late 1980s. Through behavioral analyses, vertebrate species such as swordtail (*Xiphophorus nigrensis*), rainbow trout (*Oncorhynchus mykiss*), anolis species (*Anolis krugi*, *A. cristatellus*, *A. pulchellus*, *A. gundlachi*, *A. evermanni*, *A. cooki*, and *A. cristatellus*), zebra finch (*Taeniopygia guttata*), starling (*Sturnus vulgaris*), blue tits (*Parus caeruleus*), kestrels (*Falco tinnunculus*), hummingbirds (*Archilochus alexandri*, *Lampornis clemenciae*, and *Eugenes fulgens*), cuckoo (*Cuculus solitarius*), and bat (*Glossophaga soricina*) have been shown to use UV vision for foraging, mating, and communication. From these studies and the characterization of UV pigments (see below), it became clear that many fish, amphibian, reptilian, and avian species, as well as some mammalian species, use UV vision (Bennett *et al*., 1996, 1997; Browman and Hawryshyn, 1994; Burkhardt, 1982, 1989; Cherry and Bennett, 2001; Church *et al*., 1998; Cummings *et al*., 2003; Fleishman *et al*., 1993; Goldsmith, 1980; Hunt *et al*., 1999; Koyanagi *et al*., 2004; Leal and Fleishman, 2002; Ma *et al*., 2001; Viitala *et al*., 1995; Winter *et al*., 2003).

UV vision and violet (or blue) vision are mediated by UV- and violet- (or blue-) sensitive visual pigments, which absorb light maximally (λ_{max}) at ~360 and 390–440 nm, respectively (Jacobs, 1992). The UV and violet pigments belong to an evolutionarily distinct short wavelength-sensitive type 1 (SWS1) pigment group (Yokoyama, 2000). The evolution of UV and violet vision in various vertebrates can be studied in two steps; we first examine how UV and violet pigments diverged from each other and then relate the evolution of SWS1 pigments to the ecological and behavioral characteristics of organisms. These analyses will reveal why and how these organisms have acquired, or abandoned, their UV vision.

2.16.2 Visual Pigments

Visual pigments represent a group of G-protein-coupled receptors that are responsible for the

capture of photons and the initiation of visual excitation. The crystal structure of the bovine rhodopsin demonstrates the existence of seven transmembrane (TM) motifs (Palczewski *et al*., 2000; Okada *et al*., 2002). Each visual pigment consists of an apoprotein, opsin, covalently linked to a conjugated polyene chromophore, either 11-*cis*-retinal or 11-*cis*-3,4-dehydroretinal. A visual pigment with 11-*cis*-3,4-dehydroretinal often absorbs longer wavelengths than a pigment with 11-*cis*-retinal. For UV pigments, however, the two chromophores do not cause any appreciable difference in the λ_{max} value. Therefore, the variable λ_{max} values among different SWS1 pigments are generated by the direct and indirect interactions between the chromophore and various types of opsins, which is referred to as the spectral tuning of visual pigments. In many amphibians, birds, and reptiles, the light sensitivity of visual pigments can also be modified by a colored oil droplet in a photoreceptor cell. In this respect, UV pigments are unique because if there are associated oil droplets, they are transparent and UV pigments mediate UV vision (Yokoyama, 2000).

To determine the λ_{max} values of visual pigments experimentally, we first isolated total RNAs from a retina or whole eye. From this RNA, the opsin cDNAs were obtained by reverse transcription-polymerase chain reaction using appropriate primers, subcloned into an expression vector, and expressed in cultured cells. These opsins were incubated with 11-*cis*-retinal in the dark, and the absorption spectra of the resulting visual pigments were recorded using a spectrophotometer. Using this *in vitro* assay, we could also evaluate the effects of virtually any single and multiple amino acid changes on the λ_{max} shift (Yokoyama *et al*., 1998; Wilkie *et al*., 1998).

2.16.3 Ancestral SWS1 Pigments

The evolutionary changes in UV and violet vision in vertebrates can be studied directly by constructing SWS1 pigments of ancestral organisms and tracing their λ_{max} values through time. For this purpose, the phylogenetic tree of 10 representative SWS1 pigments from a wide range of vertebrate species was examined. Based on this tree topology, the amino acid sequences of visual pigments at all ancestral nodes have been inferred by using statistical methods. Then, eight ancestral pigments were engineered by introducing all necessary amino acid changes into selected contemporary pigments and their λ_{max} values were determined using an *in vitro* assay (Shi and Yokoyama, 2003).

The results show that most SWS1 pigments had λ_{max} values of ~360 nm and were UV sensitive; i.e., most ancestral vertebrate species used UV vision (e.g., Figure 1). In Figure 1, the evolutionary changes in the avian lineage are of particular interest; the avian ancestor developed violet vision, but some descendants regained UV vision. Thus, with the exception of the avian UV pigments, contemporary UV pigments inherited their UV sensitivities directly from the vertebrate ancestor, whereas violet pigments evolved from the ancestral UV pigment (Shi and Yokoyama, 2003).

2.16.4 Molecular Bases of UV and Violet Vision

The genetic analyses of UV vision began only in approximately the year 2000 by comparing the SWS1 pigments of zebra finch, budgerigar, canary, chicken, pigeon, and a wide range of other vertebrates. The comparative analysis of their amino acid sequences revealed that the cysteine at site 90 (C90) is restricted to the UV pigments of zebra finch, budgerigar, and canary, whereas all other SWS1 pigments, including those of pigeon and chicken, have serine at the same position (S90). Thus, it was hypothesized that this amino acid difference was responsible for the differentiation of UV and violet pigments. Such hypotheses can be tested rather easily by introducing specific amino acid changes in the visual pigments and evaluating whether or not the mutant pigments cause any λ_{max} shifts. Indeed, the amino acid change S90C in the pigeon pigment shifted the λ_{max} value from 393 to 359 nm and in the chicken pigment it shifted the value from 415 to 369 nm, decreasing the λ_{max} values by some 35–45 nm, whereas the reverse change, C90S, in the zebra finch and budgerigar pigments increased the λ_{max} values by ~40 nm (Yokoyama *et al*., 2000; Wilkie *et al*., 2000; Dukkipati *et al*., 2001; Table 1). Therefore, UV and violet sensitivities in birds can be interchanged by one amino acid change.

When these analyses were extended to mammalian pigments, things suddenly became complicated. For example, the mouse and human pigments have λ_{max} values of 359 and 414 nm, respectively (Figure 1). By constructing a series of chimeric pigments between these two pigments, the cause of their λ_{max} difference was traced to 19 amino acid sites in TM helices I–III. Surprisingly, none of these mutations caused any λ_{max} shift individually (Yokoyama and Shi, 2000; Shi *et al*., 2001; Table 1). By constructing additional chimeric pigments and conducting extensive mutagenesis analyses, it was shown that seven amino acid replacements, F46T/F49L/T52F/F86L/T93P/A114G/S118T, were responsible for the functional differentiation of

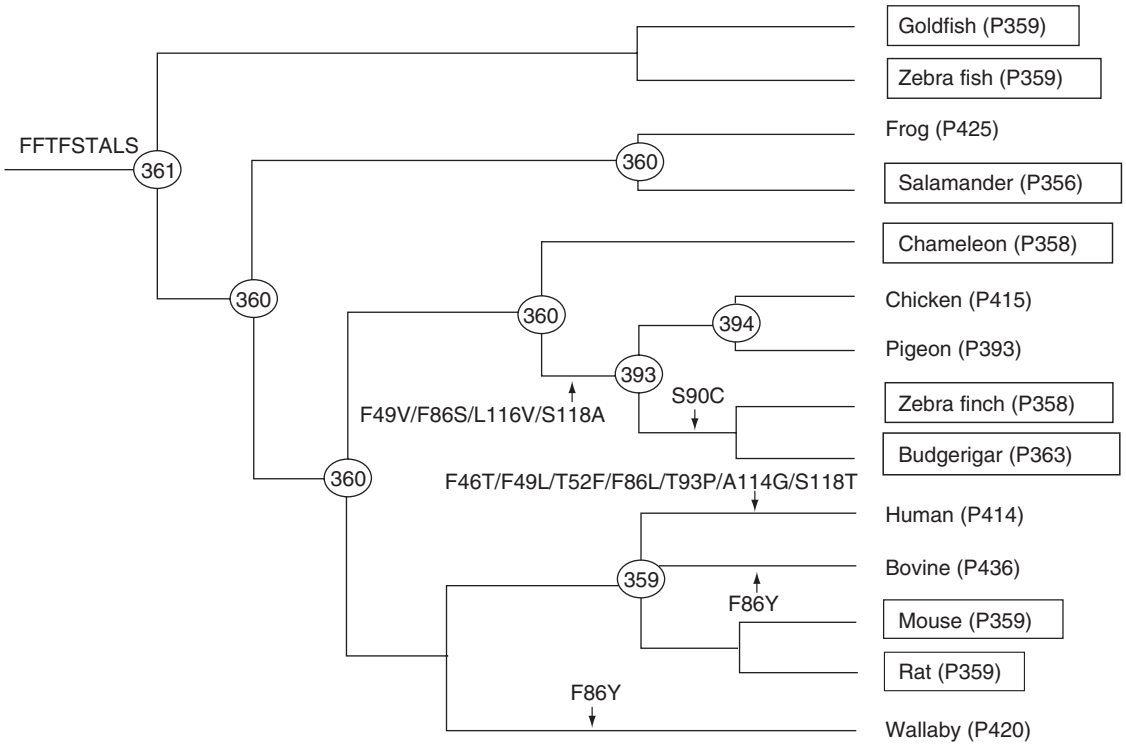

Figure 1 An evolutionary tree of 14 SWS1 pigments. FFTFSTALS are the amino acids at the critical sites 46, 49, 52, 86, 90, 93, 114, 116, and 118 for the ancestral pigment. The UV pigments are shown in rectangles. The numbers after P refer to λ_{max} values. Species considered are goldfish (*Carassius auratus*), zebra fish (*Danio rerio*), frog (*Xenopus laevis*), salamander (*Ambystoma triginum*), chameleon (*Anolis carolinensis*), chicken (*Gallus gallus*), pigeon (*Columba livia*), zebra finch (*Taeniopygia guttata*), budgerigar (*Melopsittacus undulates*), human (*Homo sapiens*), bovine (*Bos taurus*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), and wallaby (*Macropus eugenii*).

Table 1 Representative amino acid changes that cause significant spectral shifts

Pigment	*Mutation*	λ_{max} *shift* (nm)
Mouse (P359)	F46T, F49L, T52F, F86L, T93P, A114G, S118T	0
Mouse (P359)	F46T/F49L/T52F/F86L/T93P/A114G/S118T	+51
Mouse (P359)	F86S	+17
Mouse (P359)	F86Y	+66
Wallaby (P420)	Y86F	−59
Mouse (P359)	S90C	−3
Pigeon (P393)	S90C	−34
Chicken (P415)	S90C	−46
Zebra fish (P359)	C90S	+38

the human pigment (Yokoyama and Shi, 2000; Shi *et al*., 2001; Table 1). For the bovine SWS1 pigment, however, F86Y explained the majority of its λ_{max} shift (Fasick *et al*., 2002; see also Cowing *et al*., 2002; Table 1). Curiously, the wallaby pigment also achieved its present λ_{max} by F86Y independently; the reverse mutation, Y86F, in the wallaby pigment decreased the λ_{max} value by 59nm (Figure 2). The effect of F86Y exhibited a stark contrast to that of F86L at the same site in the human pigment (Table 1). Despite the significant contribution of F86Y in the differentiation of these violet pigments, the total λ_{max} shift must be explained by complex amino acid interactions at several critical sites. The details of such interactions remain to be elucidated.

We have seen that S90C decreased the λ_{max} values of the avian pigment by ~50nm. However, when the same mutation was introduced into the mouse pigment, the mutant pigment caused virtually no λ_{max} shift (Shi *et al*., 2001; Table 1), again showing strong synergistic interactions among different amino acids in the UV pigment. To understand such interactions at the critical sites, extensive mutagenesis experiments were required. Furthermore, additional mutagenesis analyses of the engineered ancestral avian pigments showed that L116V is

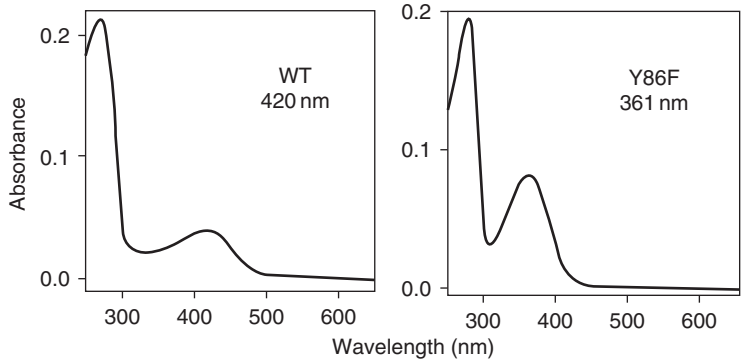

Figure 2 Absorption spectra of wallaby SWS1 pigment. WT, wild-type pigment; Y86F, mutant pigment with Y86F.

also involved in the evolution of the violet pigment. Thus far, therefore, a total of nine amino acid sites have been shown to be involved in the spectral tuning of SWS1 pigments. As more SWS1 pigments are analyzed, this number is expected to increase.

2.16.5 Ecology and Physiology of UV Vision

UV vision is useful for organisms only when they are exposed to UV light. Thus, knowing that many diurnal animals, living under ample UV exposure, abandoned their UV vision, it is not immediately clear why nocturnal rodents, such as mouse and rat, have maintained their UV vision. For example, voles (*Microtus agrestis*) mark their runaways with urine and feces, which can be detected more easily under UV light than under visible light (Viitala *et al.*, 1995). To understand the maintenance of UV vision in these rodents, it seems important to realize that a high proportion of light available at approximately dawn and dusk is of short wavelength. Therefore, these and other nocturnal animals that are active at these times of the day can perform a wide range of tasks better using UV vision. Similarly, the restoration of UV vision in some avian species might have occurred because they migrate and use UV vision as the 'sun compass'.

Since many species have switched from UV vision to violet vision, the advantage of organisms having UV vision must be limited to certain circumstances. We can identify at least three major reasons why so many species abandoned UV vision. First, UV vision is not useful when organisms do not receive UV light in their habitats. Under such conditions, organisms will probably abandon UV vision. For example, living at the depth of 200 m, coelacanths (*Latimeria chalumnae*) receive only a narrow range of light at approximately 480 nm (Yokoyama, 2000). In this species, both UV pigments and longer wavelength-sensitive pigments have become nonfunctional. It should also be noted that many fish species have UV vision, but this does not mean that they use UV vision throughout their entire lives. In fact, UV vision in many fishes declines as they grow older. This change in gene expression makes sense because young fishes may live in shallow water and feed on plankton, where UV light is essential, whereas adults live in deeper water and do not receive much UV light (e.g., Browman and Hawryshyn, 1994). Second, UV light can damage retinal tissues. To prevent such injuries, yellow pigments in the lenses or corneas in human and many other species are devised to absorb most UV light before it reaches the retina. This makes UV vision unattainable even with UV pigments. Third, by abandoning UV vision, organisms can improve their visual acuity and subtle contrast detection (see The Evolution of Vertebrate Eyes).

2.16.6 Perspectives

The use of UV vision is strongly associated with the photic environment and behavior of organisms. Compared with organisms with violet vision, those with UV vision have the advantage of recognizing certain UV-reflecting objects much more quickly, but they lack acuity in viewing their surroundings and are also subjected to a higher chance of developing retinal damage from UV light. Thus, whether organisms use UV vision or violet vision depends on the relative importance of these and other conflicting characteristics associated with UV vision (see The Evolution of Vertebrate Eyes). To appreciate the evolution of UV vision, it is necessary to study the roles of the UV and violet pigments of many species in different photic environments. The molecular basis of the functional differentiation of SWS1 pigments is unique, showing strong synergistic interactions among critical amino acids (Babu *et al.*, 2001; Dukkipati *et al.*, 2002; Fasick *et al.*, 2002; Kusnetzow *et al.*, 2001, 2004; Shi *et al.*, 2001). The nature of these interactions is still poorly understood and remains to be elucidated.

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2.17 Evolution of Vertebrate Olfactory Subsystems

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Glossary

olfactory system	The olfactory system in any animal is the primary sensory system that responds to chemical stimuli emanating from a distant source.
pheromone	A chemical cue that, when released by an individual, elicits specific behavioral or physiological responses from conspecifics.
terminal nerve	The most anterior cranial nerve in vertebrates. The terminal nerve releases compounds into the nasal epithelia, modulating activity of sensory receptor cells.
vomeronasal system	A discrete olfactory subsystem present in tetrapods that differs morphologically from the olfactory system. Its function is unclear.

2.17.1 Introduction

2.17.1.1 What is Olfaction?

The term olfaction is commonly applied to chemosensory systems that detect chemicals emanating from a distant source. Other chemosensory systems generally require physical contact with the source for detection, and this sensory modality is called gustation. The vertebrate olfactory and gustatory systems are anatomically distinct; the latter is discussed in a separate article (Evolution of Taste). Fibers of the trigeminal nerve also detect chemical stimuli, as dochemosensors in the respiratory, circulatory, and digestive systems that detect gasses, ions, and nutrients. In general, these chemosensory systems consist of isolated sensory cells that project to the spinal cord or hindbrain. These systems will not be discussed here.

In this article, we will consider three interrelated olfactory subsystems: the main olfactory system, or olfactory system proper; the vomeronasal system; and the terminal nerve. The main olfactory system comprises receptor neurons located in a specialized sensory epithelium in the nasal cavity, as well as the central projections of these neurons. The receptor cells of the olfactory epithelium develop from the nasal placode, and the ingrowing fibers of the developing sensory neurons are involved in development of the olfactory bulb at the rostral pole of the prospective telencephalon (Gong and Shipley, 1995; Graziadei and Monti-Graziadei, 1992; Long *et al.*, 2003).

The vomeronasal system, or accessory olfactory system, also develops from the nasal placode, and is present as a discrete sensory system only in tetrapods (see The Evolution of the Vomeronasal System). The vomeronasal epithelium is sequestered in the vomeronasal organ, also known as Jacobson's organ (Figure 1). The sensory epithelium contains some what different cell types than does the main olfactory epithelium, and the vomeronasal receptor neurons terminate in microvilli, whereas olfactory receptor neurons can terminate in cilia or microvilli or both. In some vertebrates,

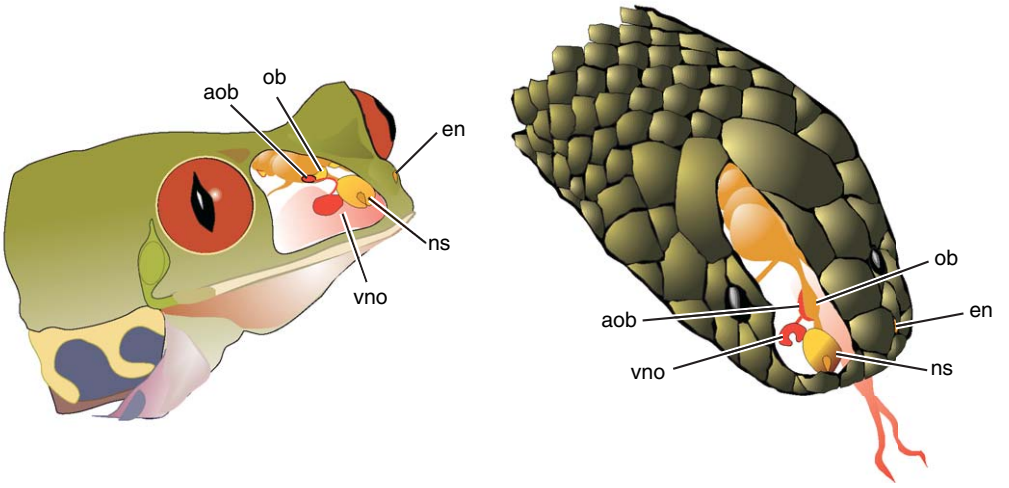

Figure 1 Illustration of the locations of the olfactory epithelium, inside the nasal sac (ns), and of the vomeronasal organ (vno) in a frog and a snake. The nasal sac opens to an external nostril (en) on the dorsal surface of the snout, whereas the vomeronasal organ opens to the nasal sac in frogs and oral cavity in snakes. The axons of the olfactory receptor neurons project to the olfactory bulb (ob), and those of the vomeronasal receptor neurons project to the accessory olfactory bulb (aob).

the axons of the vomeronasal receptor neurons form a separate cranial nerve, the vomeronasal nerve, but in others these axons run alongside the olfactory nerve and the two cannot be distinguished using conventional light microscopy. Vomeronasal receptor cell axons project to the accessory olfactory bulb, a structure that is histologically distinct from the main olfactory bulb (Figure 1). The secondary projections of the olfactory and accessory olfactory bulbs differ. The molecular, physiological, and anatomical differences between the vomeronasal and olfactory system suggest that the two subsystems serve different behavioral functions, but the nature of this difference is unclear. Although the vomeronasal system is often presumed to be specialized for detecting pheromones, it responds to a variety of odorants with differing behavioral significance in all groups of tetrapods, and this presumption is unwarranted (see The Evolution of the Vomeronasal System; Baxi *et al.*, 2006; Halpern and Martínez-Marcos, 2003; Restrepo *et al.*, 2004; Shepherd, 2006). A different hypothesis posits that the vomeronasal system is specialized for detecting nonvolatile molecules. Although better supported than the pheromone hypothesis, this hypothesis is still problematic (Baxi *et al.*, 2006).

Throughout this article, we will refer to the nasal chemosensory system present in all vertebrates as the 'olfactory system' rather than the 'main olfactory system'. Similarly, although it is common to use the term 'main olfactory bulb' to refer to the primary target of the olfactory receptor cells in animals that possess a separate vomeronasal system, we will refer to this structure simply as the 'olfactory bulb' to facilitate comparisons across vertebrates.

The terminal nerve, or nervus terminalis, is the most anterior of the cranial nerves. It extends between the nasal cavity and basal forebrain, and, because of its anatomy, has been thought to serve sensory function (e.g., Demski and Northcutt, 1983; Rossi *et al.*, 1972). Nevertheless, recordings from the terminal nerve have failed to detect sensory activity (Bullock and Northcutt, 1984; White and Meredith, 1995), and the terminal nerve is now thought to function in modulating activity in the olfactory epithelium (reviewed in Oka, 1992; Wirsig-Wiechmann *et al.*, 2002a). The terminal nerve usually contains a ganglion, the location of which varies across groups of vertebrates. The neurites extending outward from these bipolar neurons sometimes comprise a separate nerve, but the fibers of the terminal nerve can also run within the olfactory or vomeronasal nerve, as illustrated in Figure 2. The cells and fibers of the terminal nerve contain neuromodulatory compounds, including gonadotropin releasing hormone (GnRH) and acetylcholine (reviewed in Wirsig-Wiechmann *et al.*, 2002a). The nerve can also be immunohistochemically labeled with antisera directed against neuropeptide Y (NPY) and the molluscan cardioexcitatory neuropeptide FMRFamide, but the two antisera may cross-react with a single peptide (Chiba, 2000). We include the terminal nerve in this article not only because of its role as a centrifugal portion of the olfactory system, but also because the primary neurons of the terminal nerve develop from the

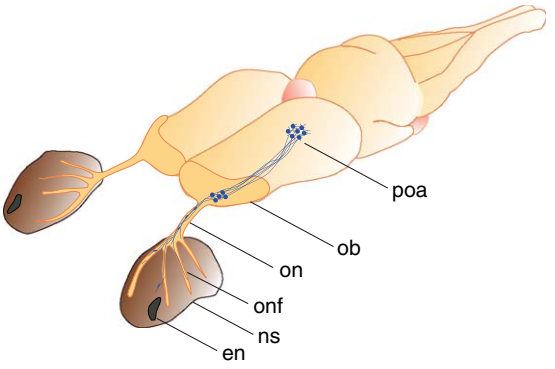

Figure 2 Location of the terminal nerve around the nasal cavities and in the brain of a salamander. Anterior is down and to the left. The fibers of the terminal nerve extend between the preoptic area and nasal cavities, wrapping around the outside of the olfactory epithelium (lower left). en, external nostril; ns, nasal sac; ob, olfactory bulb; onf, olfactory nerve fascicles; on, olfactory nerve; poa, preoptic area.

nasal placode (Schwanzel-Fukuda and Pfaff, 1989), as do the olfactory and vomeronasal receptor neurons.

2.17.1.2 Components of the Vertebrate Olfactory System

Elements of the olfactory system have been described from an evolutionary or comparative perspective in several reviews (Ache and Young, 2005; Eisthen, 1992, 1997, 2002; Hildebrand and Shepherd, 1997; Nieuwenhuys, 1967). Here, we will describe the general features of the olfactory system in nonmammalian vertebrates, illustrated in Figure 3, to provide a context for understanding the changes that have occurred over the course of vertebrate evolution, as well as variations that occur within specific lineages. In the sections that follow, we will survey the structure and function of the olfactory system in each class of vertebrates, noting features that are new, unusual, or taxon-specific. We will not describe the results of neurobiological studies designed to investigate general features of olfactory system function in vertebrates unless the results have interesting implications for understanding olfaction in the group under investigation. At the end of the article, we will discuss innovations and variations, and their possible functional implications.

In all vertebrates, the olfactory epithelium is sequestered inside the nasal cavity. Because adaptation occurs fairly quickly, the odorant-containing medium must be kept moving over the surface of the sensory epithelium. A variety of mechanisms are employed to achieve this end, including nasal cavities that allow water or air to flow through when the animal is locomoting or breathing, specialized pumping mechanisms, and nonsensory cells with motile cilia that create constant movement. Within the nasal cavity, the pseudostratified sensory epithelium contains three basic cell types (Figure 3b): olfactory receptor cells; sustentacular cells, a class of supporting cells; and basal cells, the progenitor cells that give rise to new receptor and sustentacular cells throughout life. The olfactory receptor cells are bipolar neurons with a dendrite that terminates in cilia or microvilli or both. The membrane-bound odorant receptors are localized to these processes, which are therefore presumed to be the site of transduction (Menco, 1997). The odorant receptors are part of the large superfamily of G-protein-coupled receptors (GPCRs) that have seven membrane-spanning domains (reviewed in Gaillard *et al.*, 2004; Mombaerts, 2004). The large family of odorant receptor genes has been suggested to contain two fundamentally different classes that differ in the size of the third extracellular loop (Freitag *et al.*, 1995, 1998), although others have suggested that a larger number of groupings better describes the evolutionary history of the gene family (Niimura and Nei, 2005). As depicted schematically in Figure 3c, the odorant receptor is coupled to an olfactory-specific G-protein ($G\alpha_{olf}$), with alpha subunits that are expressed in few other tissues; when stimulated, the G-protein activates type III adenylyl cyclase (Nakamura, 2000; Ronnett and Moon, 2002). The details of olfactory transduction are well understood for only a small number of vertebrate species, and involve myriad mechanisms (Firestein, 2001; Schild and Restrepo, 1998). One interesting feature of olfactory transduction is that it often involves several steps, in which ions entering through one channel gate another (Eisthen, 2002).

Even before odorants contact receptors, they interact with other molecules in the nasal cavity. The dendrites of the receptor neurons protrude into a specialized mucus produced by a combination of glands and secretory cells, including goblet and sustentacular cells as well as Bowman's glands. This mucus contains a variety of compounds, including mucopolysaccharides, peptides, and amines (Getchell and Getchell, 1992; Getchell *et al.*, 1993; Zancanaro *et al.*, 1997). In some vertebrates, it also contains specialized odorant binding proteins, soluble lipocalins produced in the lateral nasal glands. The role of odorant binding proteins in olfactory processing is unresolved (Pelosi, 2001; Tegoni *et al.*, 2000).

At the opposite pole of the olfactory receptor neuron, the unmyelinated axon projects to the

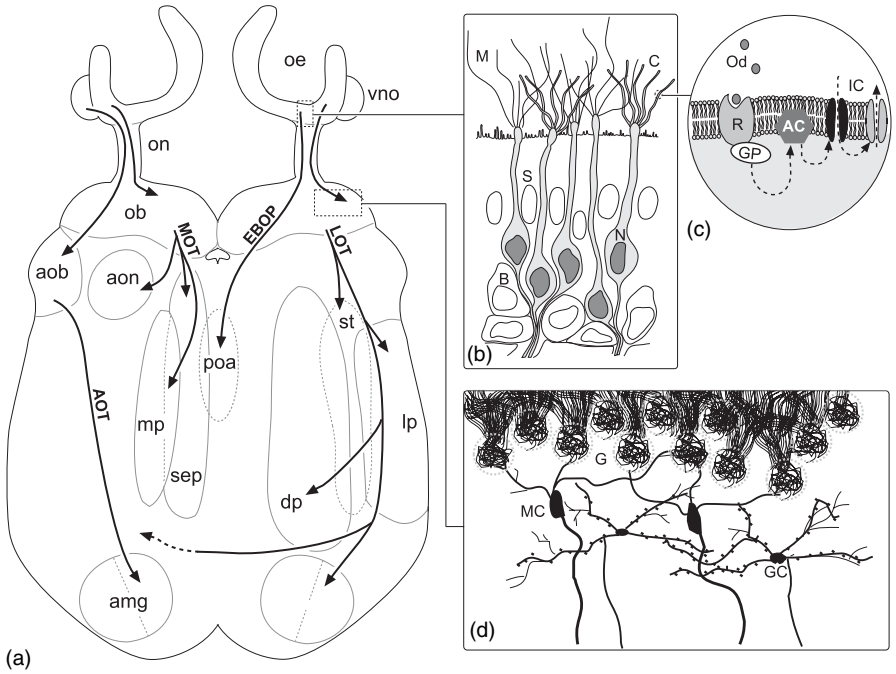

Figure 3 Diagram illustrating the basic elements of the olfactory system in nonmammalian vertebrates. a, Schematic dorsal view of the forebrain and nasal sensory epithelia of a generalized nonmammalian vertebrate. The olfactory epithelium (oe) and vomeronasal organ (vno) lie rostral to the brain, and the axons of the receptor neurons project through the olfactory nerves (on) to the olfactory bulb (ob) and accessory olfactory bulb (aob), respectively. In general, four tracts project centrally. The accessory olfactory tract (AOT) projects from the accessory olfactory bulb to the amygdala (amg). The medial olfactory tract (MOT) projects to the anterior olfactory nucleus (aon), septum (sep), and medial pallium (mp). The extrabulbar olfactory pathway (EBOP) contains axons of primary olfactory receptor neurons that bypass the olfactory bulbs, projecting directly to the preoptic area (poa). The lateral olfactory tract (LOT) projects bilaterally to the striatum (st), lateral pallium (lp), dorsal pallium (dp), and the amygdala. b, The olfactory sensory epithelium consists of three types of cells: receptor neurons (N), sustentacular cells (S), and basal cells (B). The receptor neurons in vertebrates terminate in either cilia (C) or microvilli (M). c, Transduction occurs on ciliary or microvillar membranes. The receptor protein (R) has seven membrane-spanning regions and is coupled to a G-protein (GP). Odorant (Od) binding activates adenylyl cyclase (AC), gating an ion channel (IC). Often, ions entering through this channel gate another ion channel. d, Organization of the olfactory bulb. The axons of olfactory receptor neurons enter glomeruli (G), where they interact with dendrites from mitral cells (MC), the large output neurons of the olfactory bulb. Granule cells (GC) are also present in a deeper layer of the olfactory bulb.

olfactory bulb in the rostral telencephalon. In the olfactory bulb, each unbranching axon forms synapses with many other cells in tangles of fibers known as glomeruli, which are characteristic of olfactory systems in a variety of animals (Eisthen, 2002). The olfactory bulb has a laminar organization. In many vertebrates these layers are not clearly differentiated, but in almost all the layers form concentric rings around the ventricular region in the center of the olfactory bulb. The outermost layer consists of the axons of the olfactory receptor cells, which course over the surface of the bulb before terminating in a single glomerulus in the subjacent layer (Figure 3d). This glomerular layer may contain periglomerular interneurons. Below this, an external plexiform layer is sometimes present, overlying a layer containing the cell bodies of the mitral cells, the output cells of the olfactory bulb. The mitre-shaped cell body that gives these cells their name is only obvious in tetrapods, leading to some confusion concerning the number of classes of output cells in nontetrapods. Mitral cells are large, and generally possess several dendrites that project into different glomeruli. Within a glomerulus, each mitral cell dendrite arborizes extensively, making large numbers of synapses with the axons of the olfactory receptor neurons. The granule cell layer lies between the mitral cell layer and the layer of ependymal cells surrounding the ventricle. The mitral and granule cell layers may be separated by an internal plexiform layer. In some vertebrates, the granule cell layer contains two classes of cells: the granule and stellate cells. Both types of cells generally have axons. Stellate cells possess multiple dendrites that arborize in the glomeruli. In contrast, the granule cells have an oval-shaped soma, and the dendrites interact with neurites of other cells below the glomerular layer or outside glomeruli. In some animals, granule cell dendrites bear spiny processes, whereas those of stellate cells do not. In the

following discussion, we will use these definitions in describing cell types present in diverse animals, regardless of the labels used by the authors of the papers cited.

The fibers projecting centrally from the olfactory bulb consist of the axons of mitral cells. In some groups of vertebrates, axons of other classes of cells, such as the granule and stellate cells, may contribute to these tracts. In most vertebrates, two tracts extend from the olfactory bulb (Figure 3a). The medial tract generally projects to ipsilateral ventral forebrain areas such as the septum and, in tetrapods, to the anterior olfactory nucleus and medial pallium/hippocampus. The lateral olfactory tract projects bilaterally to lateral and dorsolateral pallial areas and to the amygdala, and ipsilaterally to the striatum. Thus, unlike other sensory systems, olfactory projections to the cortex are not routed through the thalamus, contributing to Edinger's (1904) famous hypothesis that the telencephalon was originally an olfactory structure that was invaded by other sensory systems over the course of vertebrate evolution. In addition to the fiber tracts that arise from output cells of the olfactory bulbs, many vertebrates possess a small extrabulbar olfactory pathway that consists of axons of primary olfactory receptor neurons that bypass the olfactory bulb and project directly to the preoptic area (Hofmann and Meyer, 1989; Szabo *et al.*, 1991). Because of their similar projections, many older papers appear to confound the terminal nerve and extrabulbar olfactory pathway (Eisthen and Northcutt, 1996). In the following discussion, we will refer the projection that arises from olfactory receptor neurons as the extrabulbar olfactory pathway, regardless of the name the authors ascribed to it.

The olfactory system does not seem to contain simple maps or even one-to-one functional relationships. Olfactory receptor neurons tend to be broadly tuned, and a given cell will respond to many different odorants, sometimes in different ways; similarly, a given odorant can evoke different responses from different receptor neurons (e.g., Dionne, 1992; Dionne and Dubin, 1994). In mammals, olfactory receptor neurons expressing the same receptor genes project to the same glomeruli, which are located in relatively stable positions within the olfactory bulb (Mombaerts *et al.*, 1996; Schaefer *et al.*, 2001). The neurons that project to a given glomerulus tend to respond to the same sets of odorant stimuli (e.g., Bozza and Kauer, 1998), but a given odorant can activate many glomeruli across the olfactory bulb (e.g., Wachowiak *et al.*, 2002). The effect of changing odorant concentration on spread of activation among glomeruli is unclear, probably in part due to differences in recording methods, odorants used, and species examined in different studies (e.g., Wachowiak and Cohen, 2003; Wachowiak *et al.*, 2002). Nevertheless, the olfactory bulb does not appear to contain a simple map in which an odorant can be identified by the location of the glomeruli that are stimulated; instead, temporal features of the response also play an important role in odorant recognition (e.g., Friedrich and Laurent, 2001; Laurent, 2002).

The vomeronasal epithelium contains the same basic cell types found in the main olfactory epithelium, but lacks Bowman's glands (Parsons, 1967). Vomeronasal receptor neurons express GPCR genes, but the two families of GPCRs found in the vomeronasal organ do not share strong sequence similarity with those expressed in the olfactory epithelium (Dulac and Axel, 1995; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997). Transduction in vomeronasal receptor neurons seems to involve different G-proteins, second messengers, and ion channels than in olfactory receptor neurons, although only a handful of species have been examined to date (reviewed in Halpern and Martínez-Marcos, 2003). The axons of vomeronasal receptor neurons project to the accessory olfactory bulb, caudal to the olfactory bulb. In general, the accessory olfactory bulb contains only mitral, granule, and periglomerular cells, and the layers are less distinct than are those in the olfactory bulb (reviewed in Lohman and Lammers, 1967; Nieuwenhuys, 1967). The accessory olfactory tract projects from the accessory olfactory bulb primarily to portions of the amygdala that differ from those receiving olfactory input via the lateral olfactory tract.

Although there are theoretical reasons for envisioning that the receptors in a chemosensory system should differ in their breadth of tuning (Hildebrand and Shepherd, 1997), it is not clear that vertebrates possess any narrowly tuned receptor neurons. To illustrate this point, we will briefly discuss two examples: ciliated versus microvillar olfactory receptor neurons in teleost fishes, and olfactory versus vomeronasal receptor neurons in mammals. Researchers studying teleost fishes have used electro-olfactogram (EOG) recordings to examine odorant responses in patches of epithelium that are dominated by one receptor cell type. The results indicate that ciliated cells respond to bile acids, which can serve as pheromones in fishes, and that microvillar cells respond to amino acids (salmonids; Thommesen, 1983); or that ciliated cells respond to

amino acids, and that bile acids, steroids, and prostaglandin pheromones are preferentially detected by microvillar cells (goldfish; Zippel *et al.*, 1997). A similar study with channel catfish indicated that both ciliated and microvillar cells respond well to both amino acids and bile acids (Erickson and Caprio, 1984), but a later study in the same species using pharmacological agents to disrupt signaling in different cell types demonstrated that ciliated olfactory receptor neurons respond to bile acids and amino acids, and that microvillar receptor neurons respond to nucleotides and amino acids (Hansen *et al.*, 2003). Whole-cell recordings from olfactory receptor neurons in rainbow trout indicate that microvillar neurons respond selectively to amino acids, and that ciliated neurons respond more broadly to amino acids, a steroid, and conspecific urine (Sato and Suzuki, 2001). Agmatine labeling suggests that a greater proportion of microvillar than ciliated olfactory receptor neurons respond to amino acids in zebra fish (Lipschitz and Michel, 2002). Thus, the relationship between cell morphology and odorant responses is unclear, and may be species-specific. To consider a different comparison for a moment, researchers often assume that vomeronasal receptor neurons in tetrapods are much more narrowly tuned than are olfactory receptor neurons. Because of this bias, few studies have investigated the breadth of tuning of vomeronasal receptor neurons (Baxi *et al.*, 2006). Nevertheless, a few studies that have examined the issue conclude that vomeronasal receptor neurons respond to a broad array of chemicals, both naturally occurring and artificial (e.g., Sam *et al.*, 2001; Tucker, 1971).

The olfactory system, broadly defined, plays an important role in the behavior of most vertebrates. Olfactory cues may be involved in orientation and homing, habitat selection, territoriality, species recognition, kin recognition, individual recognition, parent–offspring interactions, mate choice, courtship and mating behavior, predator avoidance, foraging, and food choice. A thorough review of the behavioral functions of the olfactory system in each class of vertebrates is far beyond the scope of this article. Instead, for each group, we will provide a brief overview in which we illustrate the behavioral significance of olfactory input.

2.17.2 Evolutionary Changes in the Vertebrate Olfactory System

2.17.2.1 Chordates and Basal Craniates

Animals in many phyla possess a sensory system for detecting chemicals at a distance, and these sensory systems are generally labeled 'olfactory'. Nevertheless, there is no evidence that the vertebrate olfactory system is homologous with those present in other phyla; instead, it appears that similar features have evolved independently several times for use in sensing odorants (Eisthen, 2002). The nearest relatives to vertebrates are the urochordates (ascidians or tunicates), cephalochordates (lancelets), and hagfishes, which are considered craniates but not vertebrates. Ascidian larvae possess unpaired anterior sense organs, including a photosensitive system and a balance sensor, but no candidate homologue of the vertebrate olfactory system (Lacalli, 2001; Nieuwenhuys, 2002). Thus, the vertebrate olfactory system may not have arisen from a system shared with the common ancestor with urochordates.

Lancelets may or may not possess an olfactory system that is homologous with that of vertebrates. Lancelets respond to various classes of sensory stimuli, including chemical cues (Parker, 1908). They lack a discrete olfactory organ, but a class of potentially chemosensory cells that occurs in the rostral epithelium has been described (Lacalli and Hou, 1999). A GPCR gene that bears some sequence similarity to vertebrate olfactory receptor genes is expressed in bipolar rostral sensory cells in *Branchiostoma belcheri* (Satoh, 2005). Given that these cells bear axons, they appear to be one of the classes of type I sensory cells described by Lacalli and Hou (1999), who argued on morphological grounds that these cells are likely mechanosensory. In addition, the sequence was not subjected to rigorous phylogenetic analysis nor compared with those of many other GPCRs. Given that the use of GPCRs in chemosensory receptor cells appears to be a trait that has evolved several times independently (Eisthen, 2002), it is difficult to accept this observation as strong support for the presence of a vertebrate-like olfactory system in lancelets.

The lancelet *Branchiostoma floridae* possesses paired anterior nerves that, based on consideration of their external topography, have been suggested to be homologues of the vertebrate olfactory nerves (Lacalli, 2002). Although the author interprets the central target of these nerves as a possible homologue of the telencephalon, this region can also be interpreted as the equivalent of the vertebrate mesencephalon or rostral rhombencephalon; if so, these fibers are probably not homologues of the olfactory nerves (Northcutt, 2005). If Northcutt and Gans's 'new head' hypothesis is correct, then many rostral structures are evolutionarily new in craniates; among these structures are the ectodermal

placodes, including the nasal placode, and portions of the forebrain, including the olfactory system (Gans and Northcutt, 1983; Northcutt, 2005; Northcutt and Gans, 1983).

The condition in hagfishes should be instructive, as features shared by hagfish and vertebrates may have been present in earliest vertebrates. Hagfish are scavengers as well as opportunistic predators (Shelton, 1978). They respond vigorously to odorants from dead fish, and can use chemical cues to find carrion (Greene, 1925; Tamburri and Barry, 1999). In addition to their olfactory system, hagfish possess Schreiner organs, unusual chemosensory organs that are distributed across the body surface (Braun, 1995; see Evolution of Taste). The relative contributions of the olfactory and Schreiner organ system to behavior have not been determined, but odorants such as L-amino acids, GABA, and hydroxyproline evoke strong physiological responses from the olfactory epithelium (Døving and Holmberg, 1974).

Hagfish possess a single midline olfactory organ, with the sensory epithelium folded across a radial array of lamellae (e.g., Døving and Holmberg, 1974). The olfactory epithelium of the Atlantic hagfish *Myxine glutinosa* contains both ciliated and microvillar receptor neurons. Instead of the 9+2 microtubule arrangement that is typical of many cilia, including those on vertebrate olfactory receptor neurons, the cilia have a 9+0 arrangement (Theisen, 1973).

In *Myxine glutinosa* and *Eptatretus burgeri*, the olfactory bulb contains mitral, stellate, and granule cells, but the layers are indistinct, with somata of each cell type occurring in several different layers (Iwahori *et al.*, 1998; Jansen, 1930; Holmgren, 1919, in Nieuwenhuys, 1967). All three cell types have axons, and the dendrites of the mitral cells extend into several glomeruli. Periglomerular cells do not seem to be present, but all authors describe intraglomerular mitral cells with a single dendrite that arborizes in one glomerulus, and an axon that presumably exits the olfactory bulb (Holmgren, 1919; Iwahori *et al.*, 1998; Jansen, 1930).

Projections of the olfactory bulb have been investigated in the Pacific hagfish, *Eptatretus stouti* (Wicht and Northcutt, 1993). A short fiber tract projects from the medial olfactory bulb to the ipsilateral septum and through a dorsal commissure to the contralateral olfactory bulb. Fibers extend from the lateral olfactory bulb project bilaterally and widely in the forebrain, with targets that include the striatum, all layers of the pallium, and the central prosencephalic nucleus. An additional group of fibers extends from the ventrolateral portion of the olfactory bulb to terminate diffusely along a path extending into the diencephalon, including the hypothalamus and dorsal thalamus (Wicht and Northcutt, 1993). Given its location and targets, this tract may constitute the extrabulbar olfactory pathway. Immunocytochemical data suggest that hagfish lack a terminal nerve (Braun *et al.*, 1995; Crim *et al.*, 1979b; Jirikowski *et al.*, 1984; Wicht and Northcutt, 1992).

2.17.2.2 Lampreys

Lampreys have a biphasic lifecycle. The ammocoete larvae hatch in streams and rivers, then live buried in sediment for years, filter feeding, before metamorphosing and swimming downstream to live in larger bodies of water. Juvenile lampreys are generally parasitic, attaching to other fish to consume their blood and flesh. When sexually mature, adult lampreys stop feeding and migrate upstream in streams and rivers to spawn, after which they usually die.

In both *Ichthyomyzon fossor* and *Petromyzon marinus*, the complexity and relative size of the nasal cavity increases greatly during metamorphosis from ammocoete to the juvenile form, suggesting that olfaction may be important for free-swimming lampreys than for larvae (Leach, 1951; VanDenbossche *et al.*, 1995, 1997). Metamorphosed *P. marinus* increase activity in response to odorants from trout and fish-derived amines, but not amino acids (Kleerekoper and Mogensen, 1960).

Adult male *P. marinus* migrate and build nests in the spawning area before females arrive. The males emit a unique bile acid that is attractive to females, and to which females are highly sensitive (Li *et al.*, 2002; Siefkes and Li, 2004). Migratory lampreys do not home to particular streams; rather, they appear to simply seek suitable habitat for spawning (Bergstedt and Seelye, 1995). The means by which they do this is wonderful in its simplicity, for adult lampreys are attracted to odorants produced by healthy ammocoetes. Specifically, adults of several species have been shown to be extremely sensitive to bile acids produced by ammocoetes (Fine *et al.*, 2004; Li and Sorensen, 1997; Li *et al.*, 1995), as well as to two unique steroids that are released by ammocoetes (Sorensen *et al.*, 2005b). Adults are highly sensitive to these compounds, and both types of cues are released in sufficient quantities to attract them (Polkinghorne *et al.*, 2001; Sorensen *et al.*, 2005b). Interestingly, the bile acids are not released by nonfeeding ammocoetes, suggesting that these cues serve as a good indicator of habitat suitability (Polkinghorne *et al.*, 2001).

Lampreys possess a single midline nostril and nasal cavity that develops from a single nasal placode. Although earlier authors suggested that monorhiny is the ancestral condition for vertebrates, the presence of paired olfactory organs during development in *P. marinus* suggests that monorhiny may be a derived condition (Kleerekoper and Van Erkel, 1960). If so, it arose independently in hagfishes and lampreys. Within the nasal cavity, the olfactory epithelia of *Lampetra fluviatilis* and *P. marinus* contain only ciliated receptor cells, indicating that lampreys lack microvillar receptor neurons (Bronshtein and Ivanov, 1965; Thornhill, 1967; VanDenbossche *et al.*, 1995).

In contrast with the numerous and large families of odorant receptor genes described in other groups of vertebrates, lampreys (*L. fluviatilis*) appear to possess only a few small families of odorant receptors (Berghard and Dryer, 1998; Freitag *et al.*, 1999). Of course, it is difficult to rule out the possibility that lampreys possess many additional receptor genes that have not yet been identified. Although the sequences possess features that are characteristic of vertebrate odorant receptor genes, including a large third extracellular loop (Berghard and Dryer, 1998; Freitag *et al.*, 1999), phylogenetic analysis indicates that the lamprey odorant receptor gene family diverged before the origin of the two main classes of odorant receptor genes present in other vertebrates (Freitag *et al.*, 1999). The odorant receptor genes from lampreys also share strong similarity with histamine receptors, indicating that the odorant receptor genes in lampreys and other vertebrates may have been independently co-opted out of the larger GPCR superfamily (Berghard and Dryer, 1998; Niimura and Nei, 2005). The genes are expressed in the olfactory epithelium of both ammocoetes and adults, suggesting that the quantitative differences in the size of the olfactory system in the two forms do not necessarily indicate qualitative differences in the odorants detected (Berghard and Dryer, 1998).

In *Petromyzon* larvae, the olfactory bulb glomeruli are histochemically heterogeneous and form six distinct territories, suggesting a rough functional specialization (Frontini *et al.*, 2003). Whether this organization is particular to ammocoetes or remains throughout the lifecycle is not known. A single layer of glomeruli encircles the bulb (Heier, 1948; Iwahori *et al.*, 1987). As in hagfish, the layers are indistinct, and the somata of some mitral cells can be found in the glomerular layer. The olfactory bulb of *L. fluviatilis* contains mitral, stellate, and granule cells, all of which have axons that exit the olfactory bulb (Heier, 1948; Nieuwenhuys, 1967). Two classes of mitral cells appear to be present: those with cell bodies in the mitral cell layer, which extend a single dendrite to arborize in one glomerulus; and those with cell bodies in the glomerular layer, which have dendrites that arborize in more than one glomerulus (Heier, 1948; Iwahori *et al.*, 1987; Nieuwenhuys, 1967). The latter class of cells may function more like periglomerular cells than like mitral cells. A more recent description of the olfactory bulb of *L. japonica* suggests that either granule or stellate cells are absent in this species (Iwahori *et al.*, 1987). The cells that Iwahori *et al.* call 'granule' approximate our description of stellate cells, above. These cells lack axons, but possess several dendrites that arborize in glomeruli, often spanning the width of the bulb (Iwahori *et al.*, 1987). It is difficult to reconcile these different descriptions of bulbar organization, particularly for two species of *Lampetra*, but perhaps the discrepancies are due to developmental or methodological differences.

The olfactory bulb gives rise to four major centripetal pathways in silver lampreys, *Ichthyomyzon unicuspis*, illustrated in Figure 4 (Northcutt and Puzdrowski, 1988). A group of fibers that may comprise a homologue of the medial olfactory tract originates from the ventrolateral olfactory bulb and projects to the ipsilateral septum, preoptic area, and possibly to the rostral portion of the striatum (Figure 4a). A lateral olfactory tract projects ipsilaterally throughout the lateral pallium and either to or through the dorsal and medial pallia, as well as to the posterior tuberculum and hypothalamus; some fibers project contralaterally to a dorsal portion of the lateral pallium, septum, striatum, and the posterior tuberculum and hypothalamus (Figure 4b). A third group of fibers extends through the ventromedial olfactory bulb to the striatum and preoptic regions, as well as to the hypothalamus and throughout the posterior tuberculum (Figure 4c; Northcutt and Puzdrowski, 1988). This tract has been interpreted as an extrabulbar olfactory pathway (Eisthen and Northcutt, 1996). Finally, silver lampreys possess an olfactory pathway through a dorsal commissure to the adjacent dorsomedial neuropil, a fibrous region that spans the length of the olfactory bulb, and to glomeruli in the contralateral olfactory bulb (Figure 4d; Northcutt and Puzdrowski, 1988). Fibers projecting to the contralateral olfactory bulb have also been described in *L. fluviatilis* (Heier, 1948) and in hagfish (Wicht and Northcutt, 1992), suggesting that a dorsal commissure carrying secondary olfactory fibers to the contralateral olfactory bulb may have been present in the earliest vertebrates.

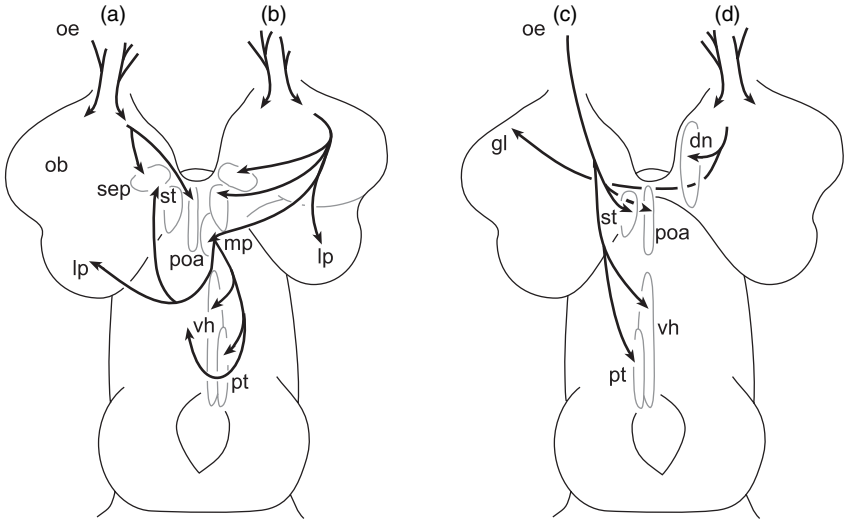

Figure 4 Schematic dorsal view of the forebrain in the silver lamprey (*Ichthyomyzon unicuspis*), illustrating the major olfactory projections. a, Projections of the medial olfactory tract. b, Projections of the lateral olfactory tract. c, Projections of the extrabulbar olfactory pathway. d, Projections through the dorsal commissure to the contralateral olfactory bulb. dn, dorsomedial neuropil; gl, glomerular layer; lp, lateral pallium; mp, medial pallium; ob, olfactory bulb; oe, olfactory epithelium; poa, preoptic area; pt, posterior tubercle; sep, septum; st, striatum; vh, ventral hypothalamus. Based on Northcutt, R. G. and Puzdrowski, R. L. 1988. Projections of the olfactory bulb and nervus terminalis in the silver lamprey. *Brain Behav. Evol.* 32, 96–107.

Heier (1948) first suggested that lampreys lack a terminal nerve, and since that time its presence in lampreys has been questionable. In other vertebrates, the terminal nerve can be labeled with antisera directed against GnRH, NPY, or FMRFamide (Wirsig-Wiechmann *et al.*, 2002a). These antisera fail to label any structures reminiscent of the terminal nerve in larval or adult *I. unicuspis*, *Lampetra japonica*, *L. planeri*, *L. richardsoni*, *Lethenteron japonica*, or *Petromyzon marinus* (Chiba, 1999; Crim *et al.*, 1979b, 1979a; Eisthen and Northcutt, 1996; King *et al.*, 1988; Meyer *et al.*, 1987; Ohtomi *et al.*, 1989; Tobet *et al.*, 1995; Wright *et al.*, 1994). Fibers that project from the nasal sac through olfactory bulb to the ventral forebrain have been labeled with injections of horseradish peroxidase or cobalt lysine into the nasal sac of larval or adult lampreys (*L. planeri*, Meyer *et al.*, 1987; *I. unicuspis*, Northcutt and Puzdrowski, 1988; von Bartheld *et al.*, 1987; von Bartheld and Meyer, 1988). Although this projection was originally interpreted as a terminal nerve, it may instead be the extrabulbar olfactory pathway, which was unknown at the time these studies were conducted (Eisthen and Northcutt, 1996).

2.17.2.3 Cartilaginous Fishes: Sharks, Skates and Rays, and Chimaeras

The class chondrichthyes consists of cartilaginous fishes (sharks, ratfish or chimaeras, and skates and rays) that are widely distributed in the world's oceans. Some species enter freshwater, and a few, like the rays *Paratrygon motoro* and *Himantura signifer*, live exclusively in freshwater (Compagno and Roberts, 1982; Müller and Henle, 1841).

Olfaction plays a major role in the life of cartilaginous fishes. Their olfactory ability is legendary, and olfaction is important for prey detection (Kleerekoper, 1978; Sheldon, 1911). Nevertheless, the popular notion that sharks can detect even a small amount of blood diluted in the ocean over many miles is an exaggeration. Electrophysiological experiments demonstrate that sharks are able to detect components of human and bovine blood, as well as other stimuli like amino acids and crab or squid extract, but responses to blood are no stronger than the responses to other stimuli (Hodgson and Mathewson, 1978; Kajiura *et al.*, 2004b; Silver, 1979; Zeiske *et al.*, 1986).

The role of olfaction in prey localization in sharks has been the subject of much study. Some species approach prey from downstream by swimming into the current (rheotaxis). Tests in the open sea indicate that lemon sharks (*Negaprion brevirostris*) use rheotaxis to find odorants from prey, but will continue to swim against the current past the stimulus source, suggesting that additional sensory cues are involved in short-range localization (Hodgson and Mathewson, 1971). Other species use a strategy called klinotaxis in which they turn in the direction of the nostril that is more strongly stimulated. Early

naris-occlusion experiments with smooth dogfish (*Mustelus canis*) demonstrated that the animals turn persistently toward the open nostril when activated by an olfactory stimulus (Sheldon, 1911). Subsequent experiments with other sharks and rays indicate that klinotaxis is common in cartilaginous fishes (reviewed in Kleerekoper, 1978).

Prey localization through klinotaxis has been suggested to constitute an important pressure driving evolution of increasing head width in hammerhead sharks (Sphyrnidae). Recent work by Kajiura *et al.* (2004a) demonstrates that the ability to localize odorants increases with increasing head width in sphyrnid sharks, although their olfactory sensitivity does not differ significantly from that of other families of sharks (Kajiura *et al.*, 2004b). In addition to its role in foraging, olfaction has also been implicated in reproductive behavior in sharks (e.g., Bleckmann and Hofmann, 1999; De Martini, 1978; Forlano *et al.*, 2000). Nevertheless, this area of research remains largely unexplored.

The olfactory organ of cartilaginous fishes consists of a chamber inside a cartilaginous capsule. Incurrent and excurrent nostrils on the ventral snout allow water to flow over the olfactory mucosa. The anterior margin of the nasal flap is sufficiently diverse that it was used in species identification until researchers learned that within-species variability is also high (Tester, 1963). In chimaeras, the two olfactory chambers are adjacent, with a cartilaginous septum that separates them down the midline. The incurrent nostrils are located medially above the mouth, and the excurrent nostrils open laterally near the edge of the mouth (Zeiske *et al.*, 1992). In pelagic sharks, water is driven over the olfactory epithelium by swimming movements, as illustrated in Figure 5. In sedentary animals, flow is driven by respiratory movements. Some species, such as spiny dogfish (*Squalus acanthias*) and the small-spotted catshark (*Scyliorhinus canicula*), use specialized valve mechanisms to regulate flow over the olfactory epithelium (Theisen *et al.*, 1986).

Inside the nasal cavity, the olfactory organ consists of a rosette composed of two rows of olfactory lamellae situated on each side of a transverse raphe. In some species, such as Oman sharks (*Iago omanensis*), secondary lamellae greatly increase the surface area of the sensory epithelium (Fishelson and Baranes, 1997). In addition to the usual receptor neurons, supporting cells, and basal cells present in all vertebrates, the olfactory epithelium of some cartilaginous fishes also contains goblet cells (Zeiske *et al.*, 1986). In sharks, the ancestral condition appears to be the presence of only microvillar olfactory receptor neurons (Reese and Brightman, 1970; Theisen *et al.*, 1986; Zeiske *et al.*, 1986, 1987). The spiny dogfish *Squalus acanthias* was originally reported to possess both ciliated and microvillar receptor cells (Bakhtin, 1976, 1977), but a later study concluded that only microvillar receptor neurons are present (Theisen *et al.*, 1986). Similarly, the olfactory epithelium of the ratfish *Chimaera monstrosa* and those of skates and rays contain only microvillar receptor cells (Holl, 1973; Meng and Yin, 1981). Nevertheless, one study with Oman sharks demonstrates the presence of unusual olfactory receptor neurons that resemble the crypt-type olfactory receptor neurons present in ray-finned fishes (Fishelson and Baranes, 1997; see discussion in Hansen and Finger, 2000).

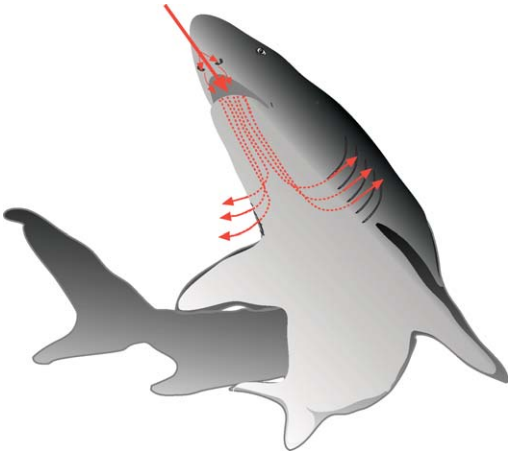

Figure 5 Schematic ventral view of a shark, showing the characteristic nostrils with prominent nasal flap, and the direction of water flow (arrows).

In cartilaginous fishes, the olfactory bulb is adjacent to the olfactory sac, and is connected by short olfactory nerves; thus, the tracts are much longer than the nerves. The length of these tracts varies across species, and in all cases they are formed by mitral cell axons with a characteristic black myelin sheath (Dryer and Graziadei, 1996). The olfactory bulb is somewhat laminated, but no plexiform layers separate the cell layers (Dryer and Graziadei, 1993; Franceschini and Ciani, 1993; Nieuwenhuys, 1967). Periglomerular cells appear to be lacking. Sterzi (1909, in Nieuwenhuys, 1967) described the olfactory bulb of spiny dogfish (*Squalus acanthias*) as containing a clear layer of mitral cells with primary dendrites that arborize in multiple glomeruli as well as secondary dendrites that interact with fibers of other cells outside the glomeruli. Granule cells have both axons and smooth dendrites, and stellate cells also appear to be present; Sterzi referred to the latter as 'triangular cells'. In contrast, a more recent study of sharks

(*Sphyrna tiburo* and *Rhizoprionodon terranovae*) and rays (*Dasyatis sabina*) found two types of mitral cells present, one with a loose dendritic arborization and the other with a more dense one (Dryer and Graziadei, 1993, 1994, 1996). The dendrites of both types arborize in a single glomerulus. Given the irregular laminar borders described by Dryer and Graziadei, it seems possible that their mitral cells with loose dendritic arbors are actually stellate cells.

The central projections of the olfactory bulb have not been examined in detail in cartilaginous fishes. The lateral olfactory tract in sharks and rays projects ipsilaterally to the area retrobulbaris, striatum, lateral pallium, and area superficialis basalis (Ebbesson and Heimer, 1970; Smeets, 1983). The existence of a medial olfactory tract is uncertain, and an earlier description of a widely projecting pathway (Smeets, 1983) has been reinterpreted as an artifact of the degeneration technique used (Northcutt, 1995). An extrabulbar olfactory pathway has not been described in this group (Hofmann and Meyer, 1995).

The terminal nerve was originally discovered in a small shark (*Mustelus asterias*) by Fritsch (1878), who referred to it as a 'supernumerary nerve'. Locy (1905) later described this structure in a variety of sharks and rays, and named it the *nervus terminalis* because the nerve enters the brain through the lamina terminalis. The exact point of entry in the brain varies somewhat among species; for example, in *Squalus acanthias* the terminal nerve enters the telencephalon dorsally (Locy, 1905). In most cartilaginous fishes, the terminal nerve is completely separate from the olfactory nerve, and includes a visible ganglion located medial and external to the nasal cavity. In some species, additional ganglia occur along the nerve (Locy, 1905).

Electron microscopic examination of the terminal nerve in sharks and rays demonstrates that the cells are heterogeneous. Most fibers are unmyelinated, but a few fibers in the proximal part of the nerve are myelinated (Demski and Schwanzel-Fukuda, 1987; White and Meredith, 1987). The ganglion consists largely of unipolar cells, with a few bipolar and multipolar cells present (White and Meredith, 1995; Wu *et al.*, 1992). Cells and fibers of the terminal nerve display GnRH-like immunoreactivity in many species of sharks and rays (Chiba, 2000; Chiba *et al.*, 1991; Demski and Schwanzel-Fukuda, 1987; Stell, 1984; White and Meredith, 1995). Data from two species of sharks (*Sphyrna tiburo* and *Scyliorhinus torazame*) suggest that in cartilaginous fishes, separate populations of terminal nerve neurons contain GnRH and FMRFamide-like compounds (Chiba, 2000; White and Meredith, 1995). Additional FMRFamide immunoreactive fibers have been described within the olfactory nerve in sharks and rays (Wu *et al.*, 1992), but it is not yet clear whether these fibers constitute part of the terminal nerve pathway.

Electrical stimulation of the terminal nerve ganglion in Atlantic stingrays (*Dasyatis sabina*) leads to an increase in GnRH levels in the brain (Moeller and Meredith, 1998), but electrical stimulation of the terminal nerve does not significantly alter activity in the olfactory bulb (Meredith and White, 1987). Recordings from the terminal nerve in sharks and rays have failed to detect sensory activity, although efferent activity from the brain has been recorded (Bullock and Northcutt, 1984; Demski and Schwanzel-Fukuda, 1987; White and Meredith, 1995). Further, activity of terminal nerve ganglion cells can be modulated by application of acetylcholine or norepinephrine, suggesting that centrifugal fibers may regulate activity of these cells (White and Meredith, 1993, 1995). Overall, the available data indicate that the terminal nerve in cartilaginous fishes is not sensory, although it has not yet been demonstrated to serve a modulatory function.

2.17.2.4 Ray-Finned Fishes

Actinopterygii, the ray-finned fishes, is the largest class of vertebrates, comprising nearly 25 000 species. One division of ray-finned fishes, the teleost fishes, contains over 23 000 species (Nelson, 1994). In addition to teleosts, the class Actinopterygii contains paddlefish and sturgeons, gars, bowfins, and bichirs or reed fishes. Ray-finned fishes occupy an enormous diversity of aquatic ecological niches and possess many specialized adaptations. We will describe here the main features of the olfactory system that are shared by the species that have been examined to date.

Olfactory cues are critical for foraging in some species of ray-finned fishes (Atema, 1980; Bateson, 1890), and electrophysiological recordings from goldfish and carp (*Carassius*) reveal a high sensitivity to food-related odorants, with thresholds for amino acids generally in the range of 10^{-6}–10^{-9} M (Goh and Tamura, 1978; Zippel *et al.*, 1993). This sensitivity is greater than that typically measured in other aquatic vertebrates (Hamdani *et al.*, 2001). In addition to locating food by following trails of extremely dilute odorants, many salmonid species return to their natal streams to spawn after spending years developing in oceans, homing in part based on olfactory cues. In anadromous species, the developing fish undergo behavioral, anatomical, and physiological changes necessary to survive the marine environment as they migrate downstream.

During this period, olfactory sensitivity increases, allowing the animals to imprint on odorants that will guide their homing behavior when they are reproductively mature (reviewed in Nevitt and Dittman, 2004).

The complex nature of pheromonal signaling during courtship is understood better in goldfish (*Carassius auratus*) than in any other vertebrate. Vitellogenic females release 17β-estradiol, which attracts males (Kobayashi *et al.*, 2002). As the female approaches ovulation, it begins to release sex steroids. During the 12 h period leading up to ovulation, the ratio of the three released steroids changes, allowing males to assess the female's reproductive state with great temporal precision (Scott and Sorensen, 1994). The steroid that dominates the mixture early in this process inhibits male responses to one of the steroids that dominates later, perhaps as a mechanism to prevent inappropriate responses to a signal that is present in small quantities throughout the ovulatory period (Stacey and Sorensen, 2002). At ovulation, the female begins to release prostaglandins, attracting males, which are extremely sensitive to this mixture (Sorensen *et al.*, 1988). Males also release a pheromone that attracts females, which has been suggested to function in sex recognition during competition for mates (Sorensen *et al.*, 2005a).

The mechanisms underlying olfactory transduction of prostaglandins and sex steroids are substantially similar in goldfish, and differ from those used to transduce amino acid odorants (Sorensen and Sato, 2005). In zebra fish (*Danio rerio*), pheromonal cues stimulate a different region of the olfactory bulb than do other odorants (Friedrich and Korsching, 1998), and processing of these two types of information may be segregated in goldfish as well (Hanson *et al.*, 1998). In addition, in goldfish and its congener the Crucian carp (*C. carassius*), the lateral olfactory tract carries information related to foraging behavior, whereas the medial tract is involved in pheromonal mediation of courtship and spawning behavior (Dulka, 1993; Hamdani *et al.*, 2001; Kyle *et al.*, 1987; Sorensen *et al.*, 1991).

Many teleost pheromones also function as hormones, or are metabolites of hormones, and may be passively released through diffusion from the blood in gills during respiration (e.g., Vermeirssen and Scott, 1996). In contrast to the common conception of pheromones as cues specifically produced as communication signals, such observations have led to the hypothesis that pheromonal communication in fishes originally evolved as a mechanism by which animals could assess the reproductive status of conspecifics through detection of cues that the releaser cannot control (e.g., Sorensen and Scott, 1994). In addition to sex pheromones, teleost fishes appear to produce alarm pheromones, which also raise interesting evolutionary issues. The phenomenon of alarm cues was first described by von Frisch (1938, 1941), who noted that a minnow, *Phoxinus phoxinus*, releases a chemical ('Schreckstoff') when injured; the release of the chemical results in a behavioral alarm response by conspecifics. This phenomenon has since been documented in many teleost species, particularly in Ostariophysi (reviewed in Døving *et al.*, 2005). Nevertheless, because its adaptive value is difficult to understand, some have questioned the very existence of the phenomenon (e.g., Magurran *et al.*, 1996). The problem is that production of alarm substances is energetically costly (Wisenden, 2000), but of questionable benefit to the animal that produces them while being consumed by a predator, even if kin are nearby (Williams, 1992). A resolution to this apparent paradox may be that release of alarm substances may attract secondary predators that will chase or consume the initial predator, allowing the injured individual to escape (Mathis *et al.*, 1995). This hypothesis has received some empirical support (Chivers *et al.*, 1996a; but see Cashner, 2004).

An interesting adaptation in some teleosts is the use of the olfactory system for detecting changes in external salinity; for example, Atlantic salmon (*Salmo salar*) have polyvalent cation-sensing receptors in the olfactory epithelium (Nearing *et al.*, 2002). Other species can compensate for changes in salinity. In sea bream (*Sparus auratus*), a euryhaline marine species, olfactory receptor neurons increase their firing rate to compensate for reduction in extracellular calcium levels (Hubbard *et al.*, 2000). Olfactory responses to odorants are similar in freshwater and seawater in rainbow trout (*Oncorhynchus mykiss*), suggesting that the compensatory mechanism resides within the olfactory epithelium (Shoji *et al.*, 1996).

Ray-finned fishes usually have two pairs of nostrils on the dorsal surface of the snout, with the anterior and posterior nares separated by a strip of skin. Water flows into the anterior (incurrent) nares, passes over the olfactory epithelium in the nasal sac, and then exits through the posterior (excurrent) nares. Water flow can be generated by swimming movements. In teleosts with an accessory nasal sac, opercular movements during respiration alternately compress and expand the accessory nasal sacs, pumping water through the nasal sac (Døving *et al.*, 1977; Nevitt, 1991).

As in other fishes, the olfactory epithelium in ray-finned fishes is organized into a rosette of lamellae

radiating outward from a central raphe. Both the shape and complexity of lamellar organization differs among species (Yamamoto, 1982; Zeiske *et al.*, 1992). The number of lamellae is also variable, increasing during development to a species-typical level, such as the 168 lamellae present in adult undulated moray eels (*Gymnothorax undulatus*) or the 230 lamellae in adult barred snappers (*Hoplopagrus guenteri*) (Fishelson, 1995; Pfeiffer, 1964). Some groups, such as salmonids, have secondary lamellae that greatly increase the size of the epithelial surface (Yamamoto and Ueda, 1977).

As illustrated in Figures 6a and 6c, the olfactory epithelium of teleost fishes contains both ciliated and microvillar receptor cells (extensively reviewed in Eisthen, 1992). Within these two broad categories, many subtypes can be distinguished; these subtypes differ morphologically and project to distinct regions of the olfactory bulb (Morita and Finger, 1998).

Ray-finned fish are the only group of vertebrates in which the olfactory epithelium clearly contains receptor neurons that do not fit into the preceding categories. Hansen *et al.* have described 'crypt' receptor neurons with a dendritic surface that bears apical microvilli as well as a large concave section that is filled with cilia (Figure 6b; Hansen and Zeiske, 1998). Another unusual feature of crypt cells is that they express two types of G-proteins (Hansen *et al.*, 2004). A recent study of the electrophysiological properties of crypt cells in the Pacific jack mackerel (*Trachurus symmetricus*) found that, among the relatively small number of neurons examined, some responded to amino acid odorants, but that none responded to polyamines or bile acids (Schmachtenberg, 2006). The axons of crypt cells terminate in the olfactory bulb, indicating that they are true olfactory receptor neurons (Hansen *et al.*, 2003). Crypt cells are present in the olfactory epithelium of many, but not all, species of teleosts that have been examined to date, and are present in a polypteriform fish, *Polypterus senegalus*, but not in shortnose gars, *Lepisosteus plastostomus* (Hansen and Finger, 2000). Like teleosts, sturgeons, and paddlefish possess ciliated, microvillar, and crypt olfactory receptor neuron (Bakhtin, 1976; Hansen and Finger, 2000; Pyatkina, 1976; Zeiske *et al.*, 2003). Overall, these data suggest that ciliated, microvillar, and crypt-type olfactory receptor neurons are broadly present in ray-finned fishes, but that the distribution of each cell type is somewhat variable.

In addition to these receptor cell types, both 'rod' and 'rodlet' cells have been proposed to function as olfactory receptor neurons in teleosts. Instead, the former are probably unhealthy or degenerating cells (Muller and Marc, 1984; Zeiske and Hansen, 2005), and the latter may be migrating secretory cells, a type of white blood cell, or even parasites (Bielek, 2005).

Both olfactory-type and vomeronasal-type odorant receptor genes are expressed in the olfactory epithelium of teleosts, although the size of the gene families appears to be smaller than in mammals. In channel catfish (*Ictalurus punctatus*) fewer than 100 members of the olfactory receptor gene family have been found (Ngai *et al.*, 1993b, 1993a). In zebra fish, the entire odorant receptor gene repertoire appears to consist of 143 genes, with even smaller numbers found in pufferfish (44 genes in *Takifugu rubripes* and 42 in *Tetraodon nigroviridis*) (Alioto and Ngai, 2005). Members of the V2R gene family have been sequenced from both pufferfish (*Takifugu*

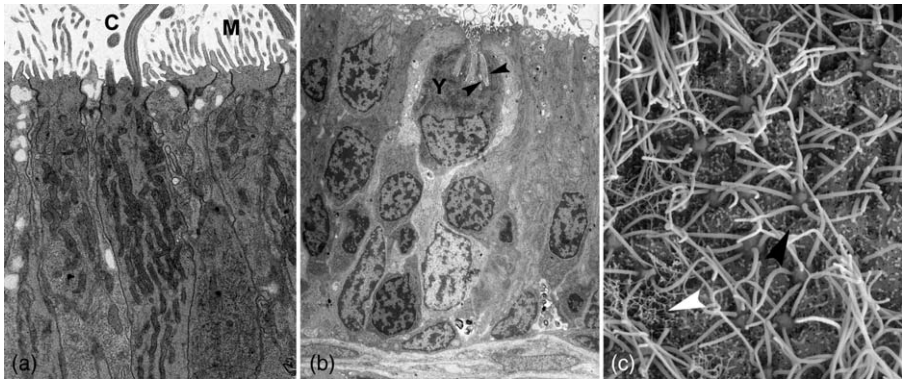

Figure 6 Electron micrographs of the three classes of olfactory receptor neurons in ray-finned fishes. a, Transmission electron micrograph of dendrites of ciliated (C) and microvillar (M) olfactory receptor neurons in goldfish, *Carassius auratus*. b, Transmission electron micrograph of a crypt cell (Y) in zebra fish, *Danio rerio*. Crypt cells are characterized by cilia sunken within the cell (arrowheads), as well as microvilli on the surface. c, Scanning electron micrograph of the dendritic surface of ciliated (black arrowhead) and microvillar (white arrowhead) olfactory receptor neurons in a fathead minnow (*Pimephales promela*). Micrographs provided courtesy of Dr. Anne Hansen.

rubripes) and goldfish (Cao *et al.*, 1998; Naito *et al.*, 1998). A single member of the V1R gene family has been sequenced in zebra fish and found to be expressed in the olfactory epithelium, and V1R-like genes with high sequence variability are also present in the genomes of several other teleost species (*Oryzias latipes*, *Danio malabaricus*, *Takifugu rubripes*, and *Tetraodon nigroviridis*) (Pfister and Rodriguez, 2005). In goldfish, the olfactory sensory neurons that bear cilia express olfactory receptor genes and $G\alpha_{olf}$, and those with microvilli express V2R genes and $G\alpha_o$, although some are immunoreactive for $G\alpha_{i\text{-}3}$ or $G\alpha_q$ instead (Hansen *et al.*, 2004). V2Rs are also expressed in crypt cells (Hansen *et al.*, 2004). In zebra fish, ciliated olfactory receptor neurons express the olfactory-type receptor genes also express olfactory marker protein and the cyclic nucleotide-gated channel A2 subunit that is typical of mammalian olfactory receptor neurons; microvillar neurons express V2R-type receptors and the TRPC2 channel that are typical of mammalian vomeronasal receptor neurons (Sato *et al.*, 2005). Taken together, these data suggest that a vomeronasal-type system is present in teleosts, but that the receptor neurons are mixed together with olfactory receptor neurons in the epithelium (discussed in Alioto and Ngai, 2005; Eisthen, 2004).

The organization of the olfactory bulb is moderately laminar. As in other fishes, distinct plexiform layers are lacking, and the cell bodies are somewhat intermingled across layers (Nieuwenhuys, 1967). Periglomerular cells may be lacking. Each mitral cell has from one to five primary dendrites, and each dendrite ends in one or more glomeruli (Nieuwenhuys, 1967). Another class of output cell is called the 'ruffed' cell, due the presence of a ruff of processes at the base of the axon (Kosaka and Hama, 1979a, 1979b). This type of cell may be broadly present in teleosts, but has not been described in other classes of vertebrates (Alonso *et al.*, 1987; Fuller and Byrd, 2005; Kosaka and Hama, 1980). In his study of the olfactory bulb of teleosts, Catois (1902) referred to an additional class of large output cells with an elongated, horizontally oriented cell body as 'fusiform' cells. Morphologically similar cells are apparent in illustrations of the olfactory bulb of sturgeons (Johnston, 1898), and may be present in other ray-finned fishes as well. Granule cells and stellate cells appear to be present in both teleosts and sturgeons (Johnston, 1898; Nieuwenhuys, 1967).

The forebrain of ray-finned fish is everted and contains many discrete cell groups, the homologies of which are difficult to establish. Because connectivity is often used by comparative neuroanatomists as a key criterion for homology, any attempt to compare the similarity of olfactory projection patterns between teleosts and other vertebrates can become circular; therefore, independent evidence, such as histochemical data, is necessary to corroborate hypotheses of homology. The central projections of the olfactory bulb have been examined in some detail in goldfish, *Carassius auratus* (Levine and Dethier, 1985; Northcutt, 2006; von Bartheld *et al.*, 1984). As depicted in Figure 7a, the medial olfactory tract projects bilaterally to the ventral forebrain areas Vs, Vl, and Vv, which may be equivalent to part of the septum (Northcutt and Braford, 1980); to Vd, which may be the equivalent of part of the striatum (Northcutt and Braford, 1980); to Dm, which may be the equivalent of the amygdala (Northcutt, 2006); and to the preoptic area, with some fibers terminating as far caudally as the hypothalamus. The lateral olfactory tract of goldfish projects mainly to dorsolateral pallial areas, as well as projecting bilaterally to the hypothalamic region and nucleus tuberis (Figure 7b). One of the targets of the lateral olfactory tract is Dl, which may be the equivalent of the medial pallium/hippocampus (Northcutt, 2006). Topographically similar projections have been described in a salmonid, *Oncorhyncus mykiss* (Folgueira *et al.*, 2004). The presence of an extra-bulbar olfactory pathway is well-established in teleosts (Anadón *et al.*, 1995; Bazer *et al.*, 1987; Hofmann and Meyer, 1995; Szabo *et al.*, 1991) as well as in *Amia*, sturgeons, and polypteriform species (Hofmann and Meyer, 1995; Huesa *et al.*, 2000). The details of the targets differ among species, but the fibers generally project to ventral forebrain areas (Figure 7c).

Among nonteleost ray-finned fishes, the olfactory projections have been most carefully examined in the bichir, *Polypterus palmas* (Braford and Northcutt, 1974; von Bartheld and Meyer, 1986). In *Polypterus*, a lateral olfactory tract projects largely to the pallial area P3 and may contain some contralaterally projecting fibers, and a medial tract projects ipsilaterally to the pallial areas P1 and bilaterally to ventral telencephalic regions, with some fibers extending as far caudal as the hypothalamus. Similar projections have been described in the sturgeon *Acipenser baeri* (Huesa *et al.*, 2000). Based on its position and its massive input from the olfactory bulb, P1 is generally considered to be the homologue of the lateral pallium (Northcutt and Davis, 1983; von Bartheld and Meyer, 1986), and topological considerations have led to the suggestion that P3 is the homologue of the medial pallium / hippocampus (Braford, 1995; Northcutt and Davis, 1983; von Bartheld and Meyer, 1986). If these interpretations

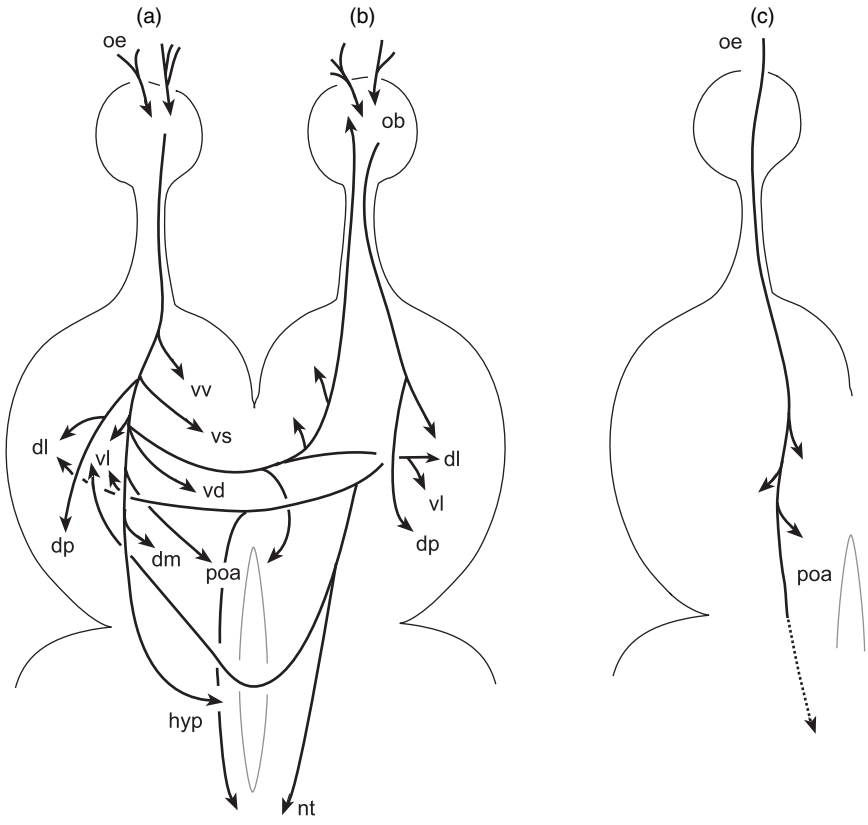

Figure 7 Schematic dorsal view of the forebrain in the goldfish, *Carassius auratus*. a, Projections of the medial olfactory tract. b, Projections of the lateral olfactory tract. c, Projections of the extrabulbar olfactory pathway. dl, lateral division of dorsal telencephalic area; dm, medial division of dorsal telencephalic area; dp, posterior division of dorsal telencephalic area; hyp, hypothalamus; nt, nucleus tuberis; ob, olfactory bulb; oe, olfactory epithelium; poa, preoptic area; vd, dorsal nucleus of the ventral telencephalic area; vl, lateral nucleus of the ventral telencephalic area; vs, supracommissural nucleus of the ventral telencephalic area; vv, ventral nucleus of the ventral telencephalic area. Based on Dulka (1993), Levine and Dethier (1985), Northcutt (2006), von Bartheld *et al.* (1984), and Polese (2004).

are correct, then the lateral olfactory tract of the bichir *Polypterus palmas* projects to the homologue of the medial pallium/hippocampus and the medial tract projects to the homologue of the lateral pallium, which is the reverse of the general vertebrate pattern. Perhaps the apparent medial and lateral tracts have been reversed in *Polypterus*, in conjunction with eversion of the telencephalon. If this were the case, then we would expect that the tracts in other ray-finned fish, such as goldfish, would be similarly reversed. Unfortunately, the data from goldfish are ambiguous: the lateral olfactory tract projects to a lateral pallial area, as is typical of the lateral tract, but also to a region that may be the equivalent of the septum, as is typical of the medial olfactory tract in tetrapods. Further, the medial olfactory tract of goldfish projects to ventral areas that are suspected homologues of portions of the septum and medial pallium, which is typical of the medial tract of other vertebrates, but also to a potential homologue of the amygdala, as it is typical of the lateral olfactory tract of other vertebrates. Thus, the relationships between the medial and lateral olfactory tracts in ray-finned fishes and other vertebrates are unresolved, and may bear no one-to-one correspondence with those in other vertebrates.

Unlike in cartilaginous fishes, the terminal nerve in ray-finned fishes projects alongside the primary olfactory fibers in the olfactory nerve, and is not visible externally. The ganglion cells of the terminal nerve develop from the nasal placode (Parhar, 2002; Whitlock and Westerfield, 2000). In most teleosts the terminal nerve ganglion consists of a cluster of cells located between the olfactory bulb and the ventral telencephalon, with fibers that extend centrally to the ventral forebrain and peripherally to both the olfactory epithelium and retina (Brookover and Jackson, 1911; Kim *et al.*, 1995; Oka *et al.*, 1986; Parhar *et al.*, 1996; Rossi *et al.*, 1972; Yamamoto *et al.*, 1995). Because of this association with both the olfactory system and retina, the terminal nerve ganglion in teleosts is also

frequently called the nucleus olfacto-retinalis (Münz *et al.*, 1981). The exact location and the morphology of the ganglion cells varies somewhat among species (reviewed in Münz and Claas, 1987). In some teleosts, such as the dwarf gourami (*Colisa lalia*), the fibers of the terminal nerve project to many disparate regions of the brain (Oka, 1992; Oka and Matsushima, 1993).

The teleost terminal nerve contains at least one form of GnRH (Yamamoto *et al.*, 1995), and many of the cells and fibers of the terminal nerve are GnRH-immunoreactive (e.g., Oka and Ichikawa, 1990; Schreibman *et al.*, 1979). Some of these cells also display immunoreactivity to NPY or to FMRFamide (Ekström *et al.*, 1988; Mathieu *et al.*, 2002; Östholm *et al.*, 1990; Pinelli *et al.*, 2000; Rama Krishna and Subhedar, 1992; Walker and Stell, 1986). The terminal nerve displays similar immunoreactivity in polypteriform fishes (*Polypterus palmas*, *P. senegalus*, and *Calamoichthys calabaricus*), sturgeons (*Acipenser ruthenus*), and gars (*Lepisosteus oculatus*) (Chiba, 1997, 2005; Pinelli *et al.*, 2000; Wright and Demski, 1996). Curiously, GnRH immunoreactivity has also been described in primary olfactory receptor neurons in the carp *Cirrhinus mrigala*; expression is seasonal, and occurs only in adult females (Biju *et al.*, 2003, 2005). Perhaps these cells are evolutionarily derived from terminal nerve cells that did not migrate properly during development, and differentiated into olfactory receptor neurons under the influence of local cues. Nevertheless, given that the neurons involved are not ganglion cells, they do not appear to be part of the terminal nerve system.

The function of the terminal nerve has been the subject of much study in teleosts, particularly in the dwarf gourami, *Colisa lalia*. Electrophysiological experiments demonstrate that the majority of the ganglion cells fire spontaneous action potentials brought about by the interaction of a tetrodotoxin-resistant, persistent sodium current that depolarizes the cell and a persistent potassium current that repolarizes the cell (Abe and Oka, 1999; Oka, 1992, 1996). The firing frequency of the cells is modulated by the same form of GnRH that is present in the nerve, causing an initial decrease in firing rate, followed by a later increase (Abe and Oka, 2000, 2002). This modulation of firing rate by GnRH may function to synchronize firing (Abe and Oka, 2000). Studies such as these were the first to suggest that the terminal nerve may function as a neuromodulatory system, rather than as a sensory system, as originally thought. Additional evidence for this hypothesis comes from studies showing that exposure to odorants does not alter the firing rate of terminal nerve ganglion cells (Fujita *et al.*, 1991), and that lesions of the terminal nerve impair initiation of nest-building behavior, but do not abolish reproduction, in dwarf gouramis (Yamamoto *et al.*, 1997). In addition, compounds present in the terminal nerve alter the activity of retinal ganglion cells, suggesting that the retinopetal branch of the terminal nerve in teleost fishes is neuromodulatory (Huang *et al.*, 2005; Maaswinkel and Li, 2003; Walker and Stell, 1986). Interestingly, a tract-tracing study with dwarf gouramis and tilapia (*Oreochromis niloticus*) indicates that the terminal nerve receives input from olfactory areas in the forebrain, as well as from the nucleus tegmento-olfactorius, a midbrain region that receives input from the reticular formation as well as areas involved in visual and somatosensory processing (Yamamoto and Ito, 2000). Taken together, the results of these studies suggest that the teleost terminal nerve functions to modulate activity in the olfactory epithelium and retina, in part in response to visual and olfactory input.

2.17.2.5 Lobe-Finned Fishes: Lungfishes and Coelacanths

Three genera of lungfishes and one of coelacanths are alive today. Although they are dispersed around the globe, each species lives in a relatively restricted area. Lungfishes live in freshwater in Africa (*Protopterus*), South America (*Lepidosiren*), and Australia (*Neoceratodus*), and the two known extant species coelacanths (*Latimeria*) live in the deep ocean near Madagascar and Indonesia. In addition to their wide geographical separation, the living lobe-finned fishes share only distant common ancestors, and each group possesses unique features that are poorly understood. *Protopterus*, for example, has a reduced olfactory system that is thought to be the result of adaptation to drought and starvation (Derivot, 1984).

In lungfish, the olfactory organ is located ventrally. The incurrent nostril opens on the dorsal surface of the snout, but, unlike other fishes the excurrent nostril opens inside the mouth (Huxley, 1876). This internal naris functionally connects the nasal cavity and respiratory system, causing water to flow across the surface of the olfactory epithelium during breathing (Huxley, 1876). The degree to which the gills and lungs are involved in respiration varies among groups.

As in other fishes, the nasal cavity of lungfishes contains a series of lamellae, on the surface of which lies the olfactory epithelium (Derivot, 1984; Pfeiffer, 1969; Theisen, 1972). The morphology of

olfactory receptor neurons varies among taxa. The African lungfish (*Protopterus annectans*) has both ciliated and microvillar olfactory receptor neurons, but the Australian lungfish (*Neoceratodus forsteri*) lacks ciliated olfactory receptor neurons (Derivot *et al.*, 1979; Theisen, 1972). The microvillar olfactory receptor neurons in these animals are unusual: the cells lack centrioles, and the microvilli contain microtubules, which have not been described in the microvilli on olfactory receptor cells in other vertebrates (Theisen, 1972). Both species lack the crypt-type olfactory receptor neurons characteristic of ray-finned fishes (Hansen and Finger, 2000).

The olfactory bulbs are located adjacent to the telencephalon in *Protoperus* and *Lepidosiren*, but those in *Neoceratodus* are connected to the telencephalon by short, hollow peduncles (Holmgren and van der Horst, 1925). The structure of the olfactory bulb in *Protoperus* has been described by Rudebeck (1945). Clear laminae are present, including plexiform layers, although the internal plexiform layer is not as robust as in *Neoceratodus* (Holmgren and van der Horst, 1925). Periglomerular cells are scattered among the glomeruli, and their morphology is unlike those in other vertebrates: each cell has a single dendrite that arborizes in a glomerulus, and the axons of most periglomerular cells form a distinct tract that passes over the dorsal surface of the olfactory bulb and extends to the anterior pallium (Rudebeck, 1945). The mitral cells have primary dendrites that arborize in the glomerular layer as well as secondary dendrites that extend through the external plexiform layer. The granule cells have spiny dendrites and unmyelinated axons. Stellate cells may not be present.

Although *Protopterus* has been suggested to possess a vomeronasal nerve and accessory olfactory bulb (Schnitzlein and Crosby, 1967), more recent studies have demonstrated that this is not the case (Derivot, 1984; Reiner and Northcutt, 1987). Thus, a discrete vomeronasal system is lacking in lobe-finned fishes.

The central projections of the olfactory system in lungfishes have not been examined using modern methods, but were described by Holmgren and van der Horst (1925) and Rudebeck (1945) based on normal material (reviewed in Nieuwenhuys, 1967). A central olfactory tract consisting of axons from both mitral and granule cells passes through the telencephalic hemisphere in the pallium and subpallium, forming internal and external fiber layers. The internal fiber layer is confined to the pallium whereas the external fiber layer extends to the striatum and the lateral parts of the olfactory tubercle, continuing posteriorly to the stria medullaris. A medial olfactory tract projects to the septum. A contralateral projection is present, but the terminations of decussating fibers has not been determined. The presence of primary olfactory fibers that project bilaterally to the di- and mesencephalon has been detected in *Protopterus* using horseradish peroxidase injections into the nasal cavity (von Bartheld and Meyer, 1988), and a similar projection has been described in *Neoceratodus* (Schober *et al.*, 1994). Thus, lungfishes appear to possess an extrabulbar olfactory pathway.

Initial studies of the anatomy of the terminal nerve in lungfish (*Protopterus annectans*; Pinkus, 1894 and *Neoceratodus forsteri*; Sewertzoff, 1902) described a discrete ganglion close to the olfactory sac with a nerve that runs independent of the olfactory nerve, entering the brain at the level of the preoptic area. Because of this topology, it was named 'nervus praeopticus' (Sewertzoff, 1902). The terminal nerve has now been examined in all three genera of lungfish, and in each it has two roots: an anterior root that runs within the olfactory nerve, consisting of a fascicle of fibers and bipolar cells, and a posterior root that corresponds to Sewertzoff's 'nervus praeopticus' (Holmgren and van der Horst, 1925; Rudebeck, 1945). In *Neoceratodus*, the two roots are joined at the level of the ganglion (Fiorentino *et al.*, 2002; Holmgren and van der Horst, 1925; Rudebeck, 1945). Experimental embryological work with larval *Neoceratodus* demonstrates that the terminal nerve ganglion and both roots originate within the olfactory placode (Fiorentino *et al.*, 2002). In *Neoceratodus* and *Protopterus*, only the posterior root displays GnRH immunoreactivity (Schober *et al.*, 1994), but in *Neoceratodus*, both roots display FMRFamide-like immunoreactivity (Fiorentino *et al.*, 2002). Perhaps the anterior root is homologous with the bundle of FMRFamide-immunoreactive fibers that run within the olfactory nerve of some sharks, described by Wu *et al.* (1992).

Two species of coelacanths are known, and because of the rarity of material the olfactory system has been examined only superficially in *Latimeria chalumnae*. This species has an incurrent nostril on the dorsal side of the snout, and a valvular excurrent nostril that opens just in front of the eye. The olfactory epithelium is radially organized around a central axis where the three dorsal and two ventral lamellar lobes converge (reviewed in Zeiske *et al.*, 1992). Although the fine structure of the olfactory epithelium has not been examined, odorant receptor genes have been sequenced from coelacanths (Freitag *et al.*, 1998). Both classes of receptor genes described by Freitag *et al.* (1995, 1998),

purportedly for odorant detection in air and in water, are present.

A bundle of receptor cell axons emerges from each lobe, and the bundles merge to become a short olfactory nerve that projects into the nearby olfactory bulb (Northcutt and Bemis, 1993). The olfactory bulb is located adjacent to the nasal cavity, and is connected to the telencephalon by a long olfactory peduncle (Nieuwenhuys, 1965). The organization of the olfactory bulb resembles that of other fishes, but clear plexiform layers are lacking (Nieuwenhuys, 1965). The olfactory tracts run through the peduncle. Some tracts terminate within the olfactory bulb (corpus rostrale), whereas the others form a central olfactory tract that becomes thinner as it proceeds caudally through the pallium. The termination sites of these fibers are unclear (Nieuwenhuys, 1965). Neither an extrabulbar olfactory pathway nor a terminal nerve has been identified in *Latimeria* (Northcutt and Bemis, 1993).

2.17.2.6 Tetrapods: Amphibians, Reptiles, and Mammals

The tetrapods include all extant amphibians, reptiles (including birds), and mammals. Early tetrapods were aquatic, and the last common ancestor of tetrapods is now generally accepted to have been fully aquatic (Lebedev and Coates, 1995; Panchen, 1991). Thus, many features in amphibians and amniotes (reptiles and mammals) that represent adaptations to terrestrial life arose independently.

In early tetrapods, the external nostril shifted position to lie low on the snout, and became enlarged (Clack, 2002). In addition, olfaction became coupled to respiration, and remains so in many extant tetrapods (Clack, 2002). The olfactory system of tetrapods is dramatically reorganized compared with that of other vertebrates, as the vomeronasal system now forms a discrete sensory system. The olfactory system of terrestrial tetrapods must contain features that facilitate functioning to detect odorants in air, instead of in water, yet few concrete examples have been identified. For example, the vomeronasal system was once thought to have arisen as an adaptation to terrestrial life (Bertmar, 1981), but this idea has since been discarded (Eisthen, 1992, 1997).

2.17.2.6.1 Amphibians Amphibians comprise three distinct groups: anurans, or frogs and toads; urodeles, or salamanders, including newts; and apodans or caecilians, legless neotropical animals that are relatively poorly understood. Amphibians lack a diaphragm, and as they draw air into and out of the lungs by expanding and contracting the buccopharyngeal cavity, air passes across the sensory epithelium in the nasal cavity (Jørgensen, 2000). Salamanders have been observed to do the same underwater, allowing for olfactory sampling of the aqueous environment (Jørgensen, 2000).

The vomeronasal system is generally present throughout life in amphibians. Among salamanders, the system is present in both aquatic and terrestrial species, including those that never metamorphose. The vomeronasal system has been described in cryptobranchids, sirenids, ambystomatids, salamandrids, amphiumids, and plethodontids, but is absent in members of the proteid family (Anton, 1908, 1911; Eisthen, 2000; Eisthen *et al.*, 1994; Saito *et al.*, 2003; Schmidt and Roth, 1990; Seydel, 1895; Stuelpnagel and Reiss, 2005). Given the phylogenetic relationships among salamander families (Frost *et al.*, 2006), either a vomeronasal-like system arose independently at least four times in salamanders, or it was present in the last common ancestor of extant salamanders and lost in proteids. Clearly, the latter is the most parsimonious hypothesis. The vomeronasal system is also present throughout life in both metamorphosing and direct-developing frogs, including those that are fully aquatic as adults (Cooper, 1943; Hansen *et al.*, 1998; Jermakowicz *et al.*, 2004; Nezlin and Schild, 2000; Reiss and Burd, 1997b, 1997a; Scalia, 1976; Zwilling, 1940), as well as in caecilians (Billo and Wake, 1987; Schmidt and Wake, 1990).

Although anurans are often considered to rely almost entirely on visual and acoustic cues, olfaction plays an important role in the lives of some species (Waldman and Bishop, 2004). For example, tadpoles reduce their activity or seek refuge when exposed to odorants from sympatric predators, injured conspecifics, or predators that have consumed conspecifics (e.g., Laurila *et al.*, 1997; Marquis *et al.*, 2004). Sustained exposure to such cues can also lead to more dramatic changes in morphology and life history (Chivers *et al.*, 2001). Juvenile toads (*Bufo cognatus* and *B. microscaphus*) avoid chemical cues from predatory garter snakes, *Thamnophis* (Flowers and Graves, 1997). Wood frog tadpoles (*Rana sylvatica*) prefer to school with kin (Waldman, 1982, 1984), and kin recognition is mediated by olfactory cues (Waldman, 1985). Some tree frogs that lay eggs in small pools of water in plants provide additional trophic eggs to ensure adequate food supplies for tadpoles. In one such species, *Chirixalus eiffingeri*, tadpoles become highly active when exposed to water conditioned by

adult females (Kam and Yang, 2002), suggesting that tadpoles associate odorants from females with food.

Olfactory cues are also used by adult frogs, particularly in relation to reproductive behavior. Olfaction is involved in orientation to breeding ponds (Ishii *et al.*, 1995), and some frogs prefer their own odorants to those from conspecifics (Waldman and Bishop, 2004). Male magnificent tree frogs, *Litoria splendida,* produce a pheromone that attracts females (Wabnitz *et al.*, 1999). Males of some other *Litoria* species have a rostrally directed spike on the tip of the snout, and the surface epithelium is rich in secretory glands (Menzies, 1993). Although the function of this spike is unknown, its sexually dimorphic distribution and glandular surface suggest that it is involved in chemical communication related to reproduction (Menzies, 1993). Adult *Xenopus* can also use odorant cues in air to find food (Shinn and Dole, 1978).

The role of olfaction in salamander behavior has been examined much more extensively than that in frogs. Like tadpoles, salamanders respond to odorants from injured conspecifics (Chivers *et al.*, 1996b, 1997). Larval tiger salamanders (*Ambystoma tigrinum*) are facultative cannibals, and larvae are more likely to become cannibals in mixed-sibship groups than when surrounded by siblings (Pfennig and Collins, 1993). Cannibals prefer to consume conspecifics that are not kin, but this discrimination disappears when the nostrils are plugged, implicating olfaction in the kin recognition process (Pfennig *et al.*, 1994).

Chemical cues are involved in social behavior in salamanders, and odorants from conspecifics are involved in aggregation (Secondi *et al.*, 2005), individual recognition (Jaeger, 1981; Ovaska, 1988), and marking territories (Chivers *et al.*, 1996b; Jaeger, 1986; Ovaska and Davis, 1992; Simons *et al.*, 1994). Salamanders also use chemical cues to discriminate the sex and reproductive condition of conspecifics (Marco *et al.*, 1998; Park *et al.*, 2004; Verrell, 1985), and the use of pheromones in courtship behavior appears to be widespread. For example, in two species of fire-belly newts (*Cynops*), males produce a species-specific peptide that attracts females (Kikuyama *et al.*, 1995; Yamamoto *et al.*, 2000). In plethodontid salamanders, (*Desmognathus ochrophaeus* and *Plethodon jordani*), male pheromones can increase female receptivity, and in red-spotted newts (*Notophthalmus viridescens*) unreceptive females can become receptive when exposed to chemical cues from a male (Houck and Reagan, 1990; Rogoff, 1927; Rollmann *et al.*, 1999). Interestingly, in red-spotted newts, males are less attracted to odorants from females engaged in courtship behavior than females that are not, even when male odorants are present in both situations (Park and Propper, 2001; Park *et al.*, 2005). Nevertheless, not all pheromone effects in salamanders are mediated by nasal chemosensory systems: in many plethodontid salamanders, males use their teeth to inject pheromones through the skin of females, thereby increasing their receptivity (Houck and Arnold, 2003).

The behavior of caecilians has not been examined in detail. Nevertheless, Himstedt and Simon (1995) showed that plugging the nostrils in *Ichthyophis kohtaoensis* disrupts foraging, suggesting that olfaction plays an important role in this behavior. Chemical cues have been shown to play a role in both aggregation and individual recognition in *Typhlonectes natans* (Warbeck and Parzefall, 2001).

The relative contributions of the olfactory and vomeronasal systems to amphibian behavior are unclear. The vomeronasal organ plays a role in predation in the red-backed salamander, *Plethodon cinereus* (Placyk and Graves, 2002). In both salamanders and caecilians, the relative size of the vomeronasal organ is larger in aquatic or semiaquatic species than in terrestrial species (Dawley, 1998; Schmidt and Wake, 1990), suggesting that aquatic amphibians may rely more on vomeronasal input than do terrestrial species. The vomeronasal organ tends to be larger in male than female plethodontid salamanders, and organ size increases during the breeding season, suggesting a role in reproductive behavior (Dawley, 1998; Dawley *et al.*, 2000). Indeed, in female salamanders (*Cynops pyrrhogaster* and *Plethodon jordani*), male-produced attraction pheromones elicit physiological responses from the vomeronasal organ, but not from the olfactory epithelium (Kikuyama and Toyoda, 1999; Wirsig-Wiechmann *et al.*, 2002b). Nevertheless, EOG recordings demonstrate that both the olfactory and vomeronasal epithelia respond to pheromones in female red-bellied newts (*C. pyrrhogaster*) and male red-spotted newts (*N. viridescens*), and that both epithelia respond more strongly to odorants from the opposite sex than the same sex in male and female axolotls, *Ambystoma mexicanum* (Park *et al.*, 2004; Park and Propper, 2002; Toyoda *et al.*, 1999).

The morphology of the nasal cavity varies considerably among amphibian groups, and often changes during metamorphosis. Many of the anatomical differences associated with life in water and on land have recently been reviewed by Reiss and Eisthen (2006). Perhaps the most dramatic changes occur during metamorphosis in frogs. For example, larval African clawed frogs, *Xenopus laevis*, possess

only one olfactory chamber, which is used for olfaction in water. This chamber contains both ciliated and microvillar receptor neurons (Hansen *et al.*, 1998). During metamorphosis, this chamber is transformed into the principal cavity, which functions for detecting odorants in air, and a new middle chamber develops (reviewed in Hansen *et al.*, 1998; Reiss and Eisthen, 2006). In other frogs, this middle cavity is nonsensory, but in *Xenopus* the middle cavity is lined with sensory epithelium and functions in olfaction underwater (Altner, 1962; Paterson, 1951). Adult *Xenopus* are almost entirely aquatic, and sample odorants in both at the surface and in water. A muscular valve shunts the medium into one chamber or the other (Altner, 1962). In all frogs studied to date, including *Xenopus*, the adult principal cavity contains only ciliated receptor neurons, but the middle cavity of *Xenopus* contains both ciliated and microvillar receptor neurons (Bloom, 1954; Hansen *et al.*, 1998; Mair *et al.*, 1982; Menco, 1980; Oikawa *et al.*, 1998; Reese, 1965; Taniguchi *et al.*, 1996). Because the principal cavity that detects waterborne odorants in larvae is transformed into the principal cavity that detect airborne odorants in adult, a clear correlation emerges: in *Xenopus*, ciliated receptor neurons are used for olfaction in air, and both ciliated and microvillar receptor neurons are used for olfaction in water (Hansen *et al.*, 1998).

In addition to these morphological differences, the sensory epithelia in the principal and middle cavities of *Xenopus* differ at the molecular level. Freitag *et al.* (1995, 1998) divide the odorant receptor genes in frogs into two classes: class I genes, with a large loop in the third extracellular domain and sequences similar to those found in teleosts, and class II genes, similar to those found in mammals. In *Xenopus*, class II genes are expressed in principal cavity and are proposed to function for detecting odorants in air, and class II are expressed in middle cavity and are proposed to function for detecting odorants in water (Freitag *et al.*, 1995; Mezler *et al.*, 2001). The principal cavity also contains a novel form of $G\alpha_s$ that is more closely related to the mammalian $G\alpha_{olf}$ than to other forms of $G\alpha_s$ in frogs, and that induces cAMP formation. In contrast, the epithelium in the middle cavity contains $G\alpha_{o1}$, which stimulates formation of IP_3 (Mezler *et al.*, 2001). Two different homologues of mammalian olfactory marker protein have been found in *Xenopus*, and they are differentially expressed in the two olfactory cavities (Rössler *et al.*, 1998).

Odorant binding proteins, suggested to be a mammalian adaptation, have been found in *Rana pipiens*, *Xenopus laevis*, and *Xenopus tropicalis* (Lee *et al.*, 1987; Millery *et al.*, 2005). As in mammals, odorant binding proteins in frogs appear to be lipocalins. Interestingly, in both species of *Xenopus*, the protein is expressed in principal cavity but not middle cavity (Millery *et al.*, 2005). This observation lends support to the hypothesis that the function of odorant binding proteins is related to physical constraints imposed by the problem of detecting odorants in air; for example, odorant binding proteins may function to transport hydrophobic molecules through aqueous lymph or mucus to the odorant receptors (Bignetti *et al.*, 1987; Vogt, 1987).

The nasal sac of salamanders is essentially a simple oval-shaped tube that is almost completely lined with sensory epithelium, which is one of the reasons why some electrophysiologists favor salamanders as model animals for olfactory research (e.g., Kauer, 2002). The olfactory epithelium of most adult salamanders, even those that are fully terrestrial, appears to contains both ciliated and microvillar receptor cells (Breipohl *et al.*, 1982; Eisthen, 2000; Eisthen *et al.*, 1994; Farbman and Gesteland, 1974; Jones *et al.*, 1994). In contrast, four types of olfactory receptor neurons have been described in *Dicamptodon tenebrosus*: those with cilia, with long microvilli, with unusual short microvilli, and those with both cilia and extremely short (<0.5 μm) microvilli (Stuelpnagel and Reiss, 2005). This finding suggests that other salamanders may possess additional types of neurons that have not been distinguished. Thirty-five putative odorant receptor genes have been sequenced from tiger salamanders (*A. tigrinum*), and all appear to be class II genes (Marchand *et al.*, 2004). Because terrestrial tiger salamanders were used, this result appears to support the Freitag *et al.* (1998) hypothesis that odorant receptors in the class II gene family function to detect odorants in air. Nevertheless, mudpuppies (*Necturus maculosus*), which are members of the fully aquatic proteid family, possess both class I and class II odorant receptor genes (Zhou *et al.*, 1996), casting doubt on the distinction. Olfactory transduction in mudpuppies also involves a novel cyclic nucleotide-dependent chloride current (Delay *et al.*, 1997). The existence of this current in mudpuppies may represent an environmental adaptation to life in freshwater, in which the ionic concentrations of the olfactory mucus may be difficult to regulate. If so, the external calcium required to gate the chloride channels widely involved in olfactory transduction may not reliably be present, leading to the use of a different gating mechanism in these animals (Delay *et al.*, 1997).

Caecilians have paired nares that open into the nasal cavity, which contains the olfactory

epithelium. Adult caecilians also have short tentacles between the naris and eye that are both chemosensory and somatosensory (Badenhorst, 1978). Caecilians are blind, and the tentacles are derived from modified eye structures (Billo and Wake, 1987). The lumen of the tentacle carries secretions from the Harderian gland and communicates with the vomeronasal organ (Badenhorst, 1978; Schmidt and Wake, 1990), an arrangement that allows the animal to detect chemicals with the vomeronasal organ even while burrowing or swimming, when the external nares are closed (Prabha *et al.*, 2000). The nasal cavity of *Typhlonectes compressicaudum* contains two morphologically distinct types of olfactory epithelium. The dorsocaudal portion of the nasal cavity contains an epithelium with only ciliated olfactory receptors neurons, whereas the anterior ventral epithelium contains both ciliated and microvillar receptor neurons (Saint Girons and Zylberberg, 1992). Given their relative locations, these observations suggest that, as in *Xenopus*, caecilians use morphologically different receptor neurons for detecting odorants in water and air (Reiss and Eisthen, 2006).

As in other tetrapods, the vomeronasal receptor neurons in frogs, salamanders, and caecilians terminate in microvilli (Eisthen, 2000; Eisthen *et al.*, 1994; Franceschini *et al.*, 1991; Kolnberger, 1971; Kolnberger and Altner, 1971; Oikawa *et al.*, 1998; Saint Girons and Zylberberg, 1992). In addition to the receptor neurons, sustentacular cells, and basal cells that are generally present in the vertebrate olfactory and vomeronasal epithelia, the vomeronasal epithelium of frogs and salamanders contains large ciliated supporting cells that may function to move fluid across the surface of the epithelium (Eisthen, 1992). The vomeronasal epithelium in *Xenopus* has been shown to express members of the V2R family of vomeronasal receptor genes as well as $G\alpha_o$, the G-protein that is co-expressed with V2Rs in mammals (Hagino-Yamagishi *et al.*, 2004). Although a portion of the principal cavity containing olfactory epithelium was reported to express V2R genes (Hagino-Yamagishi *et al.*, 2004), the authors appear to have misidentified the posterior portion of the vomeronasal organ, which is longer in *Xenopus* than in other frogs (Reiss and Eisthen, 2006).

The olfactory bulbs of amphibians display a moderate degree of lamination, and include both internal and external plexiform layers in all groups (Herrick, 1948; Hoffman, 1963; Nieuwenhuys, 1967). Unlike in any other group of vertebrates, the olfactory bulb of salamanders does not consist of concentric layers; rather, the olfactory nerve enters from the rostrolateral pole, and the layers progress in wide bands from rostral to caudal (e.g., Herrick, 1948). In frogs, the layers are not completely concentric (Scalia *et al.*, 1991b).

In addition, in frogs the two olfactory bulbs are fused across the midline (Hoffman, 1963), a condition also observed in some teleost fishes and bird species (Nieuwenhuys, 1967). Morphologically, the two bulbs in frogs appear to be organized almost as a single unit. For example, the axons of some olfactory receptor neurons cross the midline to project to the contralateral bulb (Hoffman, 1963). The laminae within the bulb are continuous across the midline (Hoffman, 1963; Scalia *et al.*, 1991b), and the processes of both mitral and granule cells cross the midline to interact with elements in the contralateral olfactory bulb (Herrick, 1921; Ramón y Cajal, 1922; Scalia *et al.*, 1991b). Field potential recordings demonstrate that the responses to unilateral stimulation of the olfactory nerve are similar in both olfactory bulbs, suggesting that signal transmission is fully bilateral (Andriason and Leveteau, 1989). However, recordings from mitral cells indicate that unilateral nerve stimulation leads to an inhibition of evoked responses on the contralateral side (Leveteau *et al.*, 1993). The authors of this study suggest that the contralateral inhibition is a mechanism for enhancing contrast between the two sides, facilitating odorant localization. If so, perhaps the joint olfactory bulbs of frogs allow them to better localize odorants than salamanders, which have separate olfactory bulbs.

In both salamanders and frogs, the axons of olfactory receptor neurons have been shown to branch upon entering the outer fiber layer of the bulb and innervate glomeruli that are widely spaced (Herrick, 1924, 1931; Nezlin and Schild, 2005). Periglomerular cells are present, although they do not surround and isolate individual glomeruli to the same extent observed in mammals (Herrick, 1924, 1931; Nezlin *et al.*, 2003; Nezlin and Schild, 2000; Ramón y Cajal, 1890). The primary dendrites of the mitral cells arborize in multiple glomeruli (Herrick, 1948; Hoffman, 1963). Mitral cells also bear secondary dendrites that do not terminate in glomeruli and appear to make synapses with processes of granule cells; these neurites are more prominent in frogs than in salamanders (Scalia *et al.*, 1991b). Herrick (1924) describes 'atypical mitral cells' in the external plexiform layer in tiger salamanders that could be homologous with the tufted cells found in mammals, and Scalia *et al.* (1991b) described candidate tufted cells in a similar location in northern leopard frogs (*Rana pipiens*). Axonless granule cells are present. The dendrites of these cells are spiny in

frogs and tiger salamanders, but smooth in mudpuppies (Herrick, 1924, 1931; Hoffman, 1963; Scalia *et al.*, 1991b). Amphibians appear to lack stellate cells. The structure of the accessory olfactory bulb is similar to that of the olfactory bulb, but plexiform layers are absent and the boundaries of the layers are less distinct (Herrick, 1924, 1931; Nieuwenhuys, 1967). In salamanders, some mitral cells have dendrites that project to glomeruli in both the olfactory and accessory olfactory bulbs (Herrick, 1924).

Central olfactory projections have been examined in several anurans, including *Xenopus* and ranid and hylid frogs, as well as in tiger salamanders, *Ambystoma tigrinum* (Kemali and Guglielmotti, 1987; Kokoros and Northcutt, 1977; Northcutt and Royce, 1975; Roden *et al.*, 2005; Scalia, 1972; Scalia *et al.*, 1991a). As shown in Figure 8a, the medial olfactory tract projects to the postolfactory eminence and medial pallium as well as to the lateral and medial septal nuclei (Northcutt and Royce, 1975; Roden *et al.*, 2005; Scalia *et al.*, 1991a). The lateral olfactory tract projects ipsilaterally to the lateral pallium, dorsal striatum, lateral amygdala, and a region interpreted as either the dorsal pallium or the dorsal portion of the lateral pallium (Figure 8b; Moreno *et al.*, 2005; Northcutt and Royce, 1975; Scalia *et al.*, 1991a). Fibers of the medial and lateral olfactory tracts project in combination to the contralateral amygdala and lateral pallium. The accessory olfactory tract projects bilaterally to the medial amygdala (Figure 8d), the rostral portion of which also receives olfactory input (Kemali and Guglielmotti, 1987; Moreno and Gonzalez, 2003; Moreno *et al.*, 2005; Scalia *et al.*, 1991a). The extrabulbar olfactory pathway of *Xenopus* has been examined in detail by Hofmann and Meyer, who have found that fibers originating in the olfactory epithelium bypass the olfactory bulb and terminate in the ipsilateral preoptic area and bilaterally in the hypothalamus (Figure 8c; Hofmann and Meyer, 1991a, 1991b; 1992). The pathway that Schmidt and colleagues (Schmidt *et al.*, 1988; Schmidt and Wake, 1990) described as the terminal nerve in salamanders and caecilians is more likely the extrabulbar olfactory

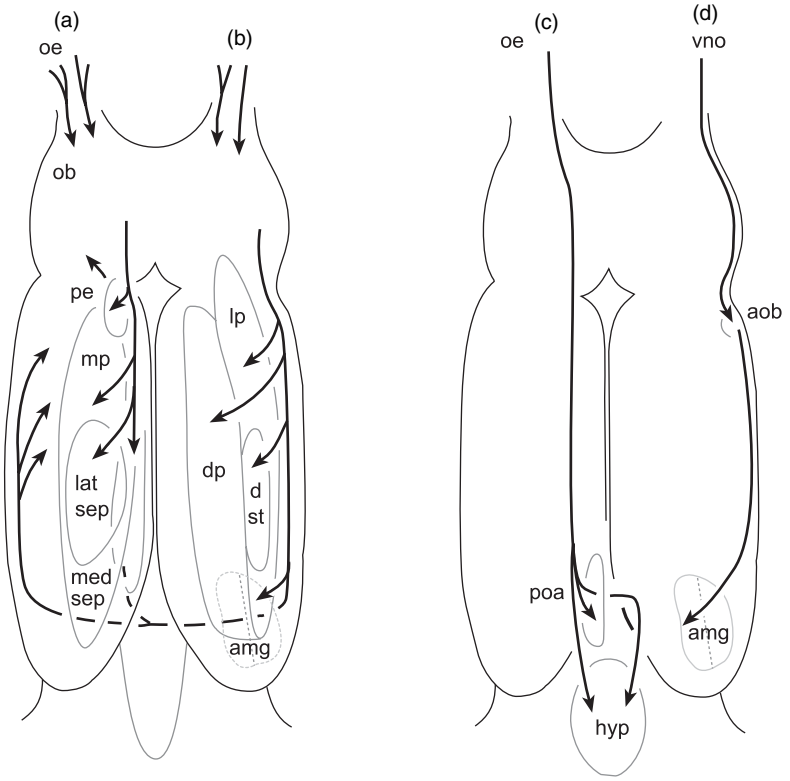

Figure 8 Schematic dorsal view of the forebrain in ranid frogs. a, Projections of the medial olfactory tract. b, Projections of the lateral olfactory tract. c, Projections of the extrabulbar olfactory pathway. d, Projections of the accessory olfactory tract. amg, amygdala; aob, accessory olfactory bulb; dp, dorsal pallium; d st, dorsal striatum; hyp, hypothalamus; lat sep, lateral septum; lp, lateral pallium; med sep, medium septum; mp, medial pallium; ob, olfactory bulb; oe, olfactory epithelium; pe, postolfactory eminence; poa, preoptic area; vno, vomeronasal organ. Based on Kemali and Guglielmotti (1987), Northcutt and Royce (1975), Scalia (1972), and Scalia *et al.* (1991a).

pathway, as it was labeled by injection of tracer into the nasal cavity but not vomeronasal organ, and ganglion cells were not found. If this interpretation is correct, the extrabulbar pathway may be more extensive in both salamanders and caecilians than in frogs (Hofmann and Meyer, 1989).

The terminal nerve of both frogs and salamanders has been examined in detail. The cells and fibers of the nerve are immunoreactive for a variety of compounds, including GnRH (D'Aniello *et al.*, 1994b, 1995; Muske and Moore, 1988; Northcutt and Muske, 1994; Sherwood *et al.*, 1986), NPY (D'Aniello *et al.*, 1996a; Lázár *et al.*, 1993; Mousley *et al.*, 2006; Tuinhof *et al.*, 1994), and FMRFamide (D'Aniello *et al.*, 1996b; Muske and Moore, 1988; Northcutt and Muske, 1994). In tiger salamanders (*Ambystoma tigrinum*), the terminal nerve also displays acetylcholinesterase activity, indicating that acetylcholine is present (Wirsig and Getchell, 1986). In frogs, the terminal nerve may send an additional projection to the retina (Uchiyama *et al.*, 1988; Wirsig-Wiechmann and Basinger, 1988).

In salamanders, terminal nerve-derived peptides alter both the odorant sensitivity and excitability of olfactory receptor neurons. The olfactory epithelium appears to contain GnRH receptors (Wirsig-Wiechmann and Jennes, 1993), and GnRH modulates both odorant responses and the voltage-dependent, tetrodotoxin-sensitive sodium current in olfactory receptor neurons (Eisthen *et al.*, 2000; Park and Eisthen, 2003). Interestingly, olfactory receptor neurons are more responsive to GnRH during the breeding season, suggesting that GnRH may play a role in regulating responses to odorants in a way that promotes reproduction (Eisthen *et al.*, 2000). Other studies show that estrogen affects GnRH concentrations in the terminal nerve in *Xenopus* (Wirsig-Wiechmann and Lee, 1999) and that concentrations are higher in courted female salamanders than in uncourted conspecifics (Propper and Moore, 1991), lending further support to the idea that the GnRH-containing fibers of the terminal nerve play a role in reproductive behavior. In addition, FMRFamide modulates both odorant responses and the sodium current in olfactory receptor neurons (Park *et al.*, 2003). Recently, we have also shown that the terminal nerve in axolotls (*Ambystoma mexicanum*) is NPY-immunoreactive, and that application of synthetic axolotl NPY modulates both odorant responses and the sodium current, but only in hungry animals (Mousley *et al.*, 2006). Taken together, these data suggest that terminal nerve modulates responsivity in the olfactory epithelium in concert with changes in internal state, perhaps to maximize sensitivity to odorants most relevant to the animal's condition.

2.17.2.6.2 Reptiles The class Reptilia includes rhynchocephalians, or tuatara; squamates, a group that consists of lizards, snakes, and amphisbaenians; crocodilians; and turtles. Because birds (Aves) form the sister group to extant crocodilians, we include birds in this group.

2.17.2.6.2.(i) Tuatara The rhynchocephalians, or tuatara, constitute a separate order of reptiles. The two living species of tuatara, *Sphenodon punctatus* and *S. guntheri*, are found only on small islands off the coast of New Zealand. Because of their phylogenetic importance as a sister group to squamate reptiles, their chemosensory systems have been studied to a limited extent.

Unlike snakes and some lizards, tuatara do not have a forked tongue (Schwenk, 1986), and they do not tongue-flick (Walls, 1981). The role of the olfactory and vomeronasal systems in tuatara feeding has been reviewed by Schwenk (2000). Much of the available evidence is anecdotal (e.g., Walls, 1981), but suggests that visual cues predominate and that chemoreception plays a secondary role in foraging and food choice (Schwenk, 2000). Tuatara will bite cotton that has been swabbed on prey, demonstrating that the animals will respond to chemical cues related to feeding (Cooper *et al.*, 2001). The role of chemical signals in social and reproductive behavior is unknown.

The nasal cavity of *Sphenodon* is fairly simple in organization, although some conchae are present. A long choana connects the ventral nasal cavity to the oral cavity. Olfactory epithelium lines the posterior portion of the dorsal half of the nasal cavity, and the vomeronasal sensory epithelium is confined to the dorsal portion of the small vomeronasal organ, which lies along the septum. The vomeronasal organ opens into the ventromedial portion of the nasal cavity, anterior to the choana. Unlike that of snakes and lizards, the vomeronasal organ has no direct connection to the oral cavity. The ultrastructure of the olfactory and vomeronasal epithelia has not been described. The axons of the vomeronasal receptor neurons join the olfactory nerve to project to a small accessory olfactory bulb dorsomedial to the much larger main olfactory bulb (Hoppe, 1934, in Parsons, 1959a; Parsons, 1967). Cairney (1926) described the olfactory projections in normal material without distinguishing among the tracts, deducing that the olfactory bulbs project to the medial pallium, septum, olfactory tubercle, olfactostriatum, lateral pallium (piriform cortex),

anterior nucleus of the amygdala, and a region he called the 'hypopallium posterius'. The nucleus sphericus of squamates has a roughly similar position and appearance, and receives a large projection from the accessory olfactory bulb. The nucleus sphericus has been thought to be present only in squamates (Northcutt, 1978), but without studying projections in tuatara using modern tract-tracing techniques, the uniqueness of this structure cannot be determined.

2.17.2.6.2.(ii) Squamates: Amphisbaenians, lizards, and snakes Squamate reptiles are diverse and widespread, and many species rely heavily on chemosensory input to mediate all aspects of their behavior. In many snakes and lizards the vomeronasal system is behaviorally more important than the olfactory system and, in some species, the neural structures that comprise the vomeronasal system are hypertrophied relative to those of the olfactory system. Although squamates are favored model animals for neurobiologists seeking to understand the structure and function of the vomeronasal system, this endeavor is paradoxically complicated by the dominance of the vomeronasal system: in many squamates, the behavioral relevance of olfactory information is not clear. This broad generalization does not apply to all squamates, however. For example, in geckos the olfactory system appears to be the dominant chemosensory system (Schwenk, 1993b).

The nasal chemosensory systems play a critical role in foraging and feeding in many squamates. For example, pygmy rattlesnakes (*Sistrurus miliarius*), which are sit-and-wait predators, aggregate in areas containing chemical cues from potential prey animals (Roth *et al.*, 1999). Snakes are well-known to follow chemical trails left by prey (Schwenk, 1994), as can some lizards, like Gould's monitor lizards, *Varanus gouldii*, and Gila monsters, *Heloderma suspectum* (Garrett *et al.*, 1996). Rattlesnakes, which strike and release their prey, use chemical cues to follow and identify envenomated animals. These cues derive from the venom itself as well as recognition of the envenomated individual (Chiszar *et al.*, 1999; Furry *et al.*, 1991; Lavin-Murcio and Kardong, 1995). Both snakes and lizards with experimentally impaired vomeronasal systems will attack but not consume prey, and garter snakes (*Thamnophis sirtalis*) with sectioned vomeronasal nerves stop following prey trails and eventually cease eating (Alving and Kardong, 1996; Graves and Halpern, 1990; Halpern *et al.*, 1997; Haverly and Kardong, 1996).

Chemical cues can facilitate predator avoidance. The Texas banded gecko, *Coleonyx brevis*, displays defensive behavior when presented with odorants from a predatory snake; changes in the rate of buccal pulsing in response to these cues suggest that the discrimination is based on input to the olfactory system (Dial and Schwenk, 1996). The ability to discriminate harmful from harmless species based solely on chemical cues has been demonstrated in lizards (Lacertidae, Iguanidae, and Anguidae) and in amphisbaenians, *Blanus cinereus* (Amo *et al.*, 2004; Bealor and Krekorian, 2002; Cabido *et al.*, 2004; Lopez and Martin, 1994, 2001; Van Damme and Quick, 2001). Vomeronasal input is critical to the ability of crotaline snakes to recognize predatory kingsnakes, *Lampropeltis getula* (Miller and Gutzke, 1999). Responsiveness to chemical cues from predators has been shown to increase the probability of surviving an encounter with a predator in garden skinks, *Lampropholis guichenoti* (Downes, 2002).

Chemical cues play a role in discrimination of the sex of conspecifics in amphisbaenians, *Blanus cinereus* (Cooper *et al.*, 1994), in recognition of familiar and unfamiliar conspecifics in lacertid lizards (Aragon *et al.*, 2003; Font and Desfilis, 2002), and in recognition of mates in snow skinks, *Niveoscincus microlepidotus* (Olsson and Shine, 1998). Male skinks (*Eulamprus heatwolei*) use chemosensory cues to assess female receptivity (Head *et al.*, 2005). Mate choice is influenced by chemical cues in snakes (*Thamnophis sirtalis*) and lizards (*Lacerta monticola*) (Lopez *et al.*, 2003; Shine *et al.*, 2003), and female Swedish sand lizards (*Lacerta agilis*) prefer odorants from males that differ from themselves at the MHC class 1 loci (Olsson *et al.*, 2003). Taken together, these observations indicate that squamates can use chemosensory information to discriminate sex, receptivity, quality as a potential mate, genotype, and individual identity of conspecifics. In squamates, pheromonal cues are not always attractants: female brown tree snakes, *Boiga irregularis*, produce a cloacal secretion that decreases courtship intensity and duration in males (Greene and Mason, 2003).

In general, squamates sniff to draw odorants across the olfactory epithelium and use their tongues to physically pick up chemical stimuli that will be deposited in the vomeronasal organ. Geckos, which depend more on olfactory than on vomeronasal input, appear to sniff via oscillations of the throat (Dial and Schwenk, 1996). Garter snakes (*Thamnophis sirtalis*) can detect prey odorants in air, without physical contact with the tongue, using the olfactory system (Halpern *et al.*, 1997).

Olfactory input appears to be important for elevating the rate of tongue-flicking, which then allows the animal to sample with the vomeronasal organ (Halpern *et al.*, 1997; Zuri and Halpern, 2003). Although tongue-flicking is widespread among squamates, not all have forked tongues (Schwenk, 1993a, 1994). Forked tongues have evolved repeatedly among squamates, particularly in groups that forage widely (Schwenk, 1994). This arrangement may be used to enhance two-point comparisons, for example to facilitate trail following by improving edge detection (Schwenk, 1994).

The vomeronasal organ of squamate reptiles opens directly into the oral cavity and has no connection with the nasal cavity, a condition that is different than in any other tetrapod (Schwenk, 1993a). Tongue-flicking brings molecules into the vomeronasal organ (reviewed in Schwenk, 1995; see also Graves and Halpern, 1989; Halpern and Kubie, 1980). Although the tongue tips are essential for vomeronasal sampling, the mechanism by which compounds move from the tongue to the vomeronasal ducts and into the organ is not understood (reviewed in Schwenk, 1994). The sublingual plicae have been suggested to play a key role, perhaps by creating suction within the vomeronasal organ or duct (Young, 1993), but snakes with cauterized plicae are able to find and consume food, and transport of molecules into the vomeronasal organs does not differ between lesioned and control animals (Halpern and Borghjid, 1997). Instead, the heavily vascularized vomeronasal organ may use a pumping mechanism to create suction, similar to a mechanism used by some mammals (Halpern and Martínez-Marcos, 2003).

The squamate nasal cavity has a relatively simple organization, and in most species is essentially an elongated sac, the posterior dorsal portion of which is lined with olfactory epithelium (Parsons, 1959b, 1967). The dorsal wall of the spherical vomeronasal organ is lined with sensory epithelium, and the lumen is made narrow by a ventral protruding structure called the mushroom body. In garter snakes (*Thamnophis sirtalis*), as in other squamates, fluid from Harderian glands flows into vomeronasal organ (Rehorek, 1997; Rehorek *et al.*, 2000a, 2000b). The products of the gland play a critical role in solubilizing a lipophilic pheromone in this species, allowing the pheromone to be detected by vomeronasal receptor neurons (Huang *et al.*, 2006). Animals from which the gland has been removed display impaired courtship behavior and prey capture, demonstrating the essential role of Harderian gland secretions in signal detection in the vomeronasal system in snakes (Mason *et al.*, 2006).

Both cilia and short microvilli are present together on the olfactory receptor cells of the blue-tongued lizard, *Tiliqua scincoides* (Kratzing, 1975), although only ciliated receptor cells were found in a scanning electron microscopic investigation of the olfactory epithelium in the garter snakes *Thamnophis sirtalis* and *T. radix* (Wang and Halpern, 1980b). The morphology of receptor neurons may vary between species, but it also seems possible that short microvilli could have been overlooked or difficult to see in the preparations from snakes. Both lizards and snakes possess only microvillar vomeronasal receptor neurons (Altner and Brachner, 1970; Altner and Müller, 1968; Bannister, 1968; Kratzing, 1975; Takami and Hirosawa, 1990; Wang and Halpern, 1980a, 1980b). The olfactory and vomeronasal receptor genes from squamates have not yet been sequenced. In snakes and lizards, the vomeronasal epithelium expresses $G\alpha_o$ and $G\alpha_{i2}$, which are also found in the vomeronasal epithelium of mammals, but lack $G\alpha_{olf}$, $G\alpha_{11}$, and $G\alpha_q$, which are found in the mammalian olfactory epithelium (Labra *et al.*, 2005; Luo *et al.*, 1994). The vomeronasal epithelium in garter snakes expresses type IV adenylyl cyclase (Liu *et al.*, 1998), as does that of rats (Rössler *et al.*, 2000).

The olfactory bulb of squamates is distinctly laminated, with both external and internal plexiform layers present. In adult lizards (*Podarcis hispanica*), periglomerular cells have a single dendrite, oriented parallel to the bulbar surface (García-Verdugo *et al.*, 1986). Each mitral cell has one primary dendrite that arborizes in a single glomerulus, as well as secondary dendrites that travel through the external plexiform layer. In contrast, in embryonic snakes (*Elaphe quadrivirgata*), the primary dendrites of mitral cells arborize in many glomeruli (Iwahori *et al.*, 1989a). In both animals, the spiny dendrites of the axonless granule cells extend into the plexiform layer, but do not arborize in the glomeruli (García-Verdugo *et al.*, 1986; Iwahori *et al.*, 1989a).

The accessory olfactory bulb lacks the clear laminar organization of the olfactory bulb, and plexiform layers are absent (Iwahori *et al.*, 1989b; Llahi and García-Verdugo, 1989). In *Podarcis*, incoming axons of vomeronasal receptor neurons branch and enter more than one glomerulus, which does not appear to be the case for olfactory receptor cell axons entering the main olfactory bulb (García-Verdugo *et al.*, 1986; Llahi and García-Verdugo, 1989). The dendrites of periglomerular cells arborize in more than glomerulus, as do those of the mitral cells (Iwahori *et al.*, 1989b; Llahi and

García-Verdugo, 1989). Mitral cells also possess secondary dendrites that extend through the external and internal plexiform layers (Iwahori *et al.*, 1989b; Llahi and García-Verdugo, 1989). A class of cells that Llahi and García-Verdugo call 'small mitral cells' have a soma in the outer mitral cell layer or external plexiform layer and a single dendrite that arborizes sparsely in the glomerular layers; these cells might be equivalent to the tufted cells present in the mammalian olfactory bulb (Llahi and García-Verdugo, 1989). The granule cells resemble those in the main olfactory bulb (Iwahori *et al.*, 1989b; Llahi and García-Verdugo, 1989). Stellate cells do not seem to be present in the main or accessory olfactory bulbs in either species.

Among squamates, the central olfactory projections have been described in most detail in garter snakes, *Thamnophis sirtalis* and *T. radix* (Halpern, 1976; Lanuza and Halpern, 1997, 1998). Three central olfactory projections are present in these animals, comprising the lateral, intermediate, and medial olfactory tracts, illustrated in Figure 9. The medial olfactory tract projects ipsilaterally to the anterior olfactory nucleus (Figure 9a). The lateral olfactory tract projects bilaterally to the lateral cortex, as well as to the external and ventral anterior amygdala (Figure 9b). The intermediate olfactory tract projects to the olfactory tubercle and olfactory gray, and joins the lateral olfactory tract in a projection to the contralateral hemisphere (Figure 9c). The accessory olfactory tract, carrying information from the vomeronasal organ, projects to three portions of the amygdala: the nucleus sphericus, medial amygdala, and nucleus of the accessory olfactory tract (Figure 9d). The homology of the nucleus sphericus with regions of the amygdala in other tetrapods is unclear, because it has been suggested to be the only amygdalar target of the accessory olfactory bulb that does not project to the hypothalamus (Bruce and Neary, 1995; Lanuza and Halpern, 1997). This interpretation is complicated by newer data demonstrating a small projection from the nucleus sphericus to the hypothalamus, and a much larger projection to the olfactostriatum (Martínez-Marcos *et al.*, 1999, 2002). The latter structure in turn projects to the lateral posterior hypothalamic nucleus, and may be homologous with the nucleus accumbens (Martínez-Marcos *et al.*, 2005a, 2005b). Thus, the nucleus sphericus may be unique to squamates, may be unique to lepidosaurs (squamates and tuatara), or may be homologous with amygdalar regions in other vertebrates but have reorganized connections in squamates.

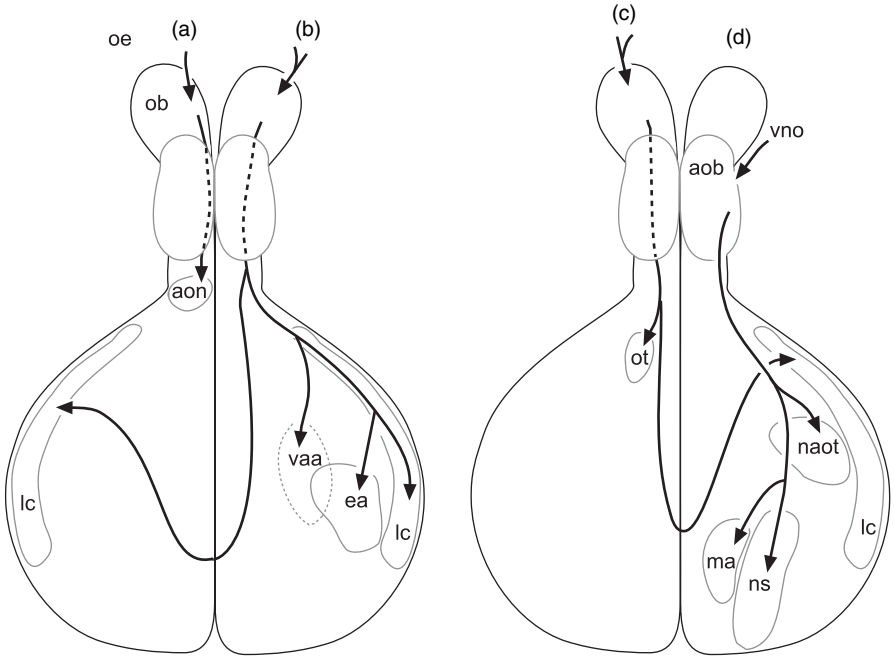

Figure 9 Schematic dorsal view of the forebrain in garter snakes, *Thamnophis sirtalis*. a, Projections of the medial olfactory tract. b, Projections of the lateral olfactory tract. c, Projections of the intermediate olfactory pathway. d, Projections of the accessory olfactory tract. aob, accessory olfactory bulb; aon, anterior olfactory nucleus; ea, external amygdala; lc, lateral cortex; ma, medial amygdala; naot, nucleus of the accessory olfactory tract; ns, nucleus sphericus; ob, olfactory bulb; oe, olfactory epithelium; ot, olfactory tubercle; vaa, ventral anterior amygdala. Based on Halpern (1976), Lanuza and Halpern (1997, 1998), and Lohman and Smeets (1993).

The medial amydala in garter snakes receives a direct projection from the accessory olfactory bulb, as well as indirect vomeronasal input via the nucleus sphericus. The olfactory bulb has an indirect projection as well, via the external and ventral anterior amygdaloid nuclei (Lanuza and Halpern, 1997, 1998; Martínez-Marcos *et al.*, 1999). Because the medial amygdala projects to the lateral posterior hypothalamic nucleus, which in turn projects to the hypoglossal nucleus, this pathway has been suggested to function in control of tongue-flicking in response to chemosensory input in snakes (Martínez-Marcos *et al.*, 2001).

Olfactory projections have also been examined in several lizards, including *Gekko gecko*. The lateral olfactory tract projects bilaterally to the entire length of the lateral cortex, as well as to the anterior olfactory nucleus, external amygdaloid nucleus, and perhaps the central amygdaloid nucleus. An intermediate olfactory tract projects to the olfactory tubercle and joins the lateral olfactory tract to project to the contralateral hemisphere. A short medial olfactory tract is present. Finally, an accessory olfactory tract projects ipsilaterally to the nucleus sphericus, external and central amygdaloid nuclei, and bed nucleus of the stria terminalis (Lohman and Smeets, 1993; Lohman *et al.*, 1988). In *Tupinambis teguixin*, the projections are similar, although the projections to the contralateral hemisphere differ somewhat between the two species (Lohman and Smeets, 1993). To our knowledge, an extrabulbar olfactory pathway has not been described in squamates.

The anti-peptide antisera often used to identify the terminal nerve in other vertebrates produce mixed results with squamates. In adult garter snakes (*Thamnophis sirtalis*), a GnRH-immunoreactive terminal nerve is present, with a discrete ganglion at the ventral border between the olfactory bulb and rostral telencephalon (Smith *et al.*, 1997). Intraventricular administration of a GnRH antagonist interferes with courtship behavior in these animals, although the relative contributions of the terminal nerve and central GnRH neurons to this effect cannot be determined (Smith and Mason, 1997). Among lizards, GnRH-immunoreactive cells and fibers are also present in the terminal nerve of adult *Eumeces laticeps* and *Sceloporus undulatus*, but not in adult *Anolis carolinensis* (Rosen *et al.*, 1997). In *Podarcis sicula*, GnRH-immunoreactive cells and fibers cannot be detected in a terminal nerve, nor in the olfactory epithelium and bulb, in either embryos or adults (D'Aniello *et al.*, 1994a; Masucci *et al.*, 1992). FMRFamide-immunoreactive cells and fibers are present in the terminal nerve during development in *Chalcides chalcides*, but not in adults (D'Aniello *et al.*, 2001). Overall, the available data indicate either that the immunoreactive characteristics of the terminal nerve in some squamate species differ from those in other jawed vertebrates, perhaps a way that varies with developmental stage, or that the nerve is not present at all developmental stages in all squamate species.

2.17.2.6.2.(iii) Crocodilians Crocodiles, alligators, caimans, and gharials are a relatively small group, comprising only 20–25 species. The nasal chemosensory systems have not been extensively studied in this group, and much of the information available concerning the role of chemoreception in behavior is reviewed by Weldon and Ferguson (1993).

The nares of crocodilians are closely situated on the dorsal portion of the snout and can protrude even while most of the animal is submerged under water, suggesting that olfactory cues may serve important functions in crocodilians. Crocodilians draw air through the nasal cavity through buccal oscillations that do not contribute to respiration (Gans and Clark, 1976). Electroencephalograph recordings from the olfactory bulb demonstrate activity coincident with buccal oscillations, demonstrating that this behavior is equivalent to sniffing in crocodilians (Huggins *et al.*, 1968; Naifeh *et al.*, 1970). Because of this demonstrated correlation, buccal pumping is used as a metric of olfactory sampling in some studies. For example, analysis of buccal pumping demonstrates that juvenile alligators (*Alligator mississippiensis*) respond to odorants from food (Weldon *et al.*, 1992). Other studies further demonstrate that olfactory cues play a role in localization of food (Scott and Weldon, 1990; Weldon *et al.*, 1990).

Crocodilians have prominent paracloacal and gular glands, which are suspected to play a role in territorial and sexual behavior (Weldon and Ferguson, 1993). Recent work has described the chemistry of the secretions from these glands (e.g., Garcia-Rubio *et al.*, 2002; Ibrahim *et al.*, 1998; Wheeler *et al.*, 1999; Yang *et al.*, 1999), but the behavioral significance of these compounds has not yet been elucidated. Behavioral observations suggest that crocodilians rub scent glands during courtship and nesting (Weldon and Ferguson (1993) and references therein), suggesting that pheromonal cues may play a role in crocodilian reproductive behavior.

The vomeronasal system has been lost in crocodilians. The vomeronasal organ begins to develop in the embryos of some alligators and crocodiles, but

regresses by the time of hatching (Parsons, 1959a). The accessory olfactory bulb is similarly present in crocodilian embryos but not adults (Parsons, 1967, 1970).

To our knowledge, the ultrastructure of the olfactory epithelium in crocodilians has not been described. The detailed anatomy of the olfactory bulb and tracts has been examined in young *A. mississippiensis* (Crosby, 1917). In these animals, clear laminae, including external and internal plexiform layers, are present. The glomeruli are surrounded by somata of periglomerular cells, which are clearly present. The mitral cells project primary dendrites into two or more glomeruli, but also possess secondary dendrites that extend laterally through the external plexiform layer. Somata in the external plexiform layer may belong to tufted cells, but could also belong to displaced mitral cells. The granule cell layer contains three classes of cells. The first are anaxonal intrinsic cells, with spiny dendrites that extend in all directions within the layer. The second are granule cells with spiny dendrites that project to the glomerular layer but do not seem to arborize inside glomeruli; Crosby (1917) called these 'stellate' cells. The axons of these cells enter the olfactory tracts and may project out of the olfactory bulb. The stellate cells have the smooth dendrites that arborize in glomeruli, and axons that project out of the olfactory bulb; Crosby (1917) called these cells "goblet" cells.

The central projections of the olfactory bulb have been described in *A. mississippiensis* based on Golgi-stained material (Crosby, 1917) and in *Caiman sklerops* based on a degenerating fiber stain (Scalia *et al.*, 1969). In *Alligator*, as in garter snakes, three centripetal tracts emerge from the olfactory bulb. The individual tracts could not be visualized in *Caiman*, but where the same target was noted in both species we will assume that the same tract carries the fibers to it. The lateral tract projects bilaterally to the lateral cortex, amygdala, and lateral portion of the olfactory peduncle. The medial tract projects to the anterior hippocampus and medial septum. The intermediate tract projects to the nucleus of the diagonal band of Broca and bilaterally to the olfactory tubercle. An additional small projection to the internal plexiform layer of the contralateral olfactory bulb was observed in *Caiman*.

Medina *et al.* (2005) report that Nile crocodiles (*Crocodylus niloticus*) possess a GnRH-immunoreactive terminal nerve, but a detailed description of the pathway has not been published. The terminal nerve of the spectacled caiman (*Caiman crocodilus*) displays FMRFamide-like immunoreactivity in embryos, but not adults (D'Aniello *et al.*, 1999, 2001).

2.17.2.6.2.(iv) Birds Although olfaction used to be considered unimportant or even absent in birds, birds possess a robust olfactory system that mediates many types of behavior. Notably, olfaction has been shown to play a role in food finding in brown kiwis (*Apteryx australis*; Wenzel, 1968, 1971), and in some, but not all, species of vultures (Graves, 1992; Houston, 1984), as well as in ravens (*Corvus corax*; Harriman and Berger, 1986), parrots (*Strigops habroptilus*; Hagelin, 2004), and procellariiforms, the group of Antarctic seabirds that includes shearwaters, petrels, and albatrosses (Hutchison and Wenzel, 1980). Some procellariid species are highly sensitive to specific odorants such as dimethyl sulfide and 3-methyl pyrazine that are associated with krill, an important and patchily distributed food source (Nevitt, 2000; Nevitt and Haberman, 2003; Nevitt *et al.*, 1995, 2004). Olfaction plays a role in both feeding and predator avoidance in chickens (reviewed in Jones and Roper, 1997).

Olfaction has also been implicated in homing and navigation by passerines, which may use stable features such as the presence of airborne hydrocarbons to orient within landscapes (reviewed in Wallraff, 2003, 2004). Nevitt and Bonadonna (2005) suggest that Procellariiforms may use dimethyl sulfide in a similar fashion for navigating to small islands in large open ocean areas.

The role of olfaction in reproduction has been examined in only a handful of species. Crested auklets (*Aethia cristatella*) produce a scent that is attractive to conspecifics, which humans perceive as resembling tangerine odor (Hagelin *et al.*, 2003). Antarctic prions, *Pachiptila desolata*, can discriminate between chemical cues from their partners and from other birds in the breeding colony, demonstrating that this species is capable of individual recognition based on odorant cues (Bonadonna and Nevitt, 2004).

Many vertebrates show learned preferences for odorants experienced before birth or hatching, as do chicks, *G. domesticus*, which could use such cues to recognize and orient to the nest (Sneddon *et al.*, 1998). Prions and petrels (Procellariidae), that nest in burrows and return to them at night, use olfactory cues to find their own burrow, but olfaction appears to be unimportant for nest recognition in diurnal and surface-nesting species (Bonadonna and Bretagnolle, 2002; Bonadonna *et al.*, 2001, 2003a, 2003b, 2004). The use of acoustic cues to recognize partners and chicks might attract avian

predators; thus, the use of chemical cues by some species may represent an adaptation for avoiding predation by other birds (Bonadonna *et al.*, 2003a). In some species, olfactory cues play an additional role in nesting: both European starlings (*Sturnus vulgaris*) and blue tits (*Parus caeruleus*) use olfactory cues to select plant leaves that are used as nest fumigants (Clark, 1991; Clark and Mason, 1985, 1987; Petit *et al.*, 2002).

Physiological studies demonstrate that the components of the olfactory system function similarly in birds and other groups of vertebrates. At the level of the olfactory epithelium and nerve, both excitatory and inhibitory responses can be observed in a wide range of odorants (Jung *et al.*, 2005; Shibuya and Tucker, 1967; Tucker, 1965). Similarly, single-unit recordings from the olfactory bulb demonstrate both excitatory and inhibitory responses to odorants (McKeegan, 2002; and references therein), and an electroencephalography study with chickens found no significant differences in responses to odorants relative to those recorded in mammals using the same technique (Oosawa *et al.*, 2000).

Birds lack a vomeronasal organ and accessory olfactory bulb (Huffman, 1963; Parsons, 1959b). The olfactory epithelium is located on a single spiral-shaped turbinate bone inside the nasal cavity, and air is drawn across it as the animal breathes in and out (Bang and Wenzel, 1985). The anatomy of the olfactory epithelium has been examined in members of five orders of birds (Anseriformes, Charadriiformes, Ciconiiformes, Columbiformes, and Galliformes), and all possess unusual olfactory receptor neurons that are capped with cilia surrounded by short microvilli (Bedini *et al.*, 1976; Brown and Beidler, 1966; Drenckhahn, 1970; Graziadei and Bannister, 1967; Matsuzaki *et al.*, 1982; Müller *et al.*, 1979; Okano and Kasuga, 1980).

Of the 20 000–23 000 genes in the genome of jungle fowl (*Gallus gallus*), 283 odorant receptor genes and ~100 odorant receptor pseudogenes have been identified (Hillier *et al.*, 2004); however, a study of domesticated chickens (*Gallus domesticus*) reported only 12 odorant receptor genes (Nef and Nef, 1997). It is not clear whether this large discrepancy is due to methodological differences, or to loss of genes as a result of domestication. The odorant receptor genes in galliforms are evolutionarily more closely related to those found in mammals than to those found in aquatic vertebrates, like teleost fishes (Niimura and Nei, 2005).

The size of the olfactory bulb relative to the brain varies considerably across birds (Bang and Cobb, 1968), and some species have small, conjoined bulbs whereas others have large, obvious olfactory bulbs (Nieuwenhuys, 1967). Lamination appears to be highly variable across taxa, with small, indistinct layers in species that have tiny olfactory bulbs (Nieuwenhuys, 1967) but clear cellular laminae with internal and external plexiform layers visible even in species with moderately sized olfactory bulbs, such as chickens and pigeons (McKeegan, 2002; Rieke and Wenzel, 1978). We are unaware of any Golgi studies of the olfactory bulbs in birds; thus, the cell types present and the details of cellular morphology are largely unknown. Rieke and Wenzel (1978) speculate that somata visible in the external plexiform layer of pigeons may belong to tufted cells.

An early study with pigeons (*Columba livia*) using a combination of electrophysiology and degenerating fiber stains demonstrated ipsilateral projections to the piriform cortex, hyperstriatum ventrale and the medial striatum, as well as projections to the contralateral globus pallidus and caudal portion of the medial striatum (Rieke and Wenzel, 1978; terminology after Reiner *et al.*, 2004). The authors of this study did not distinguish among the different olfactory tracts. The results of later study with the same species indicate that the medial olfactory tract projects ipsilaterally to the septum and to a region dorsal to this, that an intermediate tract projects to the olfactory tubercle and medial striatum, and that the lateral olfactory tract projects bilaterally to the piriform cortex and nucleus taeniae of the amygdala (Reiner and Karten, 1985; terminology after Reiner *et al.*, 2004). The authors note that the projection to the amygdala in birds is more restricted than in some other groups, including turtles, which could be related to the lack of a vomeronasal system in birds. The differences in results obtained in the two studies are difficult to understand, as they do not appear to be attributable to simple differences in nomenclature or mistaken identification of cell groups. In young ducks (*Anas platyrhynchos*), the medial olfactory tract projects to the dorsomedial hippocampus and superior frontal lamina, whereas the lateral tract terminates in the pallial–subpallial lamina, medial striatum, dorsomedial telencephalic wall, and posterior pallial amygdala (Teuchert *et al.*, 1986; terminology after Reiner *et al.*, 2004). An intermediate olfactory tract has not been described in this species.

The existence of an extrabulbar olfactory pathway in birds has not been directly demonstrated, but a study in which horseradish peroxidase was injected into the nasal cavity in ducks (*Anas platyrhynchos*) labeled a pathway that proceeded through the ventral olfactory bulb (Meyer *et al.*, 1987; von

Bartheld *et al.*, 1987). The terminations of these fibers were not observed, but ganglion cell bodies were not found and the injection is likely to have labeled primary olfactory receptor neurons.

As in other jawed vertebrates, the terminal nerve in birds arises from the nasal placode and, during development, demonstrates immunoreactivity to both GnRH and FMRFamide (Norgren and Lehman, 1991; Norgren *et al.*, 1992; Wirsig-Wiechmann, 1990; Yamamoto *et al.*, 1996). A FMRFamide-immunoreactive terminal nerve has been described in adult Japanese quail (*Coturnix japonica*), but the anatomy and chemical characteristics of the terminal nerve have not been studied in detail in adult birds (Fujii and Kobayashi, 1992).

2.17.2.6.2.(v) Turtles Turtles live in diverse habitats, ranging from completely terrestrial tortoises to fully aquatic sea turtles. Many freshwater species are semi-aquatic. Turtles sample the olfactory environment both on land and under water, and in both cases, sniffing involves throat movements that resemble those used by crocodilians and amphibians (Belkin, 1968; McCutcheon, 1943; Root, 1949). As an adaptation for prolonged diving, many species are remarkably tolerant of anoxia (Lutz and Milton, 2004). This trait makes turtles well suited for electrophysiological experiments, and many studies have used turtles as model animals for understanding general principles of olfactory system function in vertebrates. These studies will not be comprehensively reviewed here.

Sea turtles can swim thousands of miles to particular nesting beaches, and olfactory cues have been suggested to play a key role in this behavior. The available data indicate that olfactory-based homing cannot account for long-distance migration in turtles (reviewed in Lohmann *et al.*, 1999). However, some studies indicate that hatchlings may imprint on odorants specific to their local environment, suggesting that such cues could play a role in short-range orientation or selection of nesting sites (Grassman, 1993; Grassman and Owens, 1987; Grassman *et al.*, 1984). In addition, freshwater turtles (*Chrysemys picta*) can use chemical cues to discriminate water from home ponds versus that from ponds with and without conspecifics (Quinn and Graves, 1998).

The importance of chemical cues in foraging and feeding has not the subject of extensive study in turtles. Nevertheless, Honigmann (1921) showed that both aquatic and semi-aquatic species will bite at a bag filled with fish, but not at one filled with sand. Olfaction has also been shown to play a role in foraging in leatherback turtles, *Dermochelys coriacea*, in a laboratory setting (Constantino and Salmon, 2003).

Olfactory cues play a role in courtship and reproduction in turtles. Many turtles are endowed with secretory glands (Ehrenfeld and Ehrenfeld, 1973), although the behavioral significance of these secretions has not been thoroughly studied. Males of many species sniff or bob their heads when stimulated by odorants from female scent glands (e.g., Auffenberg, 1978; Kaufmann, 1992; Rose, 1970). Some species, such as the musk turtle *Sternotherus odoratus*, produce chemicals that probably serve as a deterrent or aposematic signal to predators, but could also function in intraspecific communication (Eisner *et al.*, 1977). The size of chin glands in male desert tortoises (*Gopherus agassizii*) is testosterone dependent, varying seasonally and with dominance status (Alberts *et al.*, 1994). Both males and females can discriminate individuals based on secretions from these glands (Alberts *et al.*, 1994). In male Berlandier's tortoise, *Gopherus berlandieri*, fatty acids from chin glands elicit aggressive behavior in males and cause females to approach and bob their heads at a model painted with these compounds (Rose, 1970; Rose *et al.*, 1969). In contrast, in desert tortoises (*Gopherus agassizii*), males prefer to use burrows scented with chin gland rubbings from conspecific males compared with untreated burrows (Bulova, 1997). Male stripe-necked terrapin (*Mauremys leprosa*) have been shown to avoid water conditioned by other males and prefer water conditioned by conspecific females, but only during the breeding season (Muñoz, 2004). The results of these studies clearly indicate that chemical signals play a role in social and reproductive behavior in some turtles.

The organization of the nasal cavity and vomeronasal organ varies considerably among species. In some, such as *Testudo* or *Emys*, the vomeronasal epithelium is not contained in a separate organ, but lies along the ventromedial wall or in grooves in the floor of the nasal cavity; the olfactory and vomeronasal regions of the nasal cavity are separated only by a slight horizontal ridge (Parsons, 1959b, 1959a). In loggerhead turtles, *Caretta caretta*, the vomeronasal epithelium is more widely distributed in the nasal cavity than is the olfactory epithelium (Saito *et al.*, 2000). On the other hand, in *Dipsochelys* and *Dermochelys coriacea*, the vomeronasal organ is a discrete structure that is encapsulated in bone and opens into the oral cavity (Gerlach, 2005). A comparison of axon counts indicates that olfactory receptor neurons outnumber vomeronasal receptor neurons in the Russian tortoise (*Testudo horsfieldii*), which is terrestrial, but

that two semi-aquatic species (*Chinemys reevesii* and *Mauremys japonica*) have more vomeronasal than olfactory receptor neurons (Hatanaka and Matsuzaki, 1993).

As in some other reptiles, the dendrites of the olfactory receptor neurons in Hermann's tortoise, *Testudo hermanni*, bear both cilia and numerous short microvilli (Delfino *et al.*, 1990). Like other tetrapods, turtles possess only microvillar vomeronasal receptor neurons (Graziadei and Tucker, 1970; Hatanaka *et al.*, 1982). Electrophysiological recordings from both the vomeronasal epithelium and accessory olfactory bulb in *Geoclemys reevesii* demonstrate that the vomeronasal system in turtles responds to general odorants with no inherent behavioral significance, such as amyl acetate, geraniol, cineole, and citral (Hatanaka and Matsuzaki, 1993; Hatanaka and Shibuya, 1989; Shoji *et al.*, 1993; Shoji and Kurihara, 1991). Recordings from dissociated vomeronasal receptor neurons in stinkpot turtles, *Sternotherus odoratus*, demonstrate that turtle vomeronasal cells also respond to a variety of complex natural odorants, including urine and musk from both males and females, as well as odorants derived from food pellets (Fadool *et al.*, 2001). $G\alpha_o$ is expressed in different vomeronasal receptor neurons than is $G\alpha_{i1-3}$, although the zonal segregation of expression seen in mammals does not occur in *Sternotherus* (Murphy *et al.*, 2001). In the same species, females show higher levels of $G\alpha_{i1-3}$ expression and lower levels of TRP2 immunoreactivity than do males (Murphy *et al.*, 2001), and odorant responses recorded from vomeronasal receptor neurons from females are larger than those from males (Fadool *et al.*, 2001). Taken together, these data demonstrate that the vomeronasal system in turtles responds to a wide range of chemicals, including cues that may be involved in intraspecific communication, and that the functioning of the system may be sexually dimorphic.

The turtle olfactory bulb is highly laminar, with both external and internal plexiform layers separating the cell layers (Johnston, 1915; Orrego, 1961). The glomeruli are surrounded by periglomerular cells (Orrego, 1961). Mitral cells usually extend primary dendrites into two glomeruli, and possess long secondary dendrites that project through the external plexiform layer (Mori *et al.*, 1981; Orrego, 1961). Tufted cells may be present; Johnston (1915) called these 'brush cells'. Two classes of granule cells are present, one of which has processes that arborize in glomeruli (Johnston, 1915; Orrego, 1961). As in frogs, the olfactory bulbs are fused in some species, with continuous layers and some neurites interacting across the midline (Skeen and Rolon, 1982).

As in other reptiles, three central olfactory tracts have been described in turtles. The medial olfactory tract projects ipsilaterally to the septum and to a medial cortical area that may be the homologue of the medial pallium/hippocampus of other vertebrates (Johnston, 1915; Reiner and Karten, 1985). The lateral olfactory tract has a massive bilateral projection to a lateral cortical area, and an intermediate olfactory tract that may be a subdivision of the lateral tract projects to the olfactory tubercle and the basal amygdaloid nucleus (Gamble, 1956; Reiner and Karten, 1985). In addition, a large olfactory projection to the entire pial surface of the amygdala was described in *Trachemys scripta* (Reiner and Karten, 1985). Given that the accessory olfactory bulb in turtles is sometimes included in injections or lesions of the main olfactory bulbs (Chkheidze and Belekhova, 2005; Gamble, 1956), the description of the large amygdalar projection in *Trachemys* may be due in part to inclusion of projections from the accessory olfactory bulb (see discussion in Eisthen, 1997). Because the separate projections of the accessory olfactory bulb in turtles have not been described, it is not clear whether the main and accessory olfactory systems project to different portions of the amygdala, as in other tetrapods (Chkheidze and Belekhova, 2005).

The development of the terminal nerve in turtles has been described by Johnston (1913) and Larsell (1917), who were able to visualize the nerve as it courses over the surface of the olfactory nerve due to its several conspicuous ganglia. To our knowledge, the histochemical characteristics of the nerve have not been examined in detail in turtles, although FMRFamide-immunoreactive cells and fibers do not appear to be present in or around peripheral olfactory structures in adult *Trachemys scripta* (D'Aniello *et al.*, 2001, 1999).

2.17.2.6.3 Mammals The comparative neurobiology of the olfactory system in mammals will not be described in detail here. Nevertheless, the textbook view of the organization of the vertebrate olfactory system typically includes many features that are unique to mammals, which should be noted by those interested in understanding the structure and function of the olfactory system in vertebrates in general. For example, the olfactory epithelium in mammals contains only ciliated olfactory receptor neurons (reviewed in Eisthen, 1992), whereas in other vertebrates, the morphology of olfactory receptor neurons varies considerably. As in other tetrapods, the vomeronasal receptor neurons in mammals are microvillar (Eisthen, 1992).

The organization of the olfactory bulb of mammals is similar to that of other tetrapods, with a distinct laminar organization that includes large plexiform layers. In mammals, the mitral cells possess a single primary dendrite that arborizes in one glomerulus, and prominent secondary dendrites that extend orthogonal to the primary dendrite through the external plexiform layer (reviewed in Eisthen, 1997; Nieuwenhuys, 1967). In addition to mitral cells, mammals possess tufted cells, a second class of output cell (Nieuwenhuys, 1967; Pinching and Powell, 1971). Although the somata of most tufted cells are found in the external plexiform layers, another group, the external tufted cells, have cell bodies in the glomerular layer; the glomerular layer also contains short-axon cells with no clear counterpart among nonmammalian vertebrates (Pinching and Powell, 1971). The lateral olfactory tract projects ipsilaterally, and not bilaterally as in other vertebrates (Skeen *et al.*, 1984). Mammals may lack a true medial olfactory tract that arises from the olfactory bulb; instead, the tract of the same name in mammals appears to arise from the anterior olfactory nucleus (Lohman and Lammers, 1967; Nieuwenhuys, 1967). The vomeronasal system is generally present in mammals, but has been lost in cetaceans as well as in some bats and primates (reviewed in The Vomeronasal Organ and Its Evolutionary Loss in Catarrhine Primates).

2.17.3 Conclusions

In this section, we integrate the preceding information, both to describe patterns of change and to consider the functional implications of these changes. In general, we will not cite references for information presented earlier, as details are provided in the sections pertaining to each taxonomic group.

2.17.3.1 Evolutionary Changes in the Organization of the Olfactory Epithelium

Before odorants contact the sensory receptor cells, they pass through a mucous layer. This mucus contains odorant binding proteins, which are known to be present in terrestrial mammals and in frogs. Interestingly, in *Xenopus*, the binding proteins are expressed in the principal cavity, which is used for detecting odorants in air, but not in the middle cavity, which is used for detecting odorants in water. Odorant binding proteins have been suggested to represent an adaptation for detecting odorants in air, to transport hydrophobic molecules through the mucous layer to the receptor neurons. Another possibility is that odorant binding proteins are too energetically expensive for use by aquatic vertebrates, as the hydrophilic proteins could easily dissolve in the water flowing over through the nasal sac (discussed in Eisthen, 2002). Although their presence in *Xenopus* and mammals suggests that odorant binding proteins should be broadly present in terrestrial tetrapods, Baldaccini *et al.* (1986) were unable to find binding proteins in birds (*Columba livia* and *Cairina moschata*) and turtles (*Testudo hermanni*), despite being able to sequence them from several mammalian species. Given that the odorant binding proteins in both *Xenopus* and mammals are lipocalins, it is surprising that these proteins have not been found in other classes of vertebrates. Perhaps different types of molecules are used as binding proteins in other groups of vertebrates, or perhaps the presence of these molecules in both *Xenopus* and mammals is convergent and not informative about tetrapods generally.

Although vomeronasal receptor neurons invariably terminate in microvilli, vertebrate olfactory receptor neurons are morphologically diverse; their phylogenetic distribution is shown in Figure 10. Researchers have long considered the possibility that the microvillar olfactory receptor

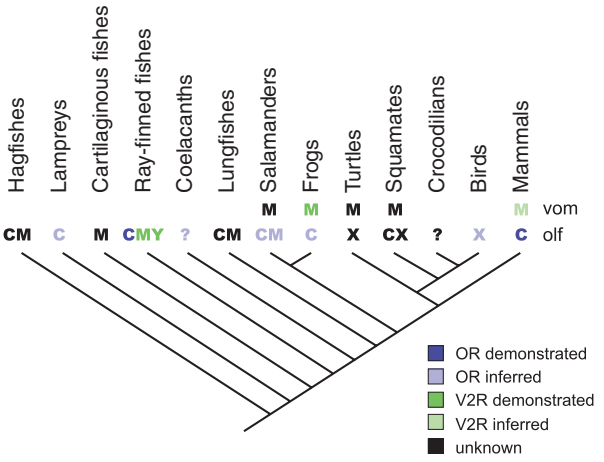

Figure 10 Phylogenetic distribution of receptor cell types and correlation with expression of olfactory (OR) and vomeronasal (V2R) receptor genes in the olfactory (olf) and vomeronasal (vom) epithelia. The expression of genes is demonstrated with electron microscopy; otherwise, it is inferred. Boldface letters indicate cell types: C, ciliated receptor neuron; M, microvillar receptor neuron; X, receptor neuron with both cilia and microvilli; Y, crypt-type receptor neuron. ? indicates that the morphology of the receptor neurons is unknown. Salamanders possess both ciliated and microvillar receptor neurons, but we do not yet know whether one or both express olfactory-type odorant receptor genes. Crocodilians, birds, and all fishes lack a vomeronasal organ. This diagram is simplified, as it assumes homogeneity within large taxonomic groups, such as teleost fishes. See text for references.

neurons in teleosts could be homologous with mammalian vomeronasal receptor neurons (discussed in Dulka, 1993; Eisthen, 1992). Recent data demonstrating that the olfactory epithelium of teleost fishes contains ciliated receptor neurons that express genes typical of olfactory receptor neurons, and microvillar neurons that express genes typical of vomeronasal receptor neurons may bolster this impression. Nevertheless, the expression of olfactory- or vomeronasal-typical genes may not correlate tightly with cell morphology (Figure 10). Further, if one assumes that the correlation applies to all craniates, then unlikely patterns are predicted to emerge. For example, although hagfish have both ciliated and microvillar olfactory receptor neurons, lampreys possess only ciliated olfactory receptor neurons, and those of sharks and skates terminate in microvilli. If the association between receptor cell morphology and gene expression arose with the earliest craniates, we would expect to see only olfactory-type genes expressed in lampreys, and only vomeronasal-type genes expressed in sharks and skates. Thus, the apparent correlation between receptor cell morphology and gene expression observed in teleosts and mammals may be a coincidence, may represent examples of convergent evolution, or may represent a derived condition that pertains only to bony vertebrates.

Alternatively, the morphological categories we are using may be too crude. For example, distinct subsets of microvillar and ciliated olfactory receptor neurons project to different regions of the olfactory bulb in channel catfish, *Ictalurus punctatus* (Morita and Finger, 1998). Similarly, in goldfish (*Carassius auratus*), the microvillar receptor neurons that are immunoreactive for $G\alpha_q$ are shorter and have more stiff microvilli than other microvillar receptor neurons (Hansen *et al.*, 2004). If 'ciliated' and 'microvillar' receptor neurons in vertebrates actually comprise several subclasses of cells, cell morphology and receptor gene expression may be tightly correlated within specific subclasses of receptor neurons. If so, some of the vomeronasal-type microvillar receptor neurons in goldfish could be homologous with vomeronasal receptor neurons in mammals. On the other hand, microvillar olfactory and vomeronasal receptor neurons often contain centrioles and basal bodies, suggesting that they derive evolutionarily from ciliated cells (Delfino *et al.*, 1990). If ciliogenesis is suppressed in microvillar cells (Kolnberger and Altner, 1971; Pyatkina, 1976), then receptor cell morphology might be evolutionarily labile, with no fixed relationship between receptor cell morphology and receptor gene expression.

2.17.3.2 Evolutionary Changes in the Organization of the Olfactory Bulbs

The organization of olfactory bulb circuits differs among vertebrate groups. Figure 11 illustrates the phylogenetic distribution of the cellular elements of the olfactory bulb. In interpreting the significance of these patterns, it is important to note that we assigned names to the different cell types based on their morphology alone, and did so independently of the names used by the authors of the source material. Thus, we use the term 'stellate cell' for any cells

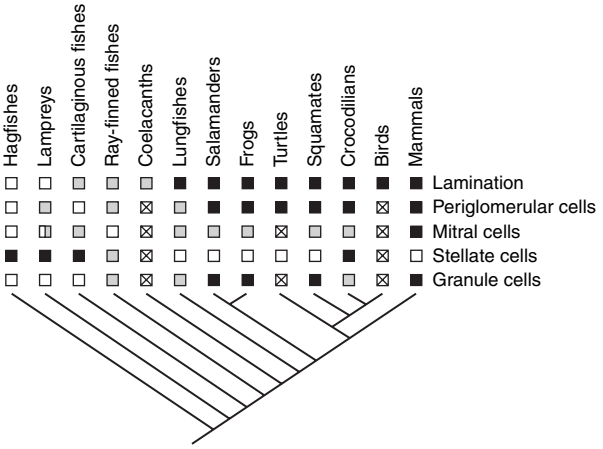

Figure 11 Phylogenetic distribution of cellular elements of olfactory bulb circuitry. The hypothesized ancestral condition, illustrated by hagfish, includes a low degree of lamination, with cell bodies frequently located across laminar boundaries and a lack of plexiform layers; periglomerular cells absent; mitral cells with primary dendrites that arborize in more than one glomerulus and no secondary (basal) dendrites; stellate cells present, with smooth dendrites that arborize in glomeruli; and granule cells bearing smooth dendrites, with an axon present. Boxes with crosses indicate cases for which information is unavailable. Lamination: white box = low degree of lamination, with cell bodies frequently located across laminar boundaries and a lack of plexiform layers; gray = moderate degree of lamination, with cell bodies confined to clear layers, and a lack of plexiform layers; black = high degree of lamination, with cell bodies confined to clear layers, separated by one or more plexiform layers. Periglomerular cells: white = absent; gray = ambiguous condition, in which candidate periglomerular cells have been described by some authors; black = periglomerular cells present. Mitral cells: white = primary dendrites arborize in more than one glomerulus, with no secondary dendrites; white/gray = primary dendrites arborize in only one glomerulus, with no secondary dendrites; gray = primary dendrites arborize in more than one glomerulus, with secondary dendrites that extend laterally; black = primary dendrites arborize in only one glomerulus, with secondary dendrites that extend laterally. Stellate cells: white = absent; gray = stellate cells present, with spiny dendrites that arborize in glomeruli; black = stellate cells present, with smooth dendrites that arborize in glomeruli. Granule cells: white = smooth dendrites, with an axon present; gray = -spiny dendrites, with axon present; black = spiny dendrites, with no axon. See text for references.

with a soma in the deep layers of the bulb, and with multiple dendrites that arborize in glomeruli. The somata of stellate cells are generally star-shaped, as the name implies. A 'granule cell' is a cell with an oval-shaped soma located in a deep layer of the bulb, and with dendrites that project upward but that do not enter or arborize in glomeruli. The dendrites of granule cells generally bear spines, whereas those of stellate cells are generally smooth. In many groups, both types of cells have a long axon. 'Periglomerular' cells have a soma in the glomerular layer, with dendrites that arborize in several glomeruli. In many groups, these cells have an axon that projects at least to deeper layers of the bulb. Ideally, additional criteria would be used to recognize cell types, such as data concerning the neurotransmitter characteristics of the cells. Unfortunately, such data are available for an extremely limited set of species, and cannot be used for broad phylogenetic comparisons. Thus, the categories we are using are quite broad, and in at least some groups probably encompass several recognizably different types of cells; for example, Golgi data indicate that multiple classes of mitral cells are present in some animals, and histochemical data indicate that several types of periglomerular cells are present in some species.

With these caveats in mind, the pattern depicted in Figure 11 indicates that the organization of the olfactory bulb did not undergo any sudden, dramatic shifts over the course of vertebrate evolution. Rather, changes in organization occurred as a series of steps. Laminar organization of brain structures is generally regarded as indicative of multiple stages of integration, with cells in one layer receiving processed input from cells in other layers and then processing these signals in more extensive ways. What are the functional consequences of the almost complete lack of lamination observed in hagfish or lampreys, or the high degree found in squamates or mammals? It is difficult to make predictions based on this feature alone, particularly because the numbers of cell types with axons that project to secondary processing areas differs considerably among these groups. Perhaps more processing occurs in the olfactory bulbs in some animals, and in secondary olfactory regions in others.

The textbook view of a mitral cell is one with a single primary dendrite that extends to one glomerulus, and prominent secondary dendrites that extend laterally to interact with dendrites of granule cells as well as secondary dendrites of other mitral cells. Nevertheless, this type of mitral cell is present only in mammals, and in most vertebrates, mitral cells have multiple primary and secondary dendrites. The breadth of inputs to mitral cells therefore differs considerably among groups. In mammals, each olfactory receptor neuron is believed to express only one odorant receptor gene, and receptor neurons that express the same gene project to the same glomerulus (reviewed in Mombaerts, 2004). Thus, mitral cells with dendrites in a single glomerulus receive inputs from a homogeneous population of receptor neurons. In contrast, olfactory receptor neurons in teleost fishes may express more than one receptor gene (Ngai *et al.*, 1993a; Speca *et al.*, 1999), and mitral cells in these animals extend dendrites to several glomeruli. Perhaps these glomeruli receive inputs from receptor neurons that express one receptor gene in common, in which case the type of coding occurring in the olfactory bulbs of teleosts and mammals may be similar. If not, the nature or location of odorant information processing in teleosts may differ considerably from that in mammals, which seems quite possible given that teleosts possess stellate cells, which mammals lack, and that the stellate and granule cells in teleosts have long axons that may project to secondary olfactory regions in the telencephalon.

Other aspects of bulbar circuitry differ among groups, although the functional consequences are not easy to predict. For example, morphologically distinct periglomerular cells are present only in tetrapods, but other cells, such as the displaced mitral cells in the glomerular layer of hagfish and lampreys, or even stellate cells, may serve similar functions. Similarly, a dorsal commissure connecting the two olfactory bulbs is unique to hagfish and lampreys, but other types of connections between the bulbs exist in other groups. For example, in frogs and some turtles and birds, the two olfactory bulbs are fused across the midline, with mitral and granule cell dendrites apparently integrating inputs from both sides. In other animals, such as the hedgehog *Erinaceus europaeus*, the olfactory bulb projects ipsilaterally to the anterior olfactory nucleus, which sends fibers to the mitral cell layer of the contralateral olfactory bulb (De Carlos *et al.*, 1989). These different anatomical arrangements may facilitate integration of inputs to the two nares, or perhaps bilateral comparison of inputs to enhance localization of odorant sources.

Granule cells lack dendritic spines in hagfish, lampreys, and cartilaginous fishes, and functionally equivalent circuitry is probably lacking. The presence of spines varies even within groups: for example, although granule cell dendrites are spiny in most amphibians, Herrick's (1931) studies indicate that the dendrites are smooth in mudpuppies

(*Necturus*). What are the functional consequences of this diversity? Given that dendritic spines are often involved in plasticity in other regions of the central nervous system, does this suggest that olfactory bulb circuits in animals lacking spines are more hard-wired? Alternatively, or in addition, perhaps less compartmentalization of processing occurs in the olfactory bulbs of animals with granule cells that lack dendritic spines (e.g., Woolf *et al.*, 1991). Finally, additional types of output cells have been described in some animals, such as the ruffed cells present in teleost olfactory bulbs, or the tufted cells present in reptiles and mammals. Without intensive electrophysiological and neurochemical studies of the olfactory bulb circuits in diverse vertebrates, the functional significance of these differences in organization are likely to remain mysterious. Given that we also lack detailed psychophysical data concerning the relative capabilities of the olfactory system in diverse vertebrates, the behavioral consequences of differences in circuitry cannot be predicted.

2.17.3.3 Evolutionary Changes in the Organization of Central Olfactory Projections

Broad patterns of change in the organization of central olfactory pathways are illustrated in Figure 12. Most vertebrates possess a medial olfactory tract that projects to the septum. The tract has been lost in mammals and may also be lost in cartilaginous fishes, although the central projections in this group must be examined using modern tract-tract methods before strong conclusions can be drawn. In tetrapods, the tract may acquire a projection to the medial pallium/hippocampus. Such a projection may also be present in ray-finned fishes, but given the confusion concerning pallial homologies in this group, we cannot reach a conclusion concerning this matter at present. Nevertheless, the medial pallium/hippocampus is generally involved in memory and spatial perception, and one might expect that olfactory input to this region would be behaviorally important for many vertebrates.

In general, a lateral olfactory tract is present and projects bilaterally to lateral and dorsal pallial or cortical areas. Although it is tempting to interpret this connectivity as a conserved feature that must be critical for processing olfactory information in all vertebrates, in many animals the homologies of pallial areas are based largely on their receipt of olfactory input. Thus, it would be circular to argue that this projection is conserved or that the lateral olfactory tract projects to homologous areas in diverse vertebrates. One clear trend is that the

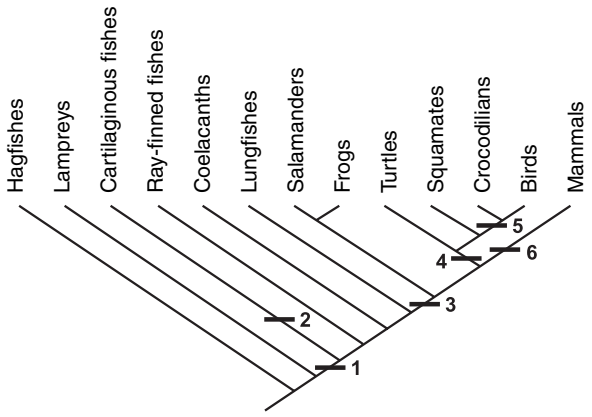

Figure 12 Evolutionary changes in the central projections of the olfactory bulbs. The hypothesized ancestral condition includes the presence of a lateral olfactory tract that projects bilaterally to lateral pallial/cortical areas and the striatum; the presence of a medial olfactory tract that projects ipsilaterally to the septum; and an extrabulbar olfactory pathway. Numbers indicate hypothesized changes in connectivity. 1, Reduction in the extent of projections of the lateral olfactory tracts. 2, Possible loss of the medial olfactory tract and bilateral projections of the lateral tract. 3, Origin of a distinct vomeronasal system, including an accessory olfactory tract that projects to the amygdala. Origin of an olfactory projection to separate portions of the amygdala via the lateral olfactory tract. Origin of an olfactory projection to the medial pallium / hippocampus via the medial olfactory tract. 4, Origin of an intermediate olfactory tract that projects to the olfactory tubercle, and loss of olfactory projection to the striatum via the lateral olfactory tract. 5, Loss of the vomeronasal system. 6, Loss of the medial olfactory tract and the contralateral projection of the lateral olfactory tract. See text for references.

lateral olfactory tract has extensive bilateral projections to both dorsal and ventral telencephalic regions in hagfish and lampreys, and that these projections are more restricted in jawed vertebrates. In addition, the lateral olfactory tract projects to the striatum in most groups discussed in this review, but not in reptiles; instead, an intermediate olfactory tract projects to the olfactory tubercle in the ventral striatum. This shift in connectivity suggests that the intermediate olfactory tract may be a branch of the lateral olfactory tract, or may have arisen from it evolutionarily. Some support for this idea comes from data demonstrating that the intermediate and lateral olfactory tracts project together to the contralateral hemisphere in snakes (Lanuza and Halpern, 1998), and that the two tracts run in partial continuity with each other through the rostral forebrain in turtles (Reiner and Karten, 1985).

A discrete vomeronasal system, including an accessory olfactory bulb and tract, is present only in tetrapods. The main central target of the accessory olfactory tract is the amygdala. The lateral

olfactory tract also projects to the amygdala, but the specific regions of the amygdala that receive input from the lateral and accessory olfactory tracts show little or no overlap in the species studied to date. Clear olfactory projections to the amygdala have been recognized only in tetrapods; thus, the origin of an olfactory bulb projection to the amygdala correlates roughly with the origin of the vomeronasal system. Given that the portions of the amygdala that receive olfactory and vomeronasal input are interconnected (reviewed in Halpern and Martínez-Marcos, 2003), perhaps this olfactory projection arose to facilitate integration of information from the two systems. If so, integration of chemosensory inputs cannot be the only current function of the olfactory projection to the amygdala, as this projection is retained in crocodilians and birds, which have lost the vomeronasal system. An olfactory projection to the amygdala may also exist in ray-finned fishes, but the homologies of possible amygdala-equivalent areas in ray-finned fishes are controversial (reviewed in Northcutt, 2006). Given that the vomeronasal-like and olfactory-like receptor neurons in teleosts project to different portions of the olfactory bulb, perhaps tracing the central projections of these two regions would provide new insight into the organization of the amygdala in teleosts.

2.17.3.4 Evolution of Vertebrate Olfactory Subsystems

A common assertion is that the olfactory system is phylogenetically ancient, or that olfaction is the oldest sensory system. The basis of such statements is unclear. Olfactory systems in animals in several phyla, including nematodes, mollusks, arthropods, and vertebrates, possess olfactory systems with similar features, but these features probably arose independently in each group, in response to similar constraints and as adaptations for similar tasks (Eisthen, 2002). Perhaps such statements simply indicate that the ability to sense chemicals in the external environment is widespread among animals, although this ability is by no means restricted to metazoans. A third possibility is that such statements are an oblique reference to a long-standing idea that among vertebrates, the forebrain was originally an olfactory structure, and that inputs from other sensory systems 'invaded' the telencephalon via thalamus over the course of vertebrate evolution (Ariëns-Kappers *et al.*, 1936; Edinger, 1904; Herrick, 1948). As described above, the olfactory projections in hagfish and lampreys distribute to larger portions of the telencephalon than do those in jawed vertebrates (Northcutt and Puzdrowski, 1988; Wicht and Northcutt, 1993). Overall, however, the available data clearly demonstrate that the invasion scenario is incorrect (reviewed in Northcutt, 1981).

Two large-scale changes in the organization of the olfactory system have occurred over the course of vertebrate evolution: the origin of the terminal nerve, and the origin of the vomeronasal system. Both hagfish and lampreys appear to lack a terminal nerve, as no projection has been described that comprises a peripheral ganglion and fibers that display the types of immunoreactivity that characterize the terminal nerve (reviewed in Wirsig-Wiechmann *et al.*, 2002a). If so, then the terminal nerve arose in jawed vertebrates. As described above, studies of the terminal nerve ganglion and retina in teleost fishes strongly indicate that the terminal nerve serves a modulatory function, and studies with salamanders demonstrate that terminal nerve-derived peptides modulate activity in the olfactory epithelium. In teleost fishes, the terminal nerve ganglion receives input from the olfactory, visual, and somatosensory systems. In amphibians, the extent to which terminal nerve peptides modulate olfactory epithelial activity depends on the animal's physiological or behavioral state, as both hunger and reproductive condition appear to play a role. Similar centrifugal modulation occurs in the retina and cochlea of many vertebrates (reviewed in Akopian, 2000; Manley, 2000, 2001). Do hagfish and lampreys lack this modulation, or do they possess alternate mechanisms for regulating olfactory responses with regard to their physiological needs? What are the overall functional consequences for animals that possess or lack such mechanisms? Perhaps the more active foraging and courting behaviors of jawed vertebrates benefit from more central control of olfactory epithelial function, which is unnecessary in hagfishes and lampreys.

A discrete vomeronasal system is present only in tetrapods, but recent work, described above, clearly indicates that the elements of the vomeronasal system are present in teleost fishes: the olfactory and vomeronasal receptor genes, as well as their associated G-proteins and ion channels, are expressed in different receptor neurons in the olfactory epithelium. In goldfish (*Carassius auratus*), the vomeronasal-type elements are expressed in microvillar olfactory receptor neurons, whereas the olfactory-type elements are expressed in ciliated receptor neurons (Hansen *et al.*, 2004). Although these morphological cell types are superficially similar to the vomeronasal and olfactory receptor neurons in mammals, as discussed above, it appears

unlikely that the two cell types in teleosts simply segregated into two epithelia to give rise to a separate vomeronasal organ (Eisthen, 2004). In zebra fish (*Danio rerio*), the two classes of receptor neurons send their axons to different portions of the olfactory bulb (Sato *et al.*, 2005), an arrangement similar to that in tetrapods, in which the olfactory and vomeronasal receptor neurons send axons to distinct olfactory and accessory olfactory bulbs. In tetrapods, the projections of the olfactory and accessory olfactory bulb differ, and the next step is to determine whether the portions of the olfactory bulb that receive input from the two cell types in teleosts also have distinct projections. If so, it would appear that teleost fishes have a complete vomeronasal system intermingled with the olfactory system. It would be interesting to know whether the same is true of other classes of fishes; perhaps the vomeronasal system has been present since the origin of vertebrates, and only became separate from the olfactory system in tetrapods. Because the functional differences between the olfactory and vomeronasal systems are unclear (reviewed in Baxi *et al.*, 2006; Halpern and Martínez-Marcos, 2003), it is difficult to speculate about the causes or consequences of the origin of a separate vomeronasal system in tetrapods.

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2.18 The Evolution of the Vomeronasal System

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Glossary

accessory olfactory bulb	A forebrain structure that is the recipient of axons from the vomeronasal sensory epithelium.
amygdala	A structure in the basal forebrain composed of multiple nuclei receiving neural information from all sensory systems including the olfactory and vomeronasal systems.
basal cells	Undifferentiated cells that undergo mitosis and eventually develop into neurons or supporting cells.
bed nucleus of the accessory olfactory tract	Neurons embedded in and surrounding the accessory olfactory tract.
bed nucleus of the stria terminalis	A nucleus closely associated with the stria terminalis.
bipolar neurons	Neurons with a single dendrite and axon at opposite poles of the cell.
Bruce effect	Failure of implantation of fertilized ova following exposure of female mice to the odor of a strange male.
cation channel	A membrane pore that allows cations to move between the intracellular and the extracellular compartments or between different intracellular compartments.
cilia	Organelles composed of extensions of the plasma membrane containing doublets of parallel microtubules.
cloaca	A posteriorly located cavity used for evacuation of intestinal and urinary wastes and for genital system activity.
cribriform plate	The bony base of the skull through which the olfactory nerve fibers pass from the nasal cavity to the brain.
decapeptide	A peptide composed of 10 amino acids.
diacylglycerol (DAG)	A second-messenger molecule that stimulates the activity of protein kinase C.
diverticulum	A pouch or sac branching from a hollow tubular or sac-like organ.
external plexiform layer	A lamina in the main and accessory olfactory bulbs just internal to the glomerular layer.
flehmen	A behavior characteristically displayed by ungulates to facilitate odorant access to the vomeronasal organ. It includes head extension, upper lip retraction, baring of the gums, and frequently opening of the mouth.
glomerular layer	A lamina in the main and accessory olfactory bulbs in which the axons of bipolar cells terminate on the dendrites of mitral and tufted cells and processes of periglomerular cells.
G-protein	A regulatory protein that binds GTP (guanosine 5′-triphosphate).
G-protein-coupled receptor	A member of the family of membrane proteins that are bound to and interact with G-proteins.
granule cell layer	A lamina in the main and accessory olfactory bulbs that contains the axonless granule cells.

head bobbing A behavior exhibited by guinea pigs in which the snout is moved forward and back across a stimulus to allow the stimulus access to the vomeronasal organ.

inositol trisphosphate (IP_3) A second-messenger molecule used in signal transduction.

internal plexiform layer A lamina in the main and accessory olfactory bulbs between the mitral cell layer and the granule cell layer.

ligand An extracellular substance that binds to cellular receptors.

main olfactory bulb A forebrain structure that is the recipient of axons from the olfactory sensory epithelium.

methyl ketones Molecules with methyl and ketone groups.

microvilli Small hair-like protuberances from epithelial cells.

microvillar cells Cells whose apical surfaces contain microvilli.

mitral cell layer A lamina in the main and accessory olfactory bulbs containing the cell bodies of mitral cells, the major projection neurons of the bulbs.

murine Relating to members of the rodent family Muridae, e.g., rats and mice.

neurogenesis The process of formation of neural tissue.

nucleus sphericus A subdivision of the amygdala of squamate reptiles that is the major recipient of direct input from the accessory olfactory bulb.

nuzzling A behavior that delivers substances to the vomeronasal organ of opossums. It consists of forward rubbing motions with the ventral aspect of the snout interspersed with rapid snout tapping motions on a substrate containing a vomeronasal stimulus.

olfactostriatum A nucleus in the basal forebrain of snakes that receives major vomeronasal input from the nucleus sphericus.

periglomerular cells Neurons in the main and accessory olfactory bulbs that are located in the glomerular layer and whose axons and dendrites enter glomeruli, providing a basis for interaction among different glomeruli.

pheromone A chemical substance secreted by one animal and responded to by another animal of the same species.

phospholipase C An enzyme that catalyzes hydrolysis of phosphatidyl choline to produce choline phosphate and 1,2-diacylglycerol (DAG).

primer pheromone A pheromone that has an endocrinological effect on other members of the same species.

pseudostratified sensory epithelium A sensory epithelium that appears to be layered, but which contains cells that extend from its apical surface to its basal lamina.

receptor cells Cells that receive and respond to external stimuli.

releaser pheromone A pheromone to which another animal of the same species responds rapidly.

respiratory epithelium Nonsensory epithelium lining the nasal cavity (and other respiratory structures).

signal transduction A process by which a cell converts one type of signal to another, e.g., a chemical stimulus into an electrical signal.

sirenids Aquatic mammals of the order Sirenia, e.g., manatee and the dugong.

sodefrin A female-attracting pheromone secreted by an abdominal gland of male newts.

supporting cells Cells in a sensory epithelium that are not receptor cells or involved in cell turnover, but act in a supporting function, e.g., removing toxic substances.

Vandenbergh effect Acceleration of puberty in female mice exposed to odors of male mice.

vomeronasal amygdala The portion of the amygdala that receives direct projections from the accessory olfactory bulb.

vomeronasal organ A sensory structure, located in close relationship to the nasal and oral cavities, that contains receptor cells that respond to chemicals, usually those produced by other animals.

Whitten effect Synchronization of estrus in female rodents exposed to the odor of a male.

The vomeronasal system is a sensory system specialized for the detection of naturally occurring chemicals, typically emitted by one animal and responded to by another animal. The system is present, as a separate entity, in virtually all terrestrial vertebrates at some time during development. Among nonmammalian vertebrates, a functional vomeronasal system is present in most terrestrial and aquatic amphibians and reptiles (see Evolution of the Amphibian Nervous System, Evolution of the Nervous System in Reptiles). It appears to be absent in crocodilians and birds, and, although precursors may be present, there is no separate vomeronasal system in fishes. Most mammals possess a vomeronasal organ although it is absent in many bats, most aquatic mammals, and Old World monkeys (see The Vomeronasal Organ and Its Evolutionary Loss in Catarrhine Primates).

2.18.1 Composition and Structure of the Vomeronasal System

In all vertebrates possessing a vomeronasal system, it is composed of a peripherally located vomeronasal organ, an accessory olfactory bulb, and forebrain structures that receive input from the accessory olfactory bulb. These forebrain structures typically contain neurons whose axons project to the hypothalamus and variably to other brain structures as well.

2.18.1.1 Vomeronasal Organ

The vomeronasal organ consists of a pseudostratified sensory epithelium that lines a lumen through which stimulating chemicals gain access to the dendritic processes of receptor cells. These receptor cells are bipolar neurons with a single dendrite terminating on the luminal surface of the organ and a single axon that projects to the accessory olfactory bulb. With few exceptions, the dendritic terminals of vomeronasal receptor cells are covered with microvillar extensions, in contrast to the ciliated dendritic knobs typical of most main olfactory system bipolar neurons (see Evolution of Vertebrate Olfactory Subsystems).

In addition to the receptor cells of the vomeronasal organ, supporting cells and basal cells are present in its sensory epithelium. In those species in which it has been studied, it is clear that the basal cells are a reservoir of undifferentiated cells that periodically undergo mitosis and differentiate into receptor cells and, perhaps, supporting cells. The receptor cells migrate through the epithelium, vertically, from base to apex in snakes (and probably in other nonmammalian species, as well), to become mature receptor cells. Eventually, these receptor cells become senescent, die, and are extruded from the epithelium. The role of the supporting cells is currently unknown.

In mammals, neurogenesis in the vomeronasal organ occurs as well; however, the process of movement of newly formed neurons, i.e., from base to apex or from margins to center, varies among different species (see Neuronal Migration).

The location, shape, and isolation (from the main olfactory chamber) of the vomeronasal organ in the different nonmammalian vertebrate species vary considerably. In amphibia, the vomeronasal organ is variably separated from the olfactory epithelium either by a short segment of respiratory epithelium or in a separate chamber lateral, medial, or ventromedial to the main chamber of the nasal cavity. In urodeles (salamanders) (Figure 1) and apodans (caecilians), the vomeronasal epithelium lines a diverticulum ventrolateral to the main nasal cavity, whereas in sirenids and anurans (frogs and toads) (Figure 2), the vomeronasal epithelium develops as a ventromedial diverticulum of the main nasal cavity. In turtles, the vomeronasal epithelium is reported to be a ventral extension of the olfactory epithelium and is isolated from the latter by respiratory epithelium. In squamate reptiles, the vomeronasal organ is completely separate from the nasal cavity, being located just dorsal to the hard palate and within the ventral-most portion of the nasal septum. It is dome-shaped and its nonsensory epithelium lines a ventral cartilaginous invagination, called the mushroom body.

The mammalian vomeronasal organ contains at least two subsets of receptor cells: those expressing $G_{i2\alpha}$ and those expressing $G_{o\alpha}$. The $G_{i2\alpha}$-expressing cells are located in more apical portions of the

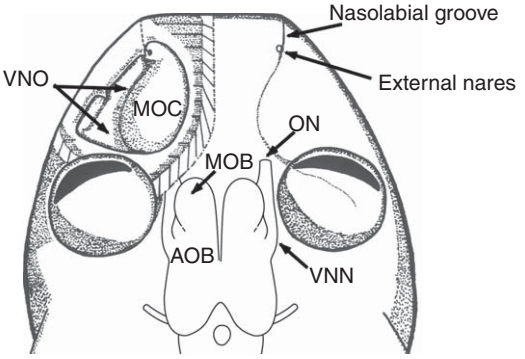

Figure 1 Drawing of the head and brain of a plethodontid salamander illustrating the position of the main olfactory chamber (MOC), vomeronasal organ (VNO), main olfactory bulb (MOB), and accessory olfactory bulb (AOB). The olfactory nerve (ON) and vomeronasal nerve (VNN) terminate in the MOB and AOB, respectively. Figure provided by Dr. Ellen Dawley.

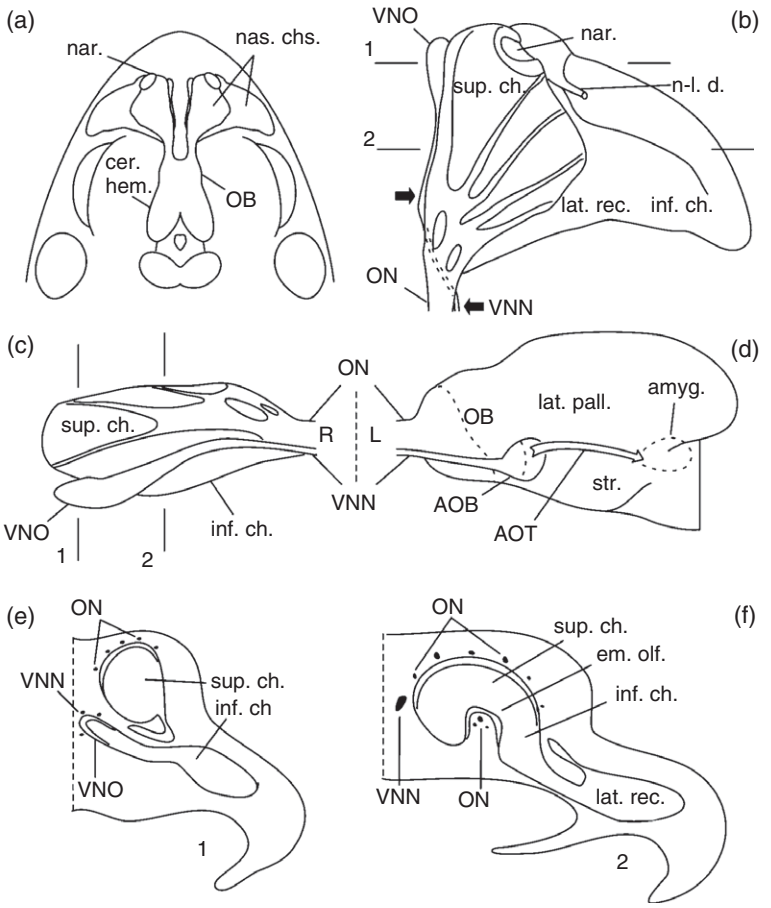

Figure 2 a, Outline of the nasal chambers and brain of a Ranid frog superimposed onto an outline of the head as seen from above. b, Dorsal aspect of the right nasal chambers. The vomeronasal organ is located in the medial recess of the inferior chamber and gives rise to the vomeronasal (accessory olfactory) nerve. The dorsal division of the main olfactory nerve is derived from multiple fascicles arising in the sensory epithelium in the roof of the superior chamber. On approaching the cranial cavity, the vomeronasal nerve (block arrows) winds across the ventral side of the ON to lie along its ventrolateral aspect before terminating in the ipsilateral accessory olfactory bulb (as in (d)). c, Medial aspect of the right nasal chambers showing the location of the vomeronasal organ and the initial course of the vomeronasal nerve. d, Lateral aspect of the left cerebral hemisphere. In this view, the left vomeronasal nerve is shown implanting into the left accessory olfactory bulb, which lies on the ventrolateral aspect of the main olfactory bulb. The MOB projects via the lateral olfactory tract (not shown) to the lateral pallium of the hemisphere, and the accessory olfactory bulb projects via the accessory olfactory tract to a distinct subdivision of the amygdala. e and f, Cross sections of the nasal chambers taken at level 1 (for (e)) and level 2 (for (f)) as indicated in (b) and (c). Part of the main olfactory epithelium is located in the inferior chamber on the dome-shaped olfactory eminence. This structure gives rise to the smaller, ventral division of the main olfactory nerve. The dorsal and ventral divisions coalesce to form a single nerve bundle as they approach the cranial cavity and join with the vomeronasal nerve. amyg., amygdala; AOB, accessory olfactory bulb; AOT, accessory olfactory tract; cer. hem., cerebral hemispheres; em. olf., eminentia olfactoria; inf. ch., inferior (nasal) chamber; lat. pall., lateral pallium; lat. rec., lateral recess (of the inferior nasal chamber); nar., nares; nas. chs., nasal chambers; n-l. d., nasolachrymal duct; OB, (main) olfactory bulb; ON, (main) olfactory nerve; str., striatum; sup. ch., superior (nasal) chamber; VNN, vomeronasal nerve; VNO, vomeronasal organ. Figure provided by Dr. Frank Scalia.

epithelium and those expressing $G_{o\alpha}$ are located closer to the basal lamina. Two families of G-protein-coupled receptor genes, V1R and V2R, have been described. Bipolar neurons expressing the V1R gene products are coextensive with $G_{i2\alpha}$-expressing cells and bipolar neurons expressing the V2R gene products are coextensive with $G_{o\alpha}$-expressing cells. Each subset of cells sends its axons to separate targets in the accessory olfactory bulb.

The lumen of the vomeronasal organ may open to the external environment through a duct into the oral cavity, as in squamate reptiles, through a groove onto the upper lip, as in plethodontid salamanders, or into the nasal cavity, as in frogs and turtles. The nature of access to the external environment is predictive of the behavioral adaptation used to deliver odorants to the vomeronasal epithelium. Thus, for example, snakes and lizards deliver odorants to the vomeronasal organ by means of tongue extensions out of the

mouth and retractions back into the mouth (tongue flicking). The exact mechanism by which the odorants are transferred from the tongue to the vomeronasal ducts is currently unknown. In plethodontid salamanders, odorants gain access to the vomeronasal organ by a head-tapping behavior that delivers the odorants to the base of the nasolabial grooves along the upper lip. The odorants then pass along the grooves to the vomeronasal epithelium, located in the lateral aspect of the nasal cavity.

In mammals, the vomeronasal organ may open into the mouth, into the nasal cavity, or into the nasopalatine duct that runs between the mouth and the nasal cavity. A variety of adaptations for delivery of odorants to the vomeronasal organ have been reported, including head bobbing in guinea pigs, flehmen and licking in a variety of mammals, and nuzzling in opossums. The autonomic nervous system, via a vomeronasal pump, has been demonstrated to control access and egress of substances to and from the rodent vomeronasal organ.

2.18.1.1.1 Signal transduction in vomeronasal receptor cells Among nonmammalian vertebrates, it is only in garter snakes that the mechanism of transduction of chemical signal to neural signal in the vomeronasal organ has been systematically investigated. Using known ligands for vomeronasal function derived from earthworms, it is now understood that the ligands bind to G-protein-coupled receptors on the luminal surface of vomeronasal bipolar neurons, initiating a cascade of events that include the activation of phospholipase C, leading to an increase in intracellular inositol trisphosphate (IP_3). The increase in IP_3 leads to a release of Ca^{2+} from intracellular stores and to an influx of Ca^{2+} from the extracellular compartment. The increase in intracellular Ca^{2+} leads to calcium-induced calcium release from ryanodine-sensitive stores. The intracellular calcium levels return to prestimulus levels through the action of a Na^{+}/Ca^{2+} exchanger. The exact mechanism by which this intracellular cascade results in a change in the membrane voltage of vomeronasal receptor cells is currently unknown.

The signal transduction pathway of the vomeronasal organ of mice appears to differ from that of snakes in important ways. Ligand binding to the G-protein-coupled receptors (V1R, V2R) initiates a cascade of events that results in the production of diacylglycerol (DAG). DAG activates a calcium-permeable cation channel in the distal portion of vomeronasal receptor dendrites, initiating a sensory current that is the basis of the receptor potentials recorded following ligand binding to vomeronasal sensory neurons. A principal subunit of this cation channel is encoded by the *TRPC2* gene, which has been shown to be involved in signal transduction to pheromonal stimuli in the vomeronasal organ of mice. The activity of diacylglycerol kinase terminates DAG signaling.

2.18.1.1.2 Primary vomeronasal pathway In salamanders (Figure 1), the axons of vomeronasal receptor cells do not aggregate, but follow the lateral edge of the olfactory nerve to the accessory olfactory bulb. In frogs, the fibers that make up the vomeronasal nerves are initially found medial to the olfactory nerve and wrap around the latter to obtain a ventral position prior to entering the cranial cavity (Figure 2).

In reptiles, the axons of vomeronasal receptor cells coalesce to form the vomeronasal nerves that travel along the nasal septum to penetrate the cribriform plate and terminate in the glomerular layer of the accessory olfactory bulb (Figures 3 and 4).

In mammals, the vomeronasal nerve coalesces posterior to the vomeronasal organ, travels along the nasal septum, enters the cranial cavity, usually medial to the main olfactory bulb, and terminates in the glomerular layer of the accessory olfactory bulb.

2.18.1.2 Accessory Olfactory Bulb

The accessory olfactory bulb is the only known site of terminations of axons of vomeronasal receptor cell axons. The bulb is a laminated structure located in the rostral telencephalon. Its relationship to the main olfactory bulb, the termination site for main olfactory receptor cell axons, is variable. In salamanders and frogs, the accessory olfactory bulb is located caudo-lateral to the main olfactory bulb; in snakes, it is caudal to the main bulb, and in turtles, it is dorso-posterior to the main bulb. In mammals, the accessory olfactory bulb is usually dorsal to the most caudal portion of the main olfactory bulb.

The size of the accessory olfactory bulb relative to the main olfactory bulb also varies, being close to two and a half times larger than the main bulb in snakes, of variable size in lizards, and smaller than the main bulb in amphibians. In virtually all mammals, the accessory olfactory bulb is a fraction of the size of the main olfactory bulb.

Similar layers are present in the accessory olfactory bulb of most vertebrates studied, although some layers may be severely reduced or absent. The most external layer is the nerve layer, which is composed of the axons of bipolar neurons whose cell bodies form the receptor cell layer of the vomeronasal sensory epithelium. These axons terminate in the glomerular layer, which is located just internal to the nerve layer. External plexiform, mitral

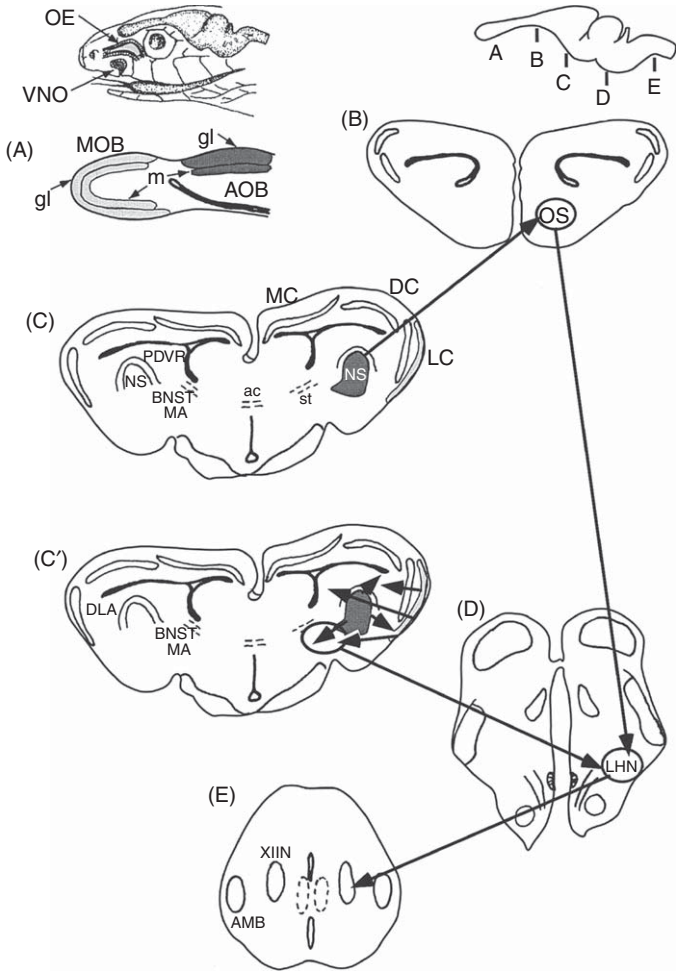

Figure 3 Top left: Drawing of snake head with vomeronasal organ (VNO), nasal cavity with olfactory epithelium (OE), and brain depicted as if the head were transparent. Top right: Drawing of brain as seen from the side, indicating the location of sections A through E. (A) Parasagittal depiction of main (MOB) and accessory (AOB) olfactory bulbs. Glomerular (gl) and mitral (m) cell layers are identified. B–E, Drawings of coronal sections through the rostral telencephalon, midcaudal telencephalon, dimesencephalic border, and hindbrain, respectively. The projections from the MOB, indicated in light gray, are primarily to the lateral cortex (LC). The projections from the AOB (dark gray shading) are primarily to the nucleus sphericus (NS) and medial amygdala (MA). The NS projects to the MA, which, in turn, projects to the lateral posterior hypothalamic nucleus (LHN), which sends its projections to the hypoglossal nucleus (XIIN), the source of efferents to the tongue musculature. Paralleling this pathway is one from the NS to the olfactostriatum (OS), which also projects to the LHN. The NS, in addition to its connections to the OS, projects to the dorsolateral amygdaloid nucleus (DLA) and LC; the LC projects to the MA and DLA. ac, anterior commissure; AMB, nucleus ambiguous; BNST, bed nucleus of the stria terminalis; DC, dorsal cortex; MC, medial cortex; st, stria terminalis; PDVR, posterior dorsal ventricular ridge. Figure provided by Dr. Alino Martínez-Marcos.

(mitral/tufted) cell, internal plexiform, and granule cell layers follow from external to internal layers. The principal dendrites of mitral/tufted cells project into the glomeruli of the glomerular layer, making synaptic contact with the axonal terminals of receptor cells. Periglomerular cells, located around the glomeruli, project dendrites and axons into the glomeruli. Short axon cells, located in the plexiform layers, make local connections; the axonless granule cells make dendro-dentritic contact with the lateral dendrites of the mitral/tufted cells. The primary dendrites of mitral/tufted cells may contact multiple glomeruli, unlike the situation in the mammalian main olfactory bulb. The mitral/tufted cells are the only projection neurons of the accessory olfactory bulb, sending their axons out of the bulb to contact a variable number of targets in the telencephalon.

2.18.1.3 Telencephalic Targets of the Accessory Olfactory Bulb

The major targets of mitral cell axons are nuclei of the amygdala (see Evolution of the Amygdala in Vertebrates). In nonmammalian vertebrates, these

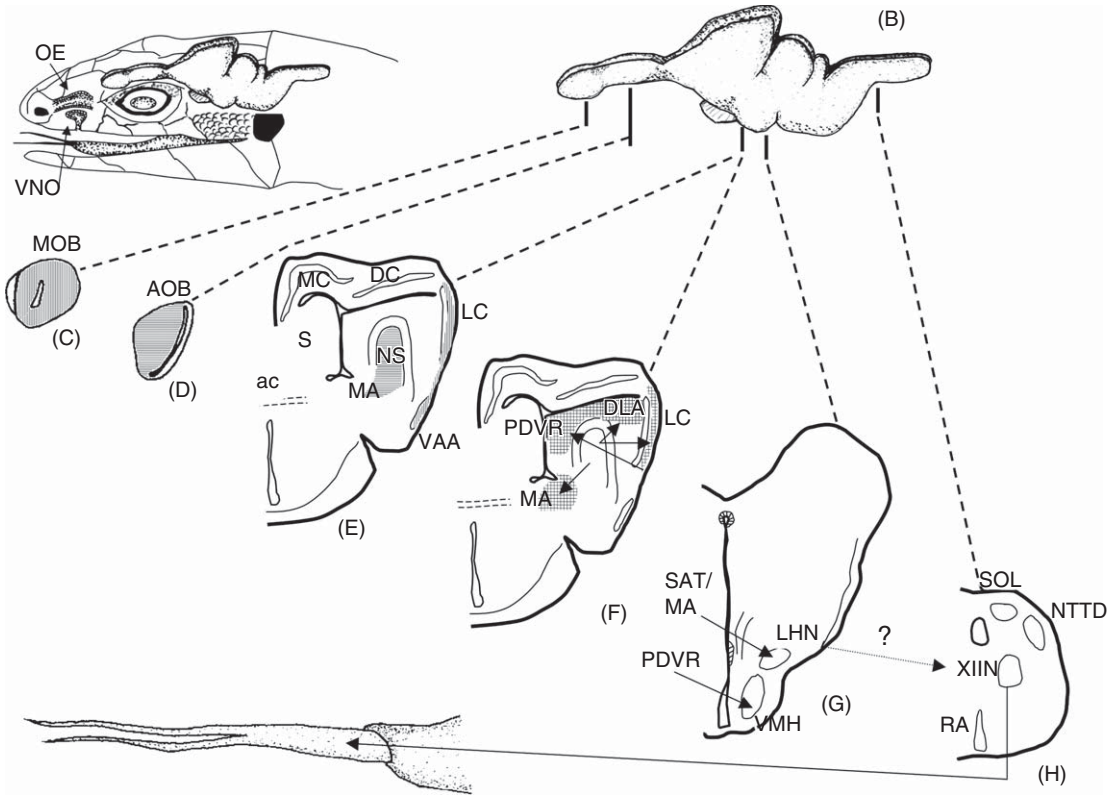

Figure 4 Vomeronasal system of the lizard, *Podarcis hispanica*. The vomeronasal organ (VNO) and olfactory epithelium (OE) are depicted *in situ* in the diagram at the top left. The brain, removed from the skull, is depicted at the top right (B) and the location of the coronal sections illustrated below are indicated with solid vertical lines. Projections from the main olfactory bulb (MOB) are indicated with vertical hatching, and projections from the accessory olfactory bulb (AOB) are indicated with horizontal hatching in section E. The primary projection of the MOB is to the lateral cortex (LC) and ventral anterior amygdala (VAA), whereas the major projection from the AOB is to the nucleus sphericus (NS) and the medial amygdaloid nucleus (MA). Further projections from the LC extend to the posterior dorsal ventricular ridge (PDVR). The nucleus sphericus projects to the dorsolateral amygdaloid nucleus (DLA), LC, and MA. The PDVR projects to the ventromedial hypothalamic nucleus (VMH) and the striatoamygdaloid transition area (SAT) and the MA projects to the lateral posterior hypothalamic nucleus (LHN). There is some unpublished evidence that LHN projects to the hypoglossal nerve nucleus (XIIN), which would control the tongue musculature. DC, dorsal cortex; MC, medial cortex; RA, raphe nuclei; S, septal nuclei. See Novejarque *et al.* (2004) for a discussion of SAT. Figure provided by Dr. Enrique Lanuza.

projections have been most extensively examined in frogs and snakes (see Evolution of the Amphibian Nervous System, Evolution of the Nervous System in Reptiles). In the frog, *Rana pipiens*, the accessory olfactory bulb projects, via the accessory olfactory tract, to the ipsilateral medial and cortical amygdaloid nuclei, with a fascicle continuing past these nuclei to cross the midline in the anterior commissure and terminate in the contralateral amygdala. In salamanders, the projection from the accessory olfactory bulb is also to the vomeronasal amygdala.

In snakes, the accessory olfactory bulb projects to the nucleus sphericus (a hypertrophied portion of the amygdala), the medial amygdala, and the nucleus of the accessory olfactory tract. The nucleus sphericus, in turn, projects to the lateral posterior hypothalamic nucleus and the olfactostriatum, a telencephalic structure. The neurons in the lateral posterior hypothalamic nucleus project their axons to the nucleus of the hypoglossal nerve, thus completing a circuit that is the anatomical substrate for vomeronasally induced tongue flicking (Figure 3).

In lizards, the accessory olfactory bulb projects to the nucleus sphericus, the bed nucleus of the accessory olfactory tract, the central and ventromedial amygdaloid nuclei, and the bed nucleus of the stria terminalis. In turn, the vomeronasal amygdala projects to the hypothalamus (Figure 4).

Early studies on the projections of the olfactory bulb in turtles assumed that there was no vomeronasal organ or accessory olfactory bulb; therefore, separate projections from the main and accessory olfactory bulb were not investigated. Therefore, at present, we do not know the targets of the accessory olfactory bulb in turtles.

The projections of the accessory olfactory bulb in mammals follow a pattern that is similar to, albeit more complex than, that in other vertebrates,

projecting primarily to amygdaloid targets that in turn project to discrete portions of the preoptic area and hypothalamus.

2.18.2 Functions of the Vomeronasal System

The best documented functions of the vomeronasal system are those related to pheromone detection and its role in reproductive behavior. Among the nonmammalian vertebrates, most information on the role of the vomeronasal system in behavior comes from snakes, although information about the role of the vomeronasal system in foraging and pheromone detection in lizards and amphibians has also become available.

A female-attracting pheromone, a decapeptide, from the abdominal gland of the cloaca of the male red-bellied newt, *Cynops pyrrhogaster*, sodefrin, has been identified, isolated, purified, sequenced, and synthesized. Sodefrin elicits marked physiological responses when applied to the vomeronasal epithelium of female newts. In other amphibian species, pheromones that increase female receptivity and attract females have also been isolated and purified, and a female-attracting aquatic sex pheromone from the parotid and rostral glands of the male magnificent tree frog, *Litoria splendida*, has also been identified. However, it has not yet been established that these latter pheromones act through stimulation of the vomeronasal system.

The vomeronasal system of snakes is involved in prey detection and response to pheromones. Garter snakes with their vomeronasal nerves severed are unable to follow prey trails and eventually stop eating earthworm prey. Garter snakes also employ the vomeronasal system for finding conspecifics for the purpose of aggregation and to respond appropriately to female pheromones. The female garter snake (*Thamnophis sirtalis parietalis*) produces a series of nonvolatile saturated and monounsaturated long-chain methyl ketones that attract male garter snakes and induce stereotypical courtship behaviors. Although the role of the vomeronasal system in response to this pheromone has not been directly tested, the fact that male garter snakes without a functional vomeronasal system fail to respond to females secreting this pheromone is supportive of this idea.

To date, the only known functions of the mammalian vomeronasal system are related to the response to conspecific chemical signals, pheromones. These pheromones may function to modify behavior immediately (signaling pheromones) or to modify activity of the hypothalamic/pituitary axis (primer pheromones). Many of the murine pheromone effects (the Bruce effect, Whitten effect, Vandenbergh effect, etc.) are mediated by primer pheromone stimulation of the vomeronasal system. Responses of mammals to signaling pheromones frequently lead to mounting and copulation, aggressive, parental, and/or marking behaviors. Not all responses to pheromones are mediated by the vomeronasal system; some are mediated by the olfactory system. For example, individual recognition in some species is not mediated by the vomeronasal system, although some neurons in the mouse vomeronasal organ respond to major histocompatibility complex peptides. In addition, not all vomeronasal ligands are pheromones.

2.18.3 Origins of the Vomeronasal System

The vomeronasal system is not a universal feature of vertebrates. The system is absent in most aquatic species, i.e., teleost fish, crocodilians, and cetaceans (whales and dolphins). Birds, some bats, and Old World primates also lack a vomeronasal system (see The Vomeronasal Organ and Its Evolutionary Loss in Catarrhine Primates). Whether humans possess a functional vomeronasal system is, at present, controversial.

The olfactory epithelium of some fish contains both ciliated and microvillar receptor cells, the latter being reminiscent of vomeronasal receptor cells. Fish live in an aquatic environment and the vomeronasal organ of terrestrial vertebrates is known to be fluid filled and to respond to nonvolatile odors, frequently in a liquid state. Therefore, it is not unreasonable to believe that the vomeronasal system might have evolved from the fish olfactory system, as vertebrates moved to a terrestrial environment.

One study suggests that the microvillar cells of the olfactory epithelium of fishes may be precursors to vomeronasal receptor cells. The microvillar cells of goldfish express G-proteins ($G_{o\alpha}$, $G_{i\text{-}3\alpha}$, and G_q) and receptor genes that are expressed in mammalian vomeronasal receptor neurons, but not in olfactory receptor neurons. The microvillar cells are distinct in these properties compared to the ciliated receptor cells, as well as in their location, separate from the ciliated olfactory receptor neurons, suggesting that segregated populations of receptor cells may have been present prior to the evolution of tetrapods and the segregation of olfactory and vomeronasal epithelia into distinct chambers (see Evolution of Vertebrate Olfactory Subsystems).

It is clear from research on fully aquatic amphibian species, e.g., aquatic salamanders, that some of these animals possess well-developed vomeronasal organs.

Since the vomeronasal system is generally present in tetrapods, appears to have originated in early amphibians, is present in amphibian larvae, and is well developed in aquatic amphibians, the proposition that the appearance of this system in evolutionary history was an adaptation to terrestrial life appears untenable. The presence of the vomeronasal system in most tetrapods and its absence in some species, e.g., arboreal lizards, birds, Old World monkeys, and some aquatic mammals, suggests that this system was lost in the past history of some species.

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2.19 Electric Fish, Electric Organ Discharges, and Electroreception

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Glossary

ampullary organ	A type of electroreceptor sensitive to low frequencies (under 20 Hz).
electric fish	Fish that have evolved electric organs. As these fish are dispersed in phylogenetically diverse groups, they have evolved independently a number of times.
EOD	The electric organ discharge.
JAR	The jamming avoidance response. When two weakly electric fish with similar frequencies of electric organ discharge meet, their electrolocation systems jam each other, impairing their ability to electrolocate. To avoid this jamming, they shift their EOD frequencies away from each other.
tuberous receptors	A type of electroreceptor sensitive in the range of hundreds of hertz, but not below 30 Hz.

2.19.1 Introduction

Conventional wisdom may be well justified in claiming that brain evolution is led to a large degree by the modalities of the senses. Species with good vision or a well-developed auditory system, olfaction, or proprioception show this in the size and degree of elaboration of the brain. This article provides another example from a group of fishes possessing an unfamiliar sense modality: electroreception. This is an abbreviated account of these systems, stressing their evolution, which shows both radiation in direction of special adaptations and parallelism in independent development of the same trait in two or more phylogenetic lines. Among taxa (animal groups) that exhibit reception of feeble electric currents, a first-order dichotomy exists between certain families of fishes, amphibians, and one mammal whose electroreception is used in a passive mode – processing currents from sources external to the receiver, whether animate or inanimate – whereby the sense organs filter and map extraneous electric fields. Other species use the system in an active form, involving an electric organ that generates pulses (efference) of current under central control, and an array of specialized sense organs that detect the animal's own field and central (brain) circuits that analyze this afference with respect to its spatial and temporal structure. In addition to discussing each of these topics, we will briefly mention the behavioral uses, ontogeny, evolution, and special aspects.

A species of catfish common in African freshwaters that can deliver a strong jolt as well as a Mediterranean species of ray that was called torpedo, presumably because it can induce torpor, were known to the ancients. Very soon after 1492, South American electric eels were brought back to Europe and their high-voltage discharges became familiar. They figured in classical experiments and controversies about animal electricity in the eighteenth century, reviewed by Brazier (1984). Not until the 1940s was it realized that many smaller relatives of the electric eel, common in neotropical freshwaters, also discharge electric organs, although feebly, usually peaking at a few millivolts measured on the skin, out of water, in one or a few millisecond pulses, several times every second, continuously night and day. Studies of the mechanisms of production, control, and use of the strongly electric discharges (EODs) were already well along when the weakly electric fish and the continuous train of weak discharges night and day by the powerful eel added questions of reception, brain analysis, use,

[†] Deceased.

function, development, and evolution. These rapidly expanding fields of research have been reviewed in two books: Bullock and Heiligenberg (1986), updated in Bullock *et al.* (2005). The following is a condensed and selective summary.

2.19.2 Electric Organs

Specialized for generating either low current, high voltage or high current, low voltage, pulses are from modified muscle cells or from branched nerve endings. Electric organ cells (electrocytes) maintain a standing electromagnetic field (EMF) between inside and outside by ion pumps, like ordinary cells. They can be discharged by brief depolarizing signals from the synchronized electromotor nerve cells in the spinal cord, under brain control. In some species, motor nerve endings by one of several arrangements first depolarize one face of the electrocytes and then the other, while the faces are anatomically patterned to add their pulses either many in series (e.g., high-voltage electric eel) or many in parallel (e.g., high-current torpedo).

Diversity is also conspicuous in the temporal pattern of EODs. Some species are called pulse fish because the EODs iterate at a low and irregular repetition rate (intervals several to many times the duration of the single pulse). Others are wave fish because the intervals are brief and regular (equal to or little longer than discharge duration), reaching regularities higher than any other known biological rhythms. Each discharge is commanded by a special brain nucleus that receives and integrates multiple inputs and is usually relayed through one or more synapses in the brainstem and again in the spinal cord, besides the last junction between efferent axon and electrocyte. The regularity of the EODs can be controlled by the brain and routinely shows a coefficient of variation (standard deviation divided by mean interval) smaller than 0.1% of the mean (i.e., SD < 1 μs). It is not yet clear what situations or states of the brain are associated with higher or lower regularity or what selective advantage such extremely low variation confers. Presumably, it lies in the domain of detecting or assessing objects that distort the instantaneous electric field of the EOD, the signal analyzed by the electroreceptive system to detect and evaluate objects, whether obstacles or passageways. Electric organs must have been invented independently at least several times, perhaps as many as seven times in various taxa of elasmobranchs and teleosts. Intermediate or early stages in the evolution of electric organs are not clear, but one suggestion is the apparently well-synchronized muscle action potentials of respiratory movements in lampreys (Kleerekoper and Sibakin, 1956).

2.19.3 The Electroreceptor System

The electroreceptor system begins with an array of many primary sensory neurons in small, widely dispersed sense organs sending afferent axons to the brain via the lateral line nerves. Two broad classes are: (1) ampullary receptors, which act as low-pass filters, insensitive to stimulus components above approximately 20 Hz. Some taxa are excited by one polarity, e.g., current entering the skin and inhibited by the other polarity. Other taxa are the opposite. (2) Tuberous receptors are high-pass filters, sensitive in the range of hundreds of hertz but not below about 30 Hz. Some of these respond by increasing the probability of firing nerve impulses as the stimulus is increased in amplitude (probability coders or P units). Others respond by shortening the latency of the single nerve impulse that follows each brief stimulus (phase coders or T units). Each fish typically has all three kinds of receptors – ampullary, T units, and P units – sometimes answering to only one kind of stimulus: social, passive, extraneous, or active distortion of its own EOD by an object of higher or lower conductance than the surrounding milieu.

2.19.4 Central Electrosensory Circuits

Centers, nuclei, and pathways processing the input from electroreceptor are found in each brain segment from medulla, midbrain, diencephalon, and cerebellum to the forebrain, hypothalamus, basal ganglia, and cerebral pallium plus the spinal cord, *in toto* a massive system. Many of these are known either from physiological responses to quasinormal electric stimuli or from pathway-tracing dyes injected into known cell groups to reveal the destination of their axons or to visualize the cells of origin of the fibers that took up the dye. Details can be found in the books cited and the original papers referenced in those chapters.

A landmark in neuroethology, the science that seeks to explain behavior in terms of neural structure and function, is the jamming avoidance response (JAR), a simple behavior observed in wave species when two or more individuals are within range of each other and have EOD frequencies close to each other. Unless there is a large difference in size, each fish will quite reliably shift its EOD frequency in the direction to increase the difference in frequency, interpreted as an avoidance

of being mutually jammed. This behavior means that each fish is solving the problem of recognizing which part of the mixed electric fields in the water comes from its own EOD and which part is from other fish. Ingenious experiments by Heiligenberg (1991) showed that they do not utilize information in the brain that commands each EOD train but depend on analysis of the sensory feedback. Certain cells in a particular layer of the tectal cortex are excited when the amplitude-sensitive P afferents are increasing their firing frequency in response to the rising phase of the beat cycle caused by the mixing of two or more EODs of different frequencies. Other cells are excited by the afferents from T units when their phase of firing is retarded during the delay part of the phase cycle that accompanies the beat cycle. Still other units are inhibited when the rising limb of the P cycle is in phase with the falling limb of the T cycle, and these exert the modulatory influence upon the pacemaker nucleus that we see as the JAR of EOD rates. Each of these cell types is in a small, localized population whose anatomy and connections in and out are known through at least 14 orders from the primary afferent neuron.

2.19.5 Ontogenetic Development

The multiple types of electroreceptors are all derived from lateral line receptors, which are endowments of all fishes, plus aquatic amphibians mediating mechanical senses associated with relative movement of animal and water in a low frequency range, 0.1–100 Hz, commonly called water flow and vibration, overlapping with acoustic reception via the inner ear and eighth cranial nerve. The sense cells in both mechanoreceptors and electroreceptors are members of a large class of sensory cells called hair cells, although some of the electric sense cells have lost their ciliary hairs. We do not know the crucial features that evolved to alter the adequate stimulus from the mechanical event to an electrical one, but the change in sensitivity was many orders of magnitude and, in each class of electroreceptor, was tuned to a best frequency that could be many octaves apart. The ontogenetic development in the young animal begins, as for lateral line sense organs in clusters of neuroblasts, in the head, called lateral line placodes that send their outgrowing central axonal processes into the brain by one of several cranial nerves, mostly the trigeminal. Their peripheral axons grow into the lateral line nerves to a species-characteristic, quite wide distribution over the skin of the developing non-nervous parts of the eventual sense organs. These non-nervous parts plus the sensory hair cells detect and transduce the stimulus event into physiological signals that can conduct along the axons. They depend on the nerve growing in from the distant neuron to steer, trigger, and control the placement, orientation, tuning, and sensitivity that develops (see Evolution of the Nervous System in Fishes).

2.19.6 Evolution

Electric organs are known in some species of skates (rajoid elasmobranchs) and electric rays (torpinoids), without clear indication whether there was only one or more than one independent invention. The EOD of skates is weak and episodic, probably mainly functioning in communication between members of the same species, for example, in reproductive behavior. The EOD of torpedo is high in current but low in voltage and, whatever else it does, certainly contributes to catching prey fish by disorienting their swimming. Electric organs are known in a number of bony fish, including several species and presumably all families of catfishes (Siluriformes), including one with a strong EOD (*Malapterurus*), the others being weak, presumably social communicating systems. Probably all of the New World knife fishes (Gymnotiformes), which includes the classical, high-voltage, low-current electric eel (*Electrophorus*), all species of the African order of Mormyriformes (elephant nose and many others). One family of stargazers (Astroscopidae) has EODs of very moderate strength and quite unknown function. It seems likely that evolution has discovered how to make and make use of electric organs at least five times, independently and possibly more, an outstanding case of parallel evolution, possibly exceeded, however, by the number of plesiomorphic features independently invented for electroreception. Diversity on the motor side includes a variety of forms of innervation of the electrocytes in mormyrids, where these cells have stalks that in some species penetrate the flat disk-like electrocyte and receive motor nerve endings at a circumscribed part of the stalk. Studies of the mitochondrial DNA sequencing of many species permit conclusions about which form of innervation was primitive and which was derived and agree very well with phylogeny based on anatomical characteristics (Alves-Gomes and Hopkins, 1997). With such high sensitivity, it becomes difficult to guess what the world must look like to such an animal, especially what the sources of noise might be, that interfere with detection of significant signals or limit resolution. One such factor must be lightning, since it has been shown to cause millisecond pulses in natural water bodies, up to hundreds of

kilometers distant (Hopkins, 1973), overlapping in duration and forming the EOD pulses of many species of weakly electric fish. As in the case of the JAR, it may well be that brain processing which compares the afference from different parts of the body surface enables the fish to distinguish such far field sources from the reafference of its own EOD. The fish are demonstrably able to use high-frequency-dependent small differences in impedance but it is still not known just what discriminations these fish make, based on complex impedance, for example, among aquatic plants.

A speculation of possible interest in ethology is that there might be patterns of texture in the electrochemical fields close to the bottom or to objects past which water is flowing that electrosensitive fish can detect and characterize as useful signs of the features of the solid surfaces. One class of experiments has shown that such fish detect or compute the plane of an invisible (transparent plastic) bottom in order to maintain a preferred tilt or angle between the fish's vertical axis and the plane of the substratum (the ventral substrate response akin to the dorsal light reaction) (Meyer *et al.*, 1976).

For many years, there was no special reason to believe that there must be a novel modality for electrosense, but when it was discovered that many species have a very-low-voltage EOD and convincingly when Machin and Lissmann (1960) demonstrated that one of these feeble electric fish can learn to distinguish objects such as two porous ceramic filters one containing a 6 mm diameter glass rod and the other a 4 mm rod, invisible through the walls of the filter and hence differing only in electrical impedance, via the active ongoing EOD and sense organs that measure the local strength of the electrical field at different parts of the fish's body surface. The relevant signals are therefore in gradients of hundredths of a microvolt per centimeter (Machin and Lissmann, 1960). Even higher sensitivity has been measured, repeatedly, by directly applied electrical pulses in the seawater around some skates and sharks, via their passive electrical receptors; $0.005\ \mu V\,cm^{-1}$ at the peak of a 1 Hz wave can elicit feeding or orienting behavior (Kalmijn, 1982, 2000). Differences between species can be large: one factor being the salinity of the normal habitat. Freshwater forms are generally much less sensitive than seawater animals, which is perhaps correlated with the greater attenuation of available signals in the high conductance of seawater.

Electrosensory receptors have evolved in, presumably, all elasmobranchs, including the truly freshwater rays, as well as in the sister group of the Holocephala (rat fish and chimaeras) and in one group of the agnathans (cyclostomes), the lampreys or petromyzontids, but not in the hagfishes (myxinoids), which are otherwise more advanced in brain differentiation. Thus, electroreception contributes to the argument that these two subdivisions of the cyclostomes are unrelated except through remote common ancestors.

The only analogue of an electric organ discharge in lampreys is the synchronized muscle action potential accompanying each cycle of respiratory movements, and one report would suggest that the lamprey uses this synchronized potential as a form of active electroreception (Kleerekoper and Sibakin, 1956). Most elasmobranchs lack any analogue of an EOD, as far as we know, but they might well detect distortions of their own standing electric fields due to DC fields from the skin, gills, and mucous membranes, like everything living in a conducting medium. The same questions are open for other groups that possess electroreception but which lack electric organs. This includes the holocephalans, some aquatic salamanders (apodan urodeles), and most of the large and diverse superorder of siluriform teleosts (catfish and others). Perhaps the best evidence of animals apparently anticipating earthquakes is that of Hatai and Abe (1932) on catfish in Japan. Several recent discoveries have enlarged the list of catfish that have small electric organs or produce EODs now and then at intervals of many hours, requiring special techniques to capture the episodes for analysis (Baron 1994; Baron *et al.*, 1994, 2003).

At one stage, it seemed that having the central and peripheral specializations for electroreception must be a characteristic of the order and would be found in all families of those orders. An exception has turned up (Notopteridae of the Osteoglossiformes), where one suborder (Xenomystinae) has and another (Notopterinae) lacks the electrosense. Also puzzling are the stargazers *Astroscopus* and *Uranoscopus* (Baron and Mikhailenko, 1976). These live buried in the sand with only the eyes and lips showing, ready to inhale prey fish by suddenly opening the mouth; hence, like the long interval catfish, above, they have EODs episodically (when a moving shadow coincides with a tap on the wall of the container, simulating a passing fish; Pickens and McFarland, 1964), but seem to lack electroreception (by the usually reliable test of evoked potentials in the midbrain tectum). The EOD is doubtfully strong enough to disorient a passing prey and doubtfully early enough to give sensory guidance to prey catching.

Since the two orders display an active electrosense (using their own EODs) – the Gymnotiformes and the Mormyriformes are not closely related and many sister groups have no electric sense – it seems probable that their systems have been independently invented in evolution. The gymnotiforms are close to the siluriforms and might have shared a common ancestral invention. Just possibly the xenomystine knife fishes inherited an invention of the mormyriforms since they both belong to the osteoglossiforms. As few as two parallel inventions within the teleosts can be argued with parallelisms remarkable in their detail. Outside the teleosts, conceivably one single invention has left its mark in the elasmobranch form of ampullary sense organs and central pathways, in the petromyzontiforms through the elasmobranchs and the chondrostei (sturgeons and paddlefish), dipnoans (lungfish), crossopterygians and brachiopterygians, to the apodan urodele amphibians. Too little, if any behavioral research has been done on most of these groups to advance learning on what use they make of the sensory system as a passive sense.

Exceptions are the elasmobranchs and the paddle fish. In the former, elegant behavioral physiology (Kalmijn, 1973, 1982, 2000) has shown under quasinatural conditions that it is used for navigation relative to the earth's magnetic field due to currents induced either by the translation of the animal in ocean currents or to self-movement of the animal relative to the surrounding water as well as to find food fish buried in the sand. Note that each of these requires that the medium around the animal be a good conductor. In young paddle fish (*Polyodon*, Acipenseriformes, Chondrostei; Wilkens *et al.*, 1997), it has been convincingly shown that the electric sense guides prey capture even when the prey are small planktonic crustaceans about a millimeter long (*Daphnia*), evoking accurately directed capture of single individuals.

In a remarkable case of parallel evolution (see The Evolution of The Evolution of Parallel Visual Pathways in the Brains of Primates), electroreception is also present in monotreme mammals, both the spiny anteater, *Echidna*, and the duck-billed platypus, *Ornithorhynchus*. The latter is semiaquatic and feeds under water in the ponds and streams of New Guinea and Australia. It is not clear as yet just which of their live prey are located by this sense or how. But they respond to pulses or sustained currents a fraction of a millivolt per centimeter with saccades of the head suggestive of foveation and have thousands of sense organs reminiscent of teleost ampullary organs on the bill in parasagittal stripes. Side-to-side head swaying probably enhances sensitivity by creating a succession of ON and OFF stimuli, which also should aid in localizing the sources of the field and in measuring the distance. Between the stripes are arrays of push-rod mechanoreceptors that project to the same somatosensory cortex as the electroreceptors. Units there are bimodal, responding to both bill modalities and facilitating certain temporal input features. The system shows that somatosensory cortex can involve significant distance reception.

Echidnas are mainly terrestrial but feed in moist soil on living prey that doubtless provide ample electrical fields to home in upon, including earthworms firing their giant fibers. A few mucous gland electroreceptors on the tip of the snout have higher thresholds than those of the platypus. No electrosensory behavior has been documented but a moist snout making a liquid junction has been proposed to aid in finding insects (Gregory *et al.*, 1989).

Patently, many of these topics are still very fluid and will disclose new discoveries with further investigation.

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2.20 Evolution of Taste

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Glossary

amphid A paired chemosensory organ at the anterior end of nematodes including *Caenorhabditis elegans*. The amphid is innervated by 11 chemosensory neurons and one associated thermosensory neuron.

auxiliary cells Nonsensory cells of invertebrate sensilla. This class of cells includes socket cells, sheath cells, tormogen cells, and thecogen cells.

Ecdysozoa One of two major groups of the protostome division of the animal kingdom (cf. Lophotrochozoa). Members of this group, including arthropods and nematodes, possess an outer cuticle rather than an internal skeleton.

ecto-ATPase An enzyme that breaks down extracellular ATP. The ecto-ATPases can be divided into several molecular families.

inner labial sensillum One of eight chemosensory organs around the mouth (stoma) of nematodes. This organ contains only two sensory cells only one of which has free access to the external surface of the worm.

labellum A fleshy ovoid pad at the end of the probiscus of a fly used as a taste organ. The labellum houses numerous taste and tactile sensilla.

labrum A chemosensory organ in flies situated at the anterior end of the oral cavity.

Lophotrochozoa One of two major groups of the protostome divisions of the animal kingdom (cf. Ecdysozoa). Members of this group, including annelids and mollusks, share common developmental forms although the adults appear quite diverse.

Merkel cell A specialized epithelial cell found in vertebrates which participates in mechanoreception. Upon stimulation, Merkel cells release serotonin and ATP to activate a closely associated sensory nerve fiber.

Merkel-like basal cell A basal cell of nonmammalian taste buds named because of their similarity with epithelial Merkel cells in terms of structure and neurotransmitter contents.

Schreiner organ A presumed epithelial chemosensory organ of hagfish. This multicellular end organ is superficially similar, but not homologous, to taste buds. Unlike taste buds, Schreiner organs can be innervated by any epithelial nerve.

sensillum A sensory organ of invertebrates in which the sensory cells extend a hair-like process out of the cuticle.

sheath cells The inner non-neuronal cell of a sensory end organ of invertebrates, especially *C. elegans*, closely surrounding the sensory neurons.

socket cells The outer non-neuronal cell of an invertebrate sensory organ.

solitary chemoreceptor cell (SCC)	Scattered specialized chemosensory cells in the epidermis of aquatic vertebrates also found in the gut and airways of terrestrial vertebrates.
T1R	A family of mammalian taste receptors that includes three members which heterodimerize to form either sweet or umami receptors.
T2R	A large family of mammalian taste receptors that form bitter-sensitive taste receptor molecules.
taste cell	An elongate specialized epithelial cell of vertebrate taste buds.
thecogen cell	The inner auxiliary (nonsensory) cell of a sensillum, cf. sheath cell.
tormogen cell	The outer auxiliary (nonsensory) cell of a sensillum, cf. socket cell.

2.20.1 Introduction

We humans recognize taste as sensation arising from the oral cavity and indicating information about the chemical quality of potential foodstuffs. The sense of taste uniquely arises from the specialized sensory end organs for this system: taste buds. In humans, taste sensations are only those that we describe as salty, sweet, sour, bitter, and 'umami' (the taste of glutamate). All other oral sensations, for example, the coolness of mint, the smoothness of fats or the hotness of pepper, arise from the general cutaneous innervation of the epithelium and should not be considered to be 'taste'. So, for humans and other vertebrates, 'taste' is a system defined by the sensory end organs mediating the sensibility. For humans, and by extension, other vertebrates, taste can be defined by the use of taste buds in the context of food selection.

When examining the evolution of the sense of taste, we are met with several difficulties. First, how can the sense of taste be defined for organisms lacking taste buds and, second, is the sense of taste evolutionarily conserved across species, and if so, across what range of species? This second question leads directly to the issue of where and at what point in phylogeny did taste buds evolve, and what tissues they may have evolved from. I will consider each of these points in the following sections leading to the conclusion that taste is a well-defined sense only in vertebrates, where taste buds are a clearly recognizable feature. Related chemical senses in other taxa may have arisen independently and are not 'taste' other than by having an analogous function.

2.20.1.1 What is Taste?

For humans and other vertebrates, taste is a sensory system that starts with taste buds as the specialized sensory end organ and deals with information concerning the chemical composition of food in contact with mouthparts. Primarily, the sense of taste is used across taxa to distinguish the edible from the inedible (Glendinning *et al.*, 2000). The important features in the above definition are that taste is a chemical sense associated with mouthparts and utilized in control of feeding. Note that the definition says nothing about the medium conveying the stimulus (e.g., air vs. water), nor does it include any description of molecular features or transduction mechanisms – neither is a defining feature of the taste system. For the vertebrate clade, taste is defined by the sensory end organ; for invertebrates, this definition fails since taste buds exist only in vertebrates.

Single-cell organisms may show positive chemotaxis toward a food source by following a concentration gradient of an attractive substance (Van Houten, 2000). Although this behavior shares some aspects of taste-mediated behaviors in more complex organisms, it is not taste. Single-cell organisms have no oral cavity and have no specialized sensory end organs. To include the positive chemotaxis of single-cell organisms under the rubric of 'taste' would necessitate extending the abilities of taste and smell to plants which exhibit positive and negative growth in response to chemical signals in the environment (Filleur *et al.*, 2005).

A more difficult situation arises when examining the invertebrates. Complex invertebrates such as crustaceans and mollusks (Ache, 1987) have specialized chemoreceptors associated with well-defined mouth parts. These chemoreceptive end organs are not homologous to taste buds although they share several features with taste buds, for example, multicellular aggregates specialized for the detection of a limited variety of chemical substances. The presence of such specialized chemoreceptor organs on mouthparts certainly makes these end organs similar to taste buds in terms of function and behavior; yet, are they taste? The difficulty in drawing a conclusion about this depends on the context in which one wishes to use the comparison. For example, if one wishes to compare the behavior of a fly with that of a rat, then referring to the feeding-related perioral chemosensors of these animals as 'taste' has some utility. However, calling both of these systems 'taste' is misleading when considering the detailed molecular or cellular features of the sensory end organs, that is, the labellar sensillae of a fly are

entirely different from the taste bud of a rat. The sensory cells in flies are bipolar neurons extending an axon into the central nervous system (CNS); the sensory cells of taste buds are axonless, modified epithelial cells that synapse onto the peripheral process of a cranial nerve ganglion cell. These systems are analogous, but clearly not derived from a common ancestral condition, that is, they are not homologous.

Even for vertebrates, including humans, the word 'taste' is confounded by common usage meaning sensations arising from the mouth. Conversationally, we use the word 'taste' to include many aspects of flavor other than salty, sweet, sour, bitter, and umami. The confusion arises because of the nasopharynx connecting our oral and nasal cavities. Vapors from food in the oral cavity pass retronasally through the nasopharynx to reach the olfactory epithelium. Thus, food in our mouth stimulates not only taste buds, but also chemoreceptors of the olfactory and trigeminal systems. A further confusion is that, even among vertebrates, taste buds are not always confined to the oral cavity. Catfish, for example, have plentiful taste buds scattered across the body surface, being especially densely distributed on the barbels and leading edges of the fins. Despite their location, these oddly situated taste buds are innervated by a gustatory nerve (facial N.) and are used in the context of finding foods (Bardach *et al.*, 1967).

2.20.2 Taste in Invertebrates

As mentioned above, taste, when applied to invertebrates, is not as clearly defined as for vertebrates. Following the definition above, I will consider the sensory end organs used by different invertebrates in detecting nutritive substances and toxins in potential food items. By definition, the sensory end organs for taste must be associated with mouthparts or other appendages used in feeding. However, in many segmentally organized invertebrates, similar end organs often occur on mouthparts and legs. This may, in part, be due to the fact that mouthparts and legs are serial homologues in many segmented invertebrates. Even in nonsegmented invertebrates, for example, octopus, apparent taste end organs occur on the legs as well as mouthparts. In these cases, the anatomical distinctions are blurred and one must rely more on the context in which the end organ is used to define the system. By analogy to vertebrates, for invertebrates, we can then extend the definition of taste to include contact, or near-range (i.e., high-threshold) chemoreceptors used in a feeding context and which are similar to the chemoreceptors of the mouthparts.

The invertebrate clade includes relatively primitive, radially symmetric groups, for example, Cnidaria and Porifera, and the Bilateria including the Protostomes (Holland, 2000). While the more basal group of animals clearly respond to a variety of chemicals (presumably via specialized chemoreceptors), it is difficult to draw distinctions between various modes of chemoreception. Also, comparatively little is known about the nature of chemosensory cells in these basal forms.

The Protostomes fall into two large groups: Ecdysozoa (including nematodes and arthropods) and Lophotrochozoa (including flatworms, annelids, and mollusks; Holland, 2000). In both groups, taste as a feeding-related sense, can be distinguished from other well-developed chemosensory modalities. This article will describe aspects of the 'taste' systems in representatives of each of these major groups; it is not meant to be a comprehensive review.

2.20.2.1 Ecdysozoa

Many Ecdysozoa have a relatively impermeable cuticle covering the outside of the body. Hence, exteroceptive end organs including chemoreceptors must have sensory processes extending beyond the cuticle or else have openings in the cuticle to permit access to the external stimuli. Sensory end organs of this clade have a common general structure in which the cell bodies of the sensory neurons lie beneath the surface cuticle and extend dendrites to reach through or near the cuticle. The apical dendrites of the sensory cells are usually associated with one or more non-neuronal accessory cells designated by a variety of names, for example, sheath, socket, auxiliary, tormogen, and thecogen cells.

The overall organizational scheme of taste-like sensory organs in Ecdysozoa is similar in many respects to vertebrate taste systems. Yet, any similarity must be attributed to convergence rather than common origin. In both major groups, each taste organ comprises a variety of sensory cells 'tuned' to different chemical stimuli. That is, although each end organ responds to many different chemical cues, the individual sensory cells within the end organ are tuned fairly narrowly. In the Ecdysozoa, each receptor cell responds either to appetitive or to aversive substances, but never both. This dichotomy is reflected in the nonoverlapping central connectivity of the receptor cells and the behaviors driven by their stimulation (Wang *et al.*, 2004).

2.20.2.1.1 Nematodes Chemoreceptor cells and their molecular receptors are well studied in *Caenorhabditis elegans*. Unfortunately, the literature in this field is confounded by the tendency to refer to chemoresponses to water-soluble compounds as 'taste' while chemoresponses to volatiles is termed 'olfaction' although the same end organ (amphid chemoreceptors) is used to mediate both responses. As discussed above, the separation of taste and smell according to chemical nature of the stimulus is not generally useful (e.g., compare taste and olfaction in catfish). The well-studied chemoreceptor of the nematode *C. elegans* consists of paired amphid organs each innervated by 12 neurons (Figure 1; Ward *et al.*, 1975). Of these, 11 are chemosensory, the other being thermoceptive (Bargmann and Mori, 1997). Eight of the chemosensory neurons (ADF, ADL, ASE, ASG, ASH, ASI, ASJ, ASK) extend dendrites through the amphid pore in the cuticle to be in fairly direct contact

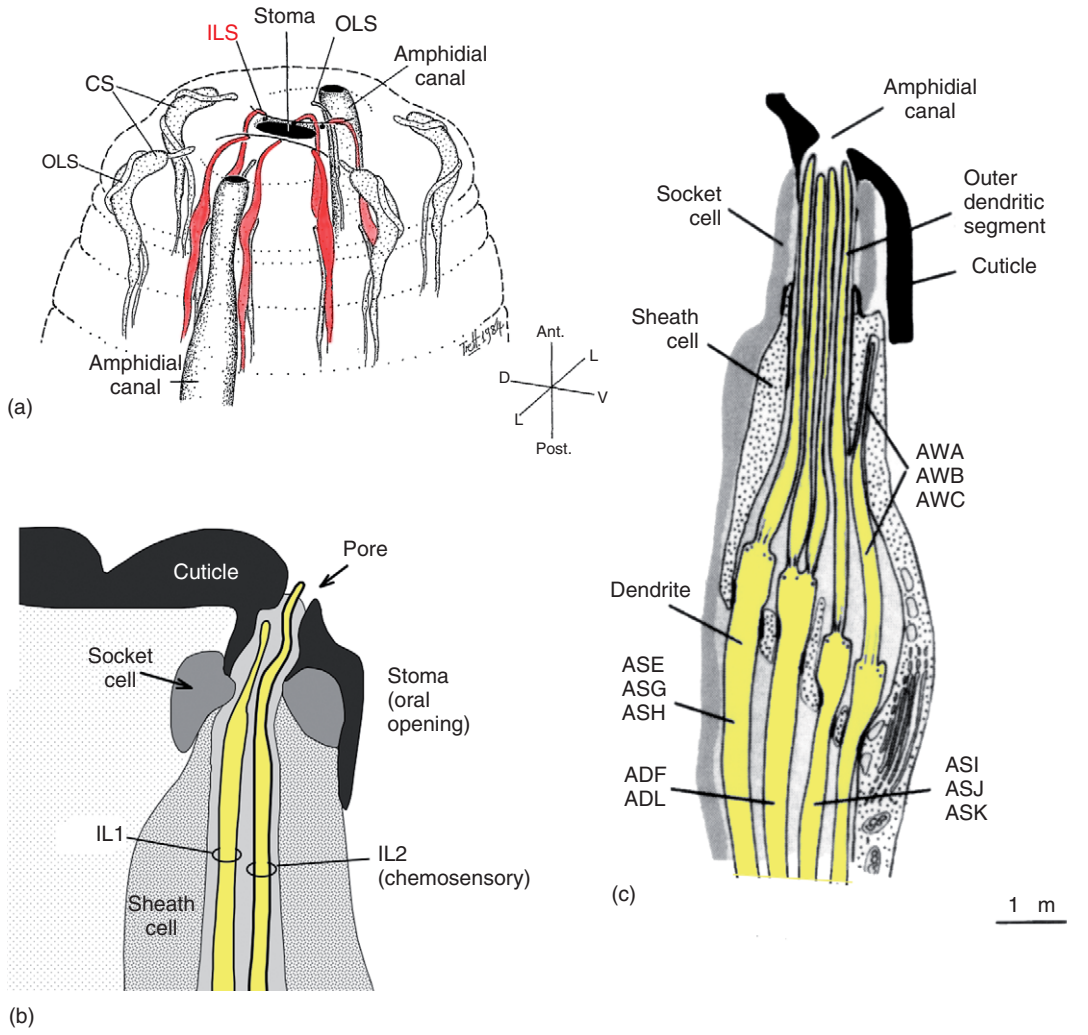

Figure 1 Chemoreceptor organs on the head of nematodes. a, Diagram showing the location of the head sensilla of *Pratylenchus* sp. CS, cephalic sensillum; ILS, inner labial sensillum; OLS, outer labial sensillum. b, Diagram of an inner labial sensillum (rendered in red in panel a). IL2, whose dendritic tip is exposed to the outside milieu, is a chemosensory neuron while IL1, whose tip is not exposed, is reported to be mechanosensory. c, The amphid contains numerous chemosensory neurons which detect either soluble (ASE, ASG, ASH, ADF, ADL, ASI, ASJ, ASK) or volatile (AWA, AWB, AWC) substances. It is not clear whether the amphid chemoreceptors should be considered 'taste' according to the definition in this chapter since stimulation of these receptor cells results in chemotaxis rather than feeding. a, Reproduced from Trett, M. W. and Perry, R. N. 1985. Functional and evolutionary implications of the anterior sensory anatomy of species of root-lesion nematode (genus *Pratylenchus*). *Revue Nematol.* 8(4), 341–355, with permission from IRD. b, Drawing based on Ward, S., Thomson, N., White, J. G., and Brenner, S. 1975. Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *J. Comp. Neurol.* 160(3), 313–337. c, Reproduced from Cell biology of olfactory epithelium. In: Neurobiology of Taste & Smell; Farbman, A.; *eds.* T. E. Finger, W. L. Silver, and D. Restrepo; Copyright © 2000, Wiley. Reprinted with permission of John Wiley & Sons, Inc.

with the environment. These cells respond to water-soluble substances. Three other amphid chemosensory neurons (AWA, AWB, AWC) have dendrites extending near the amphid pore, but are encapsulated by the 'sheath' or 'wing' cell and thus do not have direct contact with the environment. The AWA, AWB, and AWC cells respond to volatile substances, presumably those capable of diffusing through or being transported across the sheath cell. The commonly studied chemotaxic behaviors are driven almost entirely by these amphid chemoreceptors (Bargmann and Mori, 1997). As described above, the nematode chemotaxic behaviors are commonly divided into 'taste' and 'smell' according to the nature of the chemical stimulus (water soluble or volatile, respectively). I suggest that all behaviors mediated by the amphids should more properly be considered 'smell' since none of the measured behaviors is concerned with palatability of a suspected food object. Rather the amphid drives locomotor behaviors, just as the olfactory sense in vertebrates drives approach/avoidance locomotor responses.

Nematodes, including *C. elegans*, have a set of lesser-known chemoreceptors, the inner labial neurons, which are situated more within the oral cavity and appear likely to mediate taste-like behaviors (Tabish *et al.*, 1995). Yet, little is known of the function or responses of these perioral presumed chemoreceptors. Trett and Perry (1985) suggest, on the basis of structure, that the IL2 neuron of inner labial sensilla serve as contact chemoreceptors (just as taste buds have been described) but this speculation has yet to be confirmed by functional or behavioral studies. Nematodes studied to date possess six radially symmetric paired inner labial sensilla with cuticular openings facing the inner side of the rostral end of the oral cavity in many species (see Figure 1). Each sensillum is innervated by two neurons (IL1 and IL2) one of which (IL2) extends a process to reach the outer environment; the other sensory dendrite terminates just below the surface beneath the opening in the cuticle (Ward *et al.*, 1975). In parasitic species, however, the inner labial sensilla may be purely mechanosensory in that their sensory processes do not have access to the surface (Fine *et al.*, 1997) but this arrangement would not preclude detection of volatile substances like the AWA, AWB, and AWC amphid neurons.

2.20.2.1.2 Arthropods Arthropods, including insects, arachnids, and crustaceans, rely on chemosensory sensilla to detect chemicals in the environment. The best-characterized system is that of the fruit fly *Drosophila* (see Figure 2) but other arthropods appear to have receptors of similar ilk. Chemosensory sensilla are present not only on the mouthparts but also on the wing margins, tarsi (feet) and some other appendages likely to contact potential foodstuffs (e.g., Dethier, 1962). The chemosensory sensilla in the perioral region and upper alimentary canal apparently mediate feeding behavior and therefore fit into the definition of a sense of taste. The chemosensory sensilla on the other appendages are structurally and molecularly similar to the oral ones and are usually used in the context of food detection. So it is not reasonable to exclude these from the taste system merely because of their location on the body. The end organ structure is right and the behavioral context is right. Including the tarsal chemoreceptors as part of the taste system is analogous to including the taste buds on the barbels and body of fishes in their taste system. In the case of the external taste buds of fishes, they are clearly part of the taste system (based on the end organ structure, innervation, and behavioral context). By analogy, we should then accept the tarsal chemoreceptors of arthropods as being part of the taste system. A similar argument can be made for the chemoreceptors on the wing margins, although their behavioral context is less well studied. In contrast, the chemosensory sensilla of other body parts, for example, antenna or ovipositor, should not be included in the taste system, regardless of expression of common receptor molecules, since they are used in other behavioral contexts, for example, navigation or egg-laying.

The basic structure of the chemosensory sensilla is similar whether the end organ be on a mouthpart, wing, or leg. These end organs contain one mechanoreceptor cell and several (2–4), physiologically distinct chemosensory cells with an apical process (outer dendritic segment) extending into the sensilla proper which is a thin, hair-like protrusion of the cuticle (Shanbhag *et al.*, 2001). One or more pores lies at the apex of the sensilla thereby permitting substances in the outside medium to come into contact with the fluid (sensillum lymph) filling the space around the dendrites within the sensillum. Potential tastants must then traverse the fluid-filled space to activate receptors on the dendrites of the sensory cells. As is typical of invertebrate sensory cells, each receptor cell of the chemosensory sensilla contributes an axon to the peripheral nerves which then enter the CNS. In addition, there are numerous taste 'pegs', which are smaller sensory sensilla that protrude little from the surface of the epithelium and which bear only one chemosensory cell along with a mechanosensory cell (Shanbhag *et al.*, 2001).

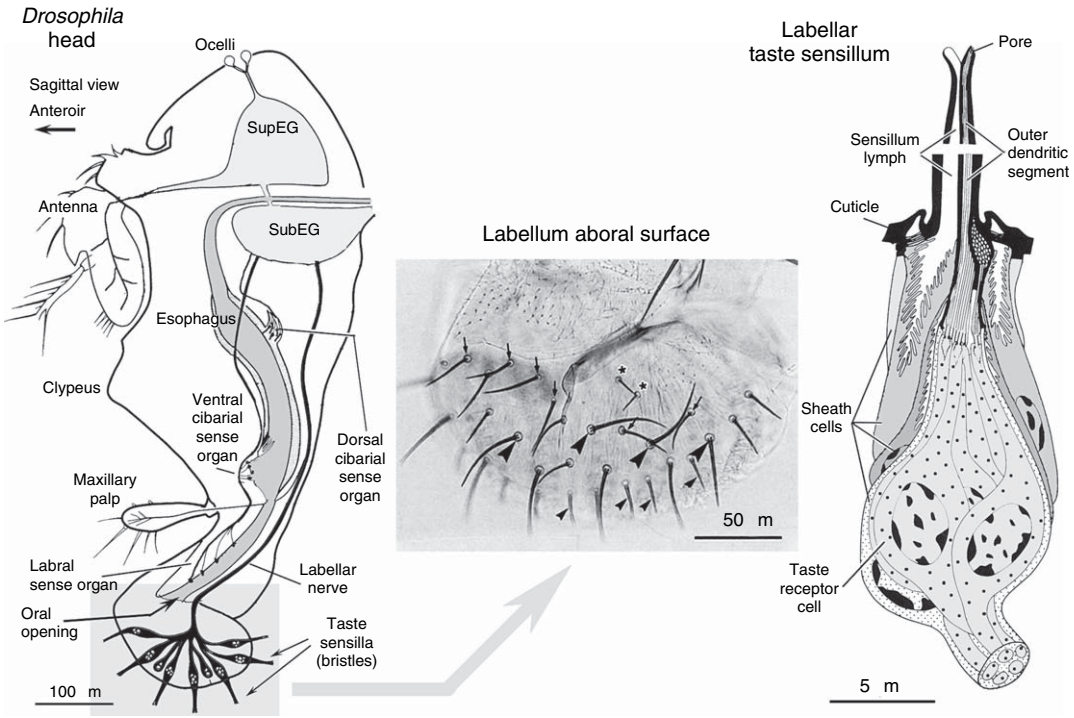

Figure 2 Taste receptors on the fly *Drosophila*. Left: Drawing of a sagittal section through the head of the fly showing the location of the major taste organs: labellum, labral sense organ, and cibarial sense organs. Center: Labial palp whole-mount preparation showing the aboral surface of left palp. Anterior is left and dorsal top. Sensilla marked with stars are purely mechanosensory and the remaining are taste bristles. Taste sensilla are divided into three sub-types: short (small arrowheads), intermediate (arrows), and large (large arrowheads). Only some sensilla of each sub-type are marked. Right: drawing of a single sensillum showing the receptor and auxiliary cells. Center panel, reproduced from *Cell Tissue Res.*, vol. 304(3), 2001, pp. 423–437, Gustatory Organs of *Drosophila melanogaster* : Fine Structure and expression of the putative odorant-binding protein PBPRP2, Shanbhag, S. R., Park, S. K., Pikielny, C. W., and Steinbrecht, R. A., figure 1b, with kind permission of Springer Science and Business Media. Left and right panels, reproduced from cell biology of taste epithelium. In: Neurobiology of Taste & Smell; Finger, T. E. and Simon, S. A.; *eds.* T. E. Finger, W. L. Silver, and D. Restrepo; Copyright © 2000, Wiley, and modified from the original work of Singh (1997). Reprinted with permission of John Wiley & Sons, Inc.

In *Drosophila*, chemosensory sensilla are especially dense on the labellum and to a lesser extent on the labrum, which sits at the entrance to the oral cavity. Intraoral chemosensory sensilla are also present in the cibarial sense organs. Both the intraoral and oral chemosensory end organs form nerves that terminate within the subesophageal ganglion, in contradistinction to the olfactory (antennal) receptors that project to the antennal lobes of the supraesophageal ganglion.

The labellar chemosensory sensilla are divisible into three morphological types according to the length of the sensillum: short (s-type), intermediate (i-type), and long (l-type) (Shanbhag *et al.*, 2001). The i-type sensilla possess only two chemosensory cells, whereas the s- and l-types have four chemosensory cells. The chemosensory cells fall into four broad functional classes according to chemoresponsiveness. The w-cells respond to water, s-cells respond to sugars, L1-cells respond to low concentrations of salt, and L2-cells to high concentrations of salt and to various bitter substances. But this formulation may be overly simple (e.g., see Hiroi *et al.*, 2002). The two chemosensory cells of the i-type sensilla consist of one cell with L2-type responses (bitter, high salt) and the other cell with a combination of S and L1 properties (Hiroi *et al.*, 2004). Water-responsive units are present only in the s-type and l-type sensilla. In summary, the sensory cells of *Drosophila* gustatory sensilla fall into one of two groups according to the behavior elicited by their activation: one group (e.g., s-units, w-units, and L1-units) drives appetitive behaviors under the right motivational conditions, while the other group (L2-units responsive to high salt and bitter substances) drives aversive behaviors.

The dichotomy in driven behaviors of the different types of receptor cells coupled with the presence of an axon extending directly from the receptor cell to the CNS, permits direct assessment of the pattern of projection into the brain of these functionally different types of receptor cells (Inoshita and

Tanimura, 2006; Wang *et al.*, 2004). Gustatory information in the CNS of *Drosophila* is organized first, according to gustatory end organ, and second, according to driven behaviors – appetitive or aversive. Thus, the taste sensilla on the labellum project to a different part of the subesophageal ganglion than do the taste organs within the oral cavity proper (Stocker and Schorderet, 1981; Wang *et al.*, 2004). Within the subesophageal ganglion, bitter-responsive cells (L2-type) map dorsomedial to the sugar-responsive (L1-type) neurons. Water-responsive receptor cells also project to the lateral neuropil of the subesophageal ganglion, perhaps overlapping or slightly lateral to the sugar-responsive group (Inoshita and Tanimura, 2006).

Some interesting similarities exist between the insect and mammalian gustatory systems. First, the gustatory end organs comprise multiple sensory cells exhibiting a limited range of chemoresponsiveness. That is, each end organ responds to a spectrum of tastants, although each sensory cell within that end organ is more limited. Second, the fundamental organizational plan in the CNS is one of organotopy, that is, each part of the body is represented in a unique part of the CNS, suggesting that the location of a chemical cue is key to gustatory-mediated behavior. Finally, within each organ-specific zone of the CNS, quality may be encoded by position within the somatotopically delineated field of neuropil. Just as different areas of neuropil are implicated in appetitive versus aversive cues in the subesophageal ganglion of the fly, different areas of neuropil appear activated by different tastants in the gustatory centers of mammals (Harrer and Travers, 1996; Sugita and Shiba, 2005).

2.20.2.2 Lophotrochozoa

2.20.2.2.1 Annelids The annelids, as represented by earthworms and leeches, have widespread chemoreceptors scattered across their body surface, but a set of these, associated with the lips (labia) control feeding behavior (Elliott, 1987). These are relatively poorly characterized, except for the labial chemoreceptors of leech which were studied both anatomically and physiologically by Elliot (1986, 1987).

The medicinal leech, *Hirudo medicinalis*, will initiate a full sequence of feeding behavior in response to human blood or plasma whether presented at room or body temperature (Elliott, 1986). The essential components of blood appear to be NaCl and arginine, which together provoke the full feeding behavior. The sensory region crucial to this behavior is the dorsal lip whose ablation results in loss of the feeding sequence in response to chemical stimulation. Likewise, in *Haemopsis marmorata*, a carnivorous leech that eats and trails earthworms, ablation of the dorsal lip abolishes their ability to track earthworm trails (Simon and Barnes, 1996).

The dorsal lips of leeches contain large and small sensilla containing unique ciliated sensory cells. As is typical of invertebrates, the sensory cells are bipolar neurons with a centrally directed axon and a dendritic process that extends to the surface of the epithelium. The sensory cells are grouped together into sensilla of two different sizes. The approximately 150 larger sensilla are arrayed ~125 μm apart in a band across the dorsal lip. Each sensillum forms a raised papilla of ~35 μm in diameter with an apical opening of ~20 μm through which extend the cilia of the underlying sensory cells (Elliott, 1987). Each sensillum contains multiple sensory cells, but the number is not specified. The 250 small lip sensilla are 8–10 μm in diameter and lie along the edges of the stripe of large sensilla. The smaller sensilla which sit flush to the surface of the surrounding epithelium can be recognized by the collection of cilia protruding from the surface. Since each sensory cell possesses a small number of cilia, a small sensillum is likely to comprise a dozen or so sensory cells (Elliott, 1987).

The nerves formed by the axons of the sensory cells assemble mostly into the dorsal cephalic nerves (Perruccio and Kleinhaus, 1996) to reach the cerebral ganglia. Stimulation of the lip region with either NaCl or arginine evokes robust neural activity (Li *et al.*, 2001). Interestingly, simultaneous stimulation with quinine or denatonium, both of which are feeding deterrents in these animals, reduces peripheral afferent activity. These findings suggest that feeding deterrents may act, at least in part, by inhibiting the neural response to appetitive cues (Li *et al.*, 2001).

2.20.2.2.2 Mollusks The taste-related chemoreceptors of mollusks have been characterized in both gastropods and cephalopods. In gastropods, as typified by *Aplysia*, feeding-related chemoreceptors are present on the lips and anterior tentacles (Jahan-Parwar, 1972). Likewise, cephalopods, especially well studied in octopus, have chemoreceptors on the tentacles as well as in the perioral region. Those associated with the suckers on the tentacles were well described in an elegant series of papers by Graziadei (Graziadei, 1964a, 1964b, 1965; Graziadei and Gagne, 1976) following studies by Emery (1975a, 1975b) on ciliated sensory cells (assumed to be chemoreceptors) on the lips of

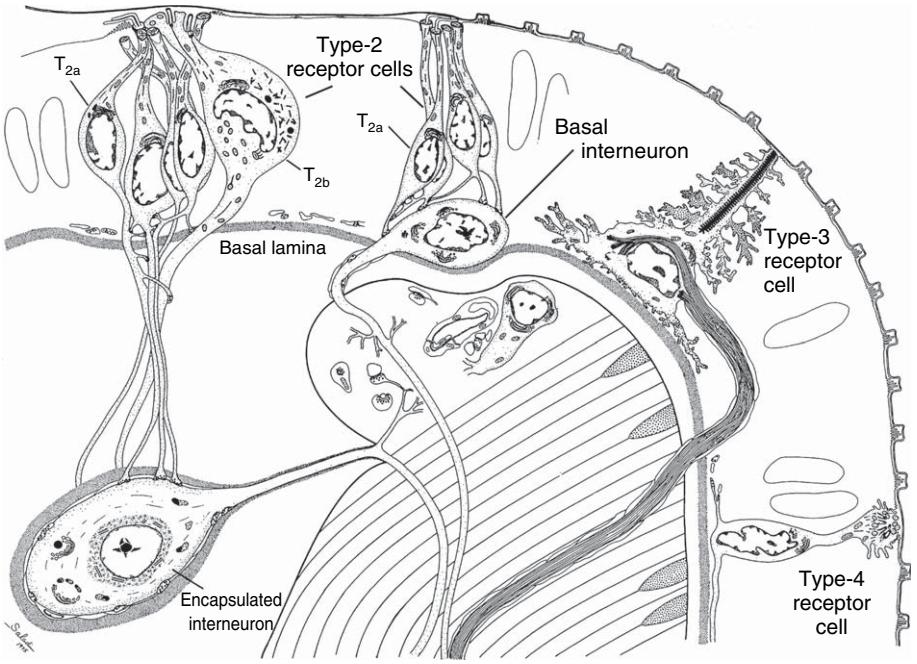

Figure 3 Diagram of sensory neurons in the rim of a sucker on the arm of an octopus. Based on structural considerations, type-2 receptors are likely to be chemoreceptors as are the type-4 cells which look similar to olfactory receptor cells in squid. Type-3 cells appear to be mechanoreceptors. The clusters of type-2 cells superficially resemble vertebrate taste buds, but obvious structural differences exist. The occasional contacts between some type-2 cells and basal interneurons is reminiscent of the relationship between elongate taste cells and Merkel-like basal cells in nonmammalian vertebrates. Reproduced from 'Sensory innervation in the rim of the octopus sucker', *J. Morphol.*; Graziadei, P. P. and Gagne, H. T.; Copyright © 1976, Wiley-Liss. Reprinted with permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.

squid and octopus. A brief summary of Graziadei's findings follows, but the reader should refer to the original papers for a complete description of the sensory apparatus of the tentacles.

Sensilla of the mollusks are similar in many ways to the sensilla of leeches. The sensory cells are bipolar neurons with an apical dendrite that extends to the surface of the epithelium, and a basal axonal process that contributes to nerves coursing to the CNS. The likely chemoreceptors of the sucker are elongate epithelial cells (termed type-2 cells by Graziadei and Gagne, 1976) which are collected into small 'apical clusters' (5–10 cells), superficially similar to taste buds in vertebrates (see Figure 3). The most common form of sensory cell in the apical cluster is a narrow elongate cell (type 2a of Graziadei and Gagne) which extends a small number (e.g., 3–8) cilia above the surface of the surrounding epithelium. The apical clusters may contain a second elongate cell type (type 2b) which is larger than the 2a cells and has somewhat different cytological features. The apical clusters also may be associated with a horizontally oriented 'basal interneuron' lying between the apical cluster and the basal lamina of the epithelium. These basal interneurons as well as 'encapsulated' interneurons apparently receive synaptic contacts from the type-2 cells of the apical cluster. The situation is reminiscent of the organization of taste buds in bony fishes where the elongate sensory cells synapse onto a Merkel-like basal cell (see below). Of course, the tentacle sensilla of octopus are not homologous to taste buds in vertebrates, hence similarities in organization must be due to convergence rather than phyletic continuity.

2.20.3 Taste in Vertebrates and Chordates

Taste buds are recognizable throughout the vertebrate lineage – from lampreys to teleosts to mammals. Although structural details can be quite varied across species, taste buds retain a host of key features that distinguish them from other end organs. The common features of taste buds include: (1) aggregates of specialized epithelial cells including both receptor and supporting cells (since the cells are epithelial, they have a limited life span and are continuously replaced through out the life span of the animal); (2) more than one type of sensory cell reaching the epithelial surface via an

opening (taste pore) in the surrounding epithelial covering; and (3) sensory (afferent) innervation from facial, glossopharyngeal, or vagus nerves which project to the viscerosensory column of the medulla. Taste buds in diverse vertebrates share other features but it is unclear whether such features are necessary as defining features, or are rather elements in common to a subset of vertebrates. Such common features include: (1) a cell type capable of concentrating and releasing serotonin (Kim and Roper, 1995; Nada and Hirata, 1977); (2) one or more cells that manifest a neuron-like phenotype (e.g., expressing NCAM: Nelson and Finger, 1993; Smith *et al.*, 1993), neuron-specific enolase (NSE) (Toyoshima *et al.*, 1991; Yoshie *et al.*, 1989), or neural differentiation markers such as Mash-1 (Kusakabe *et al.*, 2002); and (3) strong ecto-ATPase activity (Iwayama and Nada, 1967; Barry, 1992) perhaps because ATP is a requisite neurotransmitter in this system (Finger *et al.*, 2005).

2.20.3.1 Epithelial Chemoreceptors in Chordates

The chordate lineage includes the invertebrate cephalochordates (e.g., *Amphioxus*) and craniates. The craniates can be subdivided into two groups: (1) hagfish and their relatives, and (2) true vertebrates, including both agnathan (lamprey) and gnathostome lineages. All extant vertebrates, from lampreys to amniotes, have clearly recognizable taste buds innervated by branches of the facial (CN VII), glossopharyngeal (CN IX), or vagus (CN X) nerves. The cells of taste buds are modified epithelial cells and, unlike most invertebrate receptors, do not possess an axon or any process extending below the basal lamina. While taste buds are clear in all vertebrates, the evolutionary origins of these end organs is obscure.

Sensory cells in nearly all invertebrates are primary sensory neurons, also called type I receptors, complete with both a sensory dendrite extending to the epithelial surface and an axon connecting to the CNS (see above). The amphioxus has many such epithelial sensory cells including type I cells of Lacalli (Lacalli and Hou, 1999). But secondary sensory neurons first make a substantial appearance in this group of organisms. The epithelial secondary sensory cells of *Amphioxus* (type II receptors; Holland and Yu, 2002) extend immotile cilia to the epithelial surface. These cilia contain numerous microtubules (Lacalli and Hou, 1999) rather than the more standard 9+2 arrangement for cilia. The apical morphology of the type II receptors is striking in that a ruff or collar of microvilli surround a central elongate cilium (Figure 4). This feature is commensurate with a chemosensory rather than mechanosensory function. The type II epithelial receptors extend two or three basal processes a short distance within the epithelium to synapse onto neural processes (Lacalli and Hou, 1999).

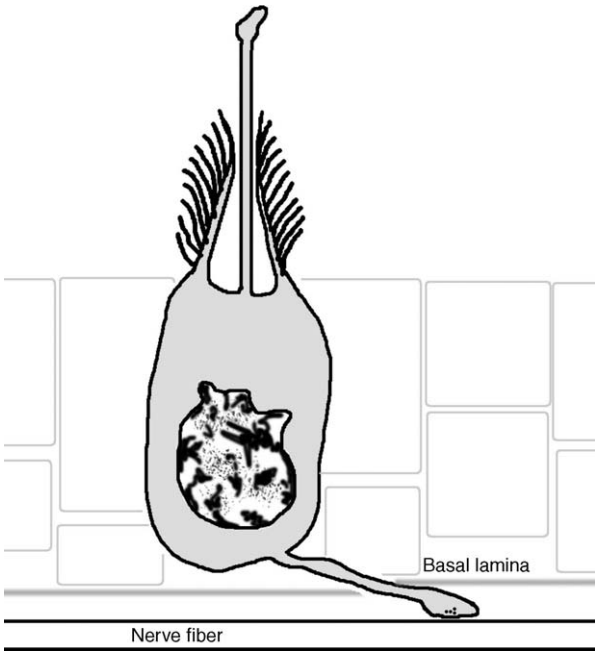

Figure 4 Schematic drawing of a type II sensory cell from *Amphioxus*. These receptor cells bear a long central cilium surrounded by a ruffle of microvilli. The numerous microvilli, which serve to expand the surface area of the cell, coupled with the lack of a 9+2 microtubule arrangement in the cilium are consonant with a chemosensory function. These sensory cells often extend a short process, sometimes through the basal lamina, to synapse on nearby nerve fibers. Based on descriptions and figures in Lacalli, T. C. and Hou, S. 1999. A re-examination of the epithelial sensory cells of amphioxus. *Acta Zool.* 80, 125–134.

2.20.3.2 Solitary Chemoreceptor Cells and Schreiner Organs

All craniates, including hagfishes, possess solitary chemoreceptor cells (SCCs) scattered within the epithelium of the gut, respiratory tract and even across the body surface (Whitear, 1992; Finger, 1997; Sbarbati and Osculati, 2003). SCCs resemble the type II sensory cells of amphioxus as well as the individual cells of taste buds in terms of being elongate, columnar epithelial cells which synapse onto cranial nerve sensory processes. The SCCs differ from taste buds in that they can be innervated by any cutaneous or visceral nerve. For example, SCCs scattered across the surface of the body of fishes are innervated by the local cutaneous nerve – either

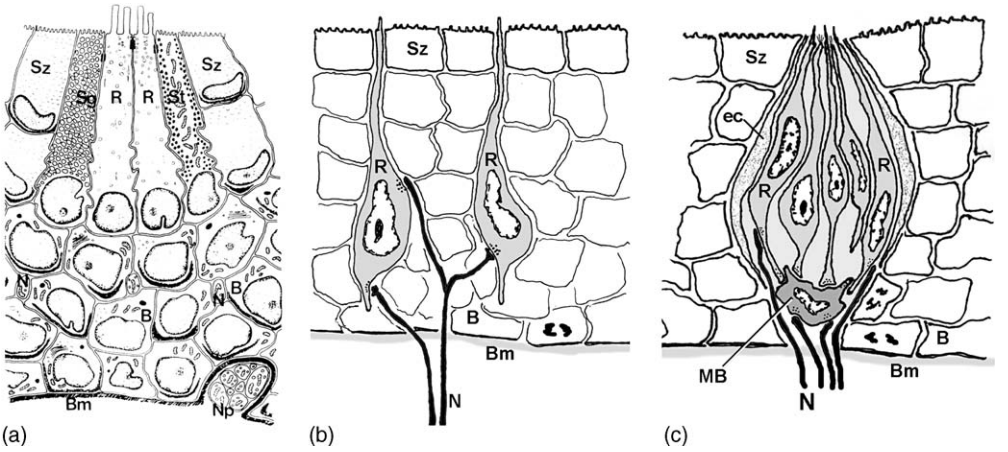

Figure 5 Schematic drawing comparing a Schreiner organ in a hagfish (a), with SCCs (b), and a taste bud in a typical teleost fish (c). In (a), the receptor cells (R) do not extend to the basal lamina and are flanked by various supporting or secretory cells. Part b is a schematic diagram of SCCs in a typical teleost. The SCCs are isolated in the epithelium appearing without associated supporting or secretory cells. Part c is a schematic diagram of a taste bud from a typical teleost. Multiple types of receptor cells are surrounded by flattened edge cells. The receptor cells reach nearly to the basal lamina where they form synapses with both nerve processes and Merkel-like basal cells. B, basal cell; Bm, basal lamina (basement membrane); ec, edge cell; MB, Merkel-like basal cell; N, nerve fiber; Np, nerve plexus; R, receptor cell; Sz, mucous cell; Sg, glandular supporting cell; St, type II supporting cell. a, Reproduced from Georgieva, V., Patzner, R., and Adam, H. 1979. Transmissions- und rasterelektronenmikroskopische Untersuchungen an den Sinnesknospen der Tentakel von *Myxine glutinosa* L. (Cyclostomata). *Zool. Scripta* 8, 61–67, with permission from Blackwell Publishing.

spinal or trigeminal according to location. In contrast, taste buds on the body are innervated by a recurrent branch of the facial nerve, not by the local spinal nerve (Herrick, 1901). Hence, taste buds always have a unique relationship with the cranial nerves associated with epibranchial placodes (Northcutt and Barlow, 1998).

Hagfish (chordates, but perhaps not vertebrates) lack taste buds as defined above, although they do possess Schreiner organs, which are multicellular aggregates of presumed chemoreceptor cells. These may simply be aggregations of SCCs, but the cells of the Schreiner organ are not identical to SCCs. Schreiner organs also have several features similar to taste buds, but do not share all of the features of taste buds, for example, Schreiner organs do not span the full thickness of the epithelium and do not possess three cytologically distinct cell types. The relationship between Schreiner organs, SCCs, and taste buds remains enigmatic (see Braun, 1998, for a nice discussion of this issue).

The ultrastructure of the Schreiner organs has been described by Georgieva *et al.* (1979), who found there to be one type of sensory cell (type I) replete with microvilli, a likely supporting cell and an associated secretory cell similar to mucus cells elsewhere in the epithelium (see Figures 5 and 6). The sensory cells of Schreiner organs appear identical to the SCCs in the same species. Further ultrastructural studies are necessary in order to determine the degree of similarity between the Schreiner organ cell types and those of taste buds. For example, the supporting cells (type II cells) of Schreiner organs are similar to type I (glial-like) cells of taste buds in that they wrap around the sensory cells. Our preliminary data indicate that Schreiner organs are not associated with high levels of ecto-ATPase, which is a key feature of the type

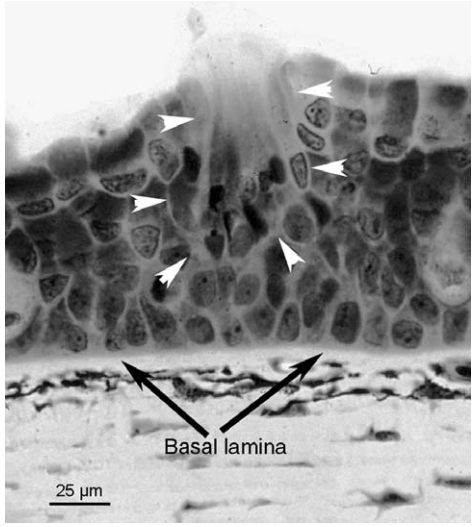

Figure 6 Photomicrograph of a Schreiner organ for the hagfish, *Eptatretus*. Note that the sensory organ (arrowheads) lies well above the basal lamina. Photomicrograph courtesy of Dr. C. Braun, Hunter College, New York, NY, USA.

I (glial-like) cells of vertebrate taste buds in which ATP serves as a neurotransmitter (Finger *et al.*, 2005; Kirino *et al.*, 2006). Thus, Schreiner organs and tastes buds are further distinguished in terms of utilizing different neurotransmitter systems.

Whatever the similarities of Schreiner organs and taste buds, it is noteworthy that SCCs themselves and taste buds share several features. Both comprise modified epithelial cells that undergo continuous replacement during the life of the animal. In the catfish, *Ictalurus punctatus*, the SCCs and taste buds react similarly to the PHA-E lectin (*Phaseolus vulgaris* agglutinin) which reacts with the arginine-binding taste receptor protein (Finger *et al.*, 1996). Thus, in these fish, it appears that SCCs and taste buds may utilize a common receptor mechanism. Similarly, in mammals, nasal and gut SCCs, like taste buds, express T2R (bitter) and T1R receptors and their associated downstream signaling components (Finger *et al.*, 2003; Sbarbati and Osculati, 2003). Thus, in both teleosts and mammals, SCCs and taste buds may utilize common receptor mechanisms. Nonetheless, differences do exist. Whereas SCCs form clear synapses with nerve fibers, the cells of taste buds that share biochemical features with SCCs (type II cells – see Section 2.20.4.3.1.(*ii*)) do not. Further studies are needed to understand the evolutionary relationships between these cutaneous chemoreceptor systems.

2.20.4 Taste Buds in Vertebrates

In this article, I present an overview of some of the different appearances of taste buds, but this is not meant to be comprehensive. An excellent comparative view of taste buds can be found in the work of Reutter and Witt (1993).

The structure of taste buds varies considerably across vertebrates (see Figure 7) but several consistent features emerge when comparing across species, as described above. These include: (1) aggregates of 50–150 specialized epithelial cells including both receptor cells and glial-like supporting cells, (2) multiple types of elongate cells reaching an opening in the epithelial surface, and (3) innervation by one of the three gustatory nerves: facial, glossopharyngeal, or vagus. Categorization of cell types within taste buds is complicated by the fact that taste buds consist not only of different functional types of cells, but also cells of different ages within each functional class. Taste buds are surrounded by specialized epithelial cells, 'edge', 'marginal', or ciliated cells (in frog), which form the outer boundary of the taste bud proper. In addition, all taste buds are closely associated with proliferative basal cells which divide to replace the aging and apoptotic cells of the taste bud. The literature on the types of cells in taste buds is extensive and complex (reviewed in Yee *et al.*, 2001). Rather than reviewing the vagaries of this literature, I will present a summary of our current understanding of the organization and structure of taste buds.

Some groups, such as frogs, have distinctive apomorphic characteristics, where taste buds take on a broad cylindrical form of large taste 'disks' spanning 100 μm. Most vertebrates have more compact taste buds organized in an onion-like configuration with an apical pore only tens of micra across. These more compact taste buds, found in all vertebrate groups, have two different plans of organization, typified in the descriptions below as the nonmammalian and mammalian schemes (the situation in birds is not clear). Whether these differences in morphology are more related to phylogeny or to habitat is unknown.

Historically, elongate taste cells in taste buds have been categorized according to their propensity to stain with acidophilic dyes or degree of osmiophilia in preparations for electron microscopy. This has led to descriptions of cells as being either 'light' or 'dark' but these descriptors may vary according to preparatory technique and particular stain utilized. Some authors have extended this classification system to imply function, characterizing the elongate taste cells as being 'sustentacular' (or 'supporting') versus 'sensory' ('receptor') cells. More careful ultrastructural analysis leads to a characterization according to structural features such as size and shape of apical specialization, presence of distinctive granules, or size and shape of the nucleus. Nonetheless, the mixed nomenclature remains in the current literature.

2.20.4.1 Taste Buds in Nonmammalian Vertebrates

Taste buds in these aquatic forms have been described in many teleosts, a few elasmobranchs, and urodeles (reviewed in Reutter and Witt, 1993) as well as in a lamprey, where the end organs have been called 'terminal buds' (Baatrup, 1983). The detailed structure of taste buds can vary substantially between species, or even within a species, between taste buds situated in different locations, for example, oral compared to extraoral (Reutter and Witt, 1993). Nonetheless, a common organizational plan can be abstracted.

2.20.4.1.1 Cell types Taste buds in this group are distinguished by containing not only elongate

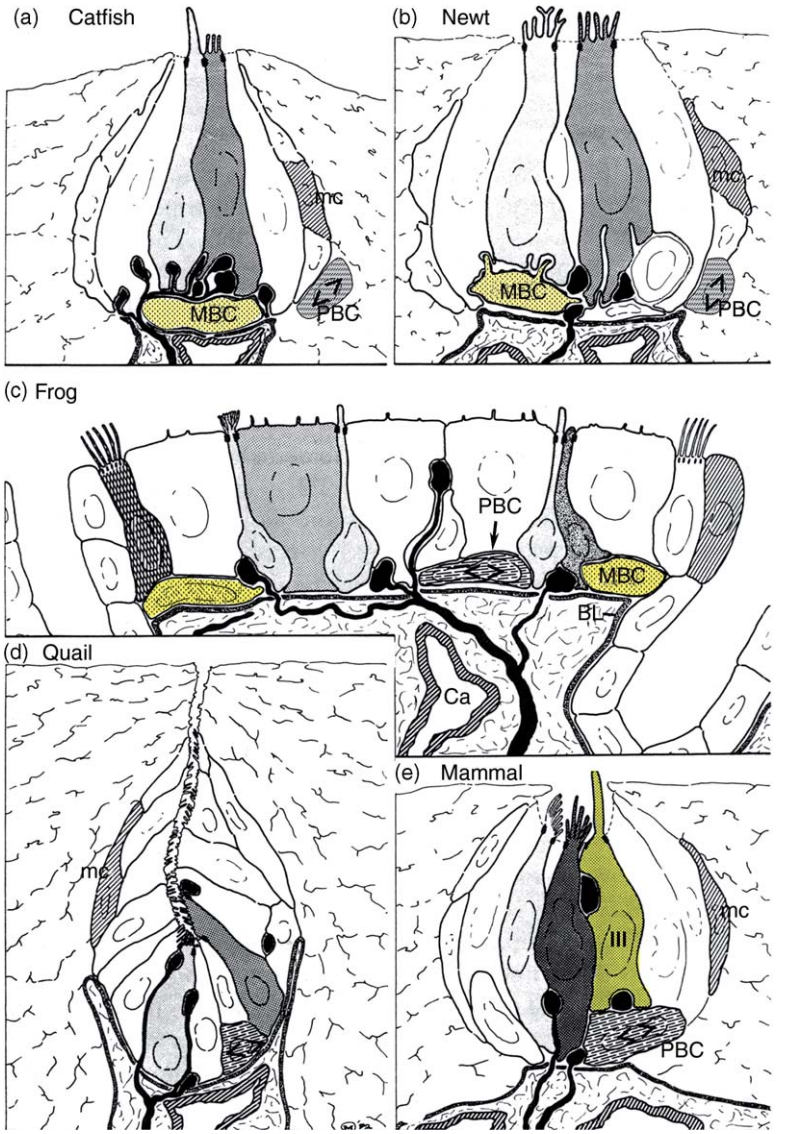

Figure 7 Schematic drawings of taste buds from various vertebrates. The area over which receptor cells gain access to taste substances (receptor area) is relatively broad in aquatic species, but narrows to a 'taste pore' in mammals and birds. Taste buds in all species contain different types of elongate cells indicated by the varied shading. Also, in all species, taste buds are bounded by specialized epithelial cells termed 'edge' cells or 'marginal' cells (mc). In all species, taste buds contain a serotonergic cell type – Merkel-like basal cells (MBCs) in nonmammalian forms, and type III taste cells (III) in mammals. All taste buds are also associated with a population of proliferative basal cells (PBCs) which undergo continuing cell division to replace the taste bud cells throughout the life span of the animal. BL, basal lamina; Ca, capillary. Copyright © 1993; From 'Morphology of vertebrate taste organs and their nerve supply'. In: Mechanisms of Taste Transduction by Reutter, K. and Witt, M.; *eds.* S. A. Simon and S. D. Roper. Reproduced by permission of Routledge/Taylor & Francis Group, LLC.

(columnar) spindle-shaped cells, but also a small number (e.g., five) of nonproliferative, 'Merkel-like' basal cells, lying in the lower half of the taste bud and which do not extend to the apical surface of the epithelium. Like cutaneous Merkel cells, the Merkel-like basal cells of taste buds concentrate biogenic amines including serotonin and are immunoreactive for NSE (Reutter and Witt, 1993). Also, like cutaneous Merkel cells, the Merkel-like cells of taste buds extend numerous spine-like processes from their cell body to form synapses on nerve fibers as well as on the elongate taste cells in the taste bud. It is likely that these Merkel-like basal cells, like cutaneous Merkel cells, serve as mechanoreceptors or perhaps in the taste bud, as integrative elements (Ewald and Roper, 1994). It is unfortunate that these Merkel-like basal cells are sometimes referred to simply as 'basal cells' in that this causes confusion with the proliferative basal cells associated with taste buds of both aquatic and terrestrial species.

The nonmammalian type of taste bud also possesses several types of elongate modified epithelial cells that extend an apical process into the region of the taste pore. In aquatic forms, including fishes and aquatic amphibians, the apex of the taste bud is a substantial opening – 10–20 μm or larger – in the surrounding epithelium through which extend the apices of the elongate taste cells (Figure 8). This opening in the epithelium is much larger than the equivalent 'taste pore' present in mammals or birds. Whether this difference in the size of the taste pore is characteristic of the clade of vertebrates (e.g., poikilothermic vs. homothermic) or of the habitat (aquatic–terrestrial) is unclear.

Elongate cells in fish and amphibia usually are characterized as being 'light' or 'dark'. These two descriptors are undoubtedly inadequate to fully characterize all of the different types of elongate cells present in these taste buds. The light cells are spindle-shaped cells with a single, large apical microvillous extending into the taste pore. Light cells extend short branches from their base to synapse with the Merkel-like basal cells and with nerve fibers. Dark taste cells are irregular in cross-sectional form and may envelop or extend interdigitating processes between the light cells (Reutter and Witt, 1993). At its apex, a dark cell extends numerous (10–25) small microvilli into the taste pore. Although dark cells apparently form synaptic contacts with the Merkel-like basal cells, they rarely do so with nerve fibers. In *Necturus*, light cells constitute only ~25% of the elongate cells within the taste bud, the remainder being dark cells. Taste buds in fish and *Necturus* also contain a less common, third cell type with a brush-like or bushy microvillous apex.

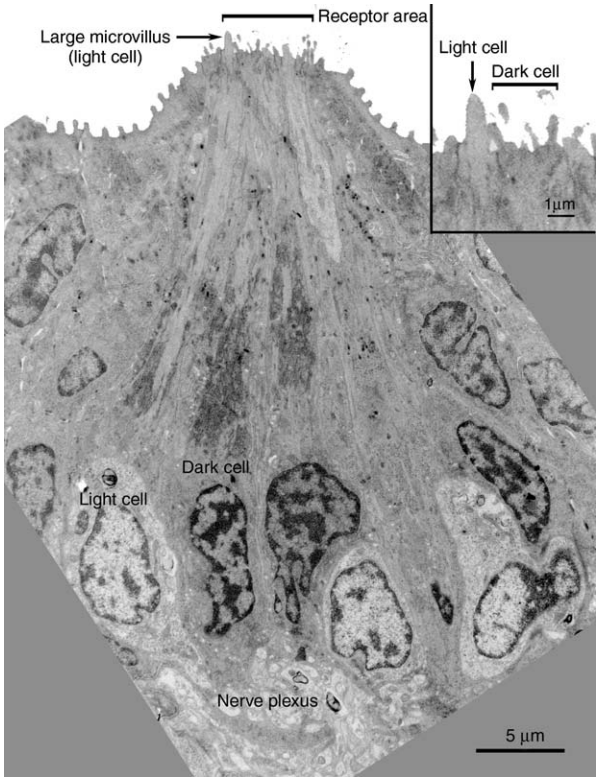

Figure 8 Electron micrograph of a taste bud from a zebra fish. Even at this low magnification, the different sizes of microvilli within the receptor area (taste pore) are evident. The large microvillus belongs to a 'light cell' while the smaller microvilli originate from a 'dark cell'. Inset (upper right) shows an enlargement of the receptor area. Courtesy of Dr. Anne Hansen, University of Colorado.

2.20.4.1.2 Proliferative cells In nonmammalian vertebrates, the taste bud is closely associated with a small number (e.g., 5) of proliferative basal or marginal cells that apparently generate daughter cells which enter into the taste bud and differentiate into the various mature cell types. These proliferative cells do not sit directly below the taste bud, where the Merkel-like basal cells reside, but rather around the basal circumference of the bud (Raderman-Little, 1979).

2.20.4.2 The Specialized Taste Organ of Frogs

The taste organs of frogs, called 'taste disks' are highly derived compared to other anamniote vertebrates (Osculati and Sbarbati, 1995) although many commonalities can be observed. In frogs, the apical opening is an expansive disk over 100 μm in diameter (see Figure 8). The taste disk is surrounded by specialized, ciliated cells. Inside this ring is a floor largely consisting of short, broad mucous cells each surrounded by the apical processes of 'wing' cells, thought to be supporting cells (Figure 9). The elongate taste (sensory) cells have their nucleus situated deeper in the taste disk than the wing and mucous cells, but extend a thin apical process to the surface of the taste organ. These cells are divided into two forms: type II cells and type III cells. Although type II cells have substantial contacts with basally situated axons, no obvious synaptic junctions occur. This situation appears similar to the type II taste cells of mammals (see below). The type III cells of the frog taste disk do exhibit clear synaptic contacts with nerve and are similar in that respect to type III cells of mammals. Glial-like sustentacular cells embrace and separate the different cells and nerve fibers in the lower half of the taste disk. This relationship is similar to the type I cells in mammalian taste buds. In addition, frogs have serotonergic Merkel-like basal cells characteristic of

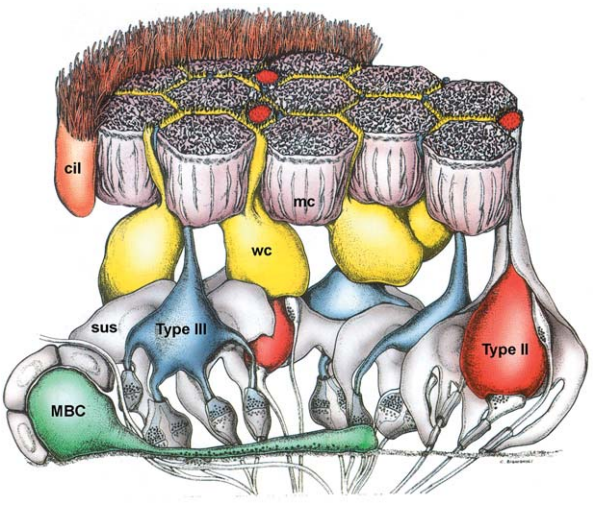

Figure 9 Drawing of the principal cell types of a frog taste disk. Ciliated cells (cil) surround the receptor surface of the taste organ. The superficial third of the organ is occupied by mucus (mc) and wing cells (wc), which are probably involved in maintenance of the mucus layer covering the taste disk. In the middle layer of the disk lie the cell bodies of the elongate receptor (type II and type III) cells which extend an apical process penetrating the surface layer. Both type II and type III cells contact afferent nerve fibers, although distinct synaptic complexes occur only between the type III cells and the nerve fibers. Sustentacular (sus = type Ic) cells wrap the other cell types and nerve fibers. Finally, MBCs lie in the deepest portion of the taste disk. Reproduced from Osculati, F. and Sbarbati, A. 1995. The frog taste disc: A prototype of the vertebrate gustatory organ. *Prog. Neurobiol.* (*Oxford*) 46(4), 351–399, with permission from Elsevier.

nonmammalian taste buds. The presence of both these Merkel-like basal cells and the type III sensory cells suggests that the transition from a nonmammalian type of taste bud to a mammalian type of taste bud is not simply the migration and transformation of the Merkel-like basal cells to an elongate morphology.

2.20.4.3 Mammalian Taste Buds

Taste buds in amniotes differ from anamniote taste buds in two respects. First, the taste pore is considerably narrower (~10 μm or less). Whether this is attributable to a drier, terrestrial lifestyle, or to phylogenetic factors is unclear. Second, mammalian taste buds lack the Merkel-like basal cell characteristic of nonmammalian taste buds. The taste buds of mammals do, however, possess a type of elongate cell which, like the Merkel-like basal cells, concentrates serotonin and forms distinctive synapses with the afferent nerve fibers. This has led many authors to speculate that the serotonin-containing elongate cells of amniote taste buds are homologous, if not functionally equivalent, to the Merkel-like basal cells (e.g., Ewald and Roper, 1994; see Evolution of Gustation).

2.20.4.3.1 Taste cells Taste buds in mammals comprise three distinct morphological types of elongate cells (type I, II, and III taste cells). These are defined according to ultrastructural criteria following the original descriptions of taste cells in rabbit foliate papillae by Murray (1986). Although the different types of taste cells are fairly distinct in rabbit foliate papillae, the morphological distinctions are less clear in other species. This has led to a great deal of confusion in the literature as to the equivalencies and distinctions between taste cell types in various mammals, especially rats and mice. In reviewing past literature on this subject, it is important to keep in mind that one author's 'type II' cell may not be the same as another author's cell of the same name. To further complicate matters, some authors have retained the older light microscopic terms: dark cell and light cell (originally based on staining properties of aniline dyes). The light–dark cell descriptors are only loosely equivalent to the morphological types as defined by electron microscopy. That is, type I cells nearly always have an electron dense cytoplasm and thus are called dark cells. Unfortunately, type III cells are more variable in staining characteristics and have been grouped by various authors into the category of 'light cell', 'dark cell', or 'intermediate cell', thereby seriously confusing the literature. With the advent of immunocytochemistry, it is possible to recognize the three distinct cytological and functional classes as originally defined by Murray.

2.20.4.3.1.(i) Type I taste cell The type I taste cells constitute over 50% of the total cells within a mature taste bud. As described by Murray and others, this cell often wraps around other taste cell types and nerve fibers. The cytoplasm is electron dense and stains heavily with acidophilic dyes, giving the cell a dark appearance in both light and electron microscopy. The nucleus is elongate with an irregular, indented nuclear membrane and substantial amounts of heterochromatin along the inner leaflet. These cells usually contain large apical granules ~100 nm in diameter and extend long, slender microvilli into the taste pore.

In many ways, the type I cells are similar to glia of the CNS. They express GLAST, a glial glutamate transporter (Lawton *et al.*, 2000) and NTPDase2, an astrocytic ecto-ATPase (Bartel *et al.*, 2006; Wink *et al.*, 2006). The processes of type I cells insinuate themselves between the other cell types and often cover a point of contact between other taste cells

and nerve fibers, just as astrocyte processes embrace synapses in the CNS. Since ATP is a crucial neurotransmitter between taste cells and the afferent nerve fibers (Finger *et al.*, 2005), these type I cell processes may serve to restrict cross-talk between cells within the taste bud by diffusion of ATP away from points of functional contact between taste cells and nerve fibers.

2.20.4.3.1.(ii) Type II taste cell Type II cells represent ~25–30% of the cells in each taste bud and are responsible for transduction of many tastants. These cells are elongate, spindle-shaped cells with short and thick apical microvilli. The cell is typified by a large, round, clear nucleus, and pale cytoplasm (Figure 10a). Type II cells express the bevy of receptor and second-messenger proteins implicated in transduction of bitter, sweet, or umami stimuli. These include the known T1R and T2R families of taste receptors, gustducin (G-protein), PLCβ2, and IP3R3 (Yang *et al.*, 2000b; Miyoshi *et al.*, 2001; Kusakabe *et al.*, 2002; Clapp *et al.*, 2004). Thus, type II cells mediate detection of these classes of tastants. Curiously, although type II cells closely contact afferent nerve fibers within taste buds (e.g., Kinnamon *et al.*, 1985; Yang *et al.*, 2000a), synapses between these elements are rare. Rather, subsurface cisternae appear at points of contact between afferent fibers and type II cells (Clapp *et al.*, 2004).

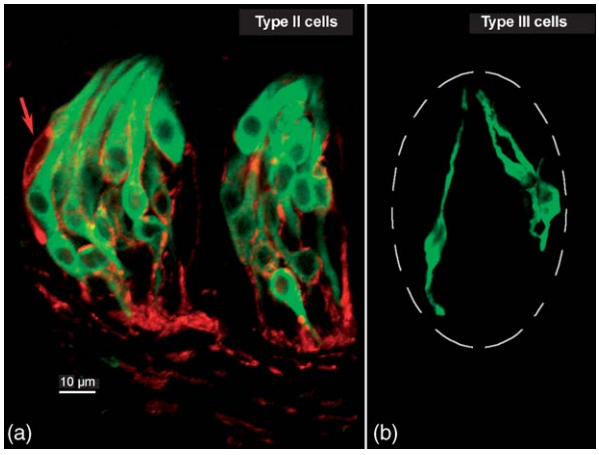

Figure 10 Fluorescence micrographs of immunocytochemically reacted taste buds of the circumvallate papilla from rodents. a, Reactivity for the inositol-trisphosphate receptor 3 (IP3R3) in a rat circumvallate papilla shows the morphology of typical type II taste receptor cells: broad, triangular cell body with a prominent, large, round nucleus. This section is also reacted for synaptobrevin revealing the numerous afferent nerve fibers and a rare taste cell (arrow), most likely a type III taste cell near the edge of the taste bud. b, A taste bud in the circumvallate papilla of a mouse, reacted for serotonin to reveal a population of type III taste cells. Note that they are more slender and less regular in shape than the type II cells illustrated in the left-hand panel. The approximate boundary of the taste bud is indicated by the dashed line. a, Photo courtesy of Drs. J. C. Kinnamon and R. Yang, Denver University, Denver, CO, USA.

Each type II taste cell is specified for detection of one class of taste substance and, therefore, expresses only one class of taste receptor, although multiple members of a class may be expressed in a single taste cell (Chandrashekar *et al.*, 2000; Zhang *et al.*, 2003). For example, a taste cell that expresses one member of the T2R family of receptors (for detecting bitter substances) will express several members of this same family (Adler *et al.*, 2000). Since each receptor molecule is responsive to only a small set of bitter substances, by expressing multiple members of the T2R family, a taste cell then exhibits broader responsiveness to many different bitter compounds. Whether type II cells also are the transduction elements for detection of sour and salty stimuli is unclear. At least some evidence implicates type III cells in these processes.

2.20.4.3.1.(iii) Type III taste cell The type III cell is relatively scarce in taste buds, comprising only 10–15% of the total population. Type III cells are sometimes called 'intermediate cells' since they share features with both type I and type II cells. What distinguishes type III cells from the others is the presence of well-formed synapses from the taste cells onto the afferent nerve fibers. Type III cells also exhibit some distinctive histochemical features which can be used to distinguish them from the other cell types. A subset of type III cells concentrates biogenic amines, including serotonin (Figure 10a). This property has led to their being likened to the Merkel-like basal cells of anamniote vertebrates (see the discussion above).

Type III taste cells are narrow, spindle-shaped cells that extend a single, thick apical process into the taste pore. Their nuclei are more elongate than those of type II cells and exhibit some degree of indentation. The cytoplasm is variable in staining density, ranging from light to dark. These features then are intermediate between the type I and II cells and have led some authors to consider type III cells to be a stage in the maturation of type II cells or to combine these cells into a single class (e.g., Delay *et al.*, 1986; Pumplin *et al.*, 1997). Recent studies suggest that type II and type III cells may arise from a common lineage (Finger, 2005; Kusakabe *et al.*, 2002; Miura *et al.*, 2005).

The function of type III cells is not established, but two possibilities are clear. First, the type III cells, being the only taste cells with prominent synapses, may serve as the only output cells of the taste bud,

receiving input from the transducing, type II taste cells and integrating this information before transmitting a signal to the afferent nerve fibers (Roper, 1992). The other possibility is that both type II and type III cells transmit information to nerve fibers, but that type II cells do so using a mechanism that does not require a conventional-looking synapse. Since type III cells exhibit voltage-gated ion channels (Medler *et al.*, 2003) and such cells respond to acidification (Richter *et al.*, 2003), then it is likely that type III cells are capable of directly transducing sour information and also passing this along the nerve fibers.

2.20.4.3.1.(iv) Other cells In addition to the three elongate types of taste cells described above, the taste buds of mammals, like those of nonmammalian vertebrates, are associated with proliferative basal cells and edge or marginal cells. Indirect evidence indicates that the taste bud progenitor cells of the basal epithelium are different than the basal cells of the general epithelium. When gustatory nerves are directed to grow into lingual epithelium that does not normally produce taste buds, the taste buds do not form despite the abundance of gustatory nerve fibers (Krimm *et al.*, 2001). The fact that taste nerves are unable to induce taste buds in anything but taste epithelium suggests that the epithelial cells in taste-bud bearing regions have a special capacity to generate these end organs. Conversely, when taste epithelia are innervated only by nongustatory nerves, then production of taste buds is limited at best (Farbman, 1971). Together, these studies indicate that basal cells of taste epithelia have a unique capacity to produce taste buds under the influence of gustatory innervation.

2.20.5 Detection and Representation of Different Tastes

The taste systems in vertebrates have the ability to respond to a variety of stimuli according to the habitat and nutritional needs of the organism. These taste stimuli are varied in chemical properties, including size, charge, hydrophobicity, and pH. Despite the diverse array of vertebrates and habitats, the taste system has a remarkable consistency in the types of compounds it can respond to. This may be due to the fact that many substances, for example, plant alkaloids, are toxic to most vertebrates and therefore all vertebrates require a food monitoring system capable of detecting potential toxins in the food supply. Conversely, different vertebrates have different nutritional needs and drives, so somewhat more divergence exists in terms of what substances can drive appetitive behaviors. Looking across all organisms, the taste system serves two primary functions: avoiding toxins and driving ingestion for nutritive substances. This means that the responses of the taste system should vary according to the diet of the particular organism. For example, the taste system of carnivores should not be driven by sugars, whereas the taste system of herbivores should be highly responsive to sugar. In contrast, most species should respond to amino acids.

Different cells in taste buds respond optimally to different taste qualities. It is interesting to note that this principle of cellular coding also occurs in taste organs of invertebrates. Since taste buds and 'taste' organs of invertebrates are not homologous, this property of encoding taste information should be viewed as convergent rather than evolutionarily conserved. Indeed, the chemosensory cells of the invertebrates seem more organized according to the behaviors they induce rather than the nature of the chemical stimulus detected. For example, a single chemosensory cell (ASE) in the amphid of *C. elegans* may respond to cAMP, biotin, and lysine despite their diverse chemical structures. But all of these substances are attractants. So, stimulation of the ASE cell will produce attraction. The taste systems in more complex organisms, for example, flies, fish, or mammals, have more complexity. Several substances may drive ingestion, but may be detected by different receptor cells. For example, both alanine and arginine drive appetitive behavior in catfish, but these amino acids appear to be detected by different receptors expressed in different taste cells (Finger *et al.*, 1996). Similarly, different taste cells in mice express receptors for glutamate and sweeteners although both drive food intake (Zhang *et al.*, 2003).

2.20.6 Evolution of Taste Preference and Taste Receptors

In order for a species to adapt to a new habitat or feeding strategy, the spectrum of substances to which its taste organs respond must change. For example, for terrestrial animals, especially those with a purely vegetarian diet, sodium is a crucial nutrient. Salt-deprived amniotes have a drive to seek out and ingest salt (Schulkin, 1991). Their taste system carries unique information about the sodium content of potential foodstuffs and detection of the sodium is regulated in part by

circulating hormones that alter the sensitivity of the sodium-detecting channels of the taste buds (Herness, 1992; Lin *et al.*, 1999). Yet, sodium is not a crucial nutrient for aquatic anamniotes, so their taste systems are not particularly responsive to sodium content of food (Caprio *et al.*, 1993). Thus, the responsiveness of taste buds had to change when vertebrates made the transition from water to land. In frogs, the entire epithelium is sensitive to sodium levels, perhaps via the same ion channel (AsNaC) used in sodium detection in many mammals (Nagai *et al.*, 1999). Thus transduction of sodium by taste may have evolved from a general epithelial property of regulated sodium transport.

The receptors for tastes can be ion channels themselves (as in the case of sodium (salty) or protons (sour), may be ligand-gated ion channels (e.g., for arginine-detection in catfish; Brand *et al.*, 1991), or can be G-protein-coupled receptors (Brand *et al.*, 1991; Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Zhang *et al.*, 2003; Ishimaru *et al.*, 2005). The G-protein-coupled receptors, T1R and T2R families, are phylogenetically old since family members have been identified in fish as well as in mammals. Yet in mammals, some of these receptors respond to sweeteners whereas in fish, sweeteners are not effective taste stimuli. Accordingly, evolutionary change of the receptor molecules is likely to correspond to evolutionary changes in the spectrum of substances to which the taste system can respond. This can be seen in the evolution of felines which are insensitive to sugars and other sweeteners unlike other carnivores. The taste system of basal carnivores most likely responds well to sweet substances since many contemporary carnivores, for example, dogs and bears, are strongly attracted to sweets, whereas cats are not (Li *et al.*, 2005). One of the genes encoding the sweet taste receptor is nonfunctional in cats, thereby rendering them insensitive to sugar and other sweeteners. Thus, a simple mutation in a single taste receptor gene is capable of altering the diet of a species.

Acknowledgments

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2.21 Shared Features of the Auditory System of Birds and Mammals

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Glossary

interaural time difference — Birds and mammals use the interaural time difference (ITD) for localization in the horizontal plane. When sound comes from one side of the body, it reaches one ear before the other, which creates an ITD. These ITDs depend upon head size, and in some cases on an interaural canal. In general, animals with large heads have larger time differences available to them.

phase-locking — The auditory system uses phase-locked spikes to encode the timing or phase of the auditory signal. Phase-locked neurons fire spikes at or near particular phase angles of sinusoidal waveform. Physiological experiments measure this spike phase with respect to the stimulus period. Spike phase is plotted in a period histogram, and is used to calculate the statistic vector strength (r). Each spike defines a vector of unit length with a measured phase angle. The vectors characterizing the spikes are plotted on a unit circle and the mean vector calculated. The length of the mean vector provides a measure of the degree of synchronization.

2.21.1 Introduction

Grothe *et al.* (2005) have pointed out that we cannot construct a comprehensive description of the evolution of the vertebrate central auditory system because we do not know what the common ancestors of terrestrial vertebrates could hear or how their ears worked. It seems likely, however, that tympanic ears have evolved independently in synapsids, lepidosauromorph diapsids, archosaurs, turtles, and amphibians (Clack, 1997). These new ears could convey responses to both vibration and airborne sound to brainstem auditory neurons, leading to the parallel evolution of the central targets of the auditory nerve. Further developments in ancestral mammals, such as moveable ears and multiple ossicles, may have had additional reorganizing effects (see Epigenetic Responses to a Changing Periphery – Wagging the Dog). In this review we will argue that the elaborated central auditory systems in the different clades of recent land vertebrates have evolved in parallel.

With the transition to airborne sound, selection might be expected to improve the auditory system's ability to encode the more rapid changes associated with higher frequency sounds. For example, precise encoding of temporal information at best frequencies above 1–2 kHz is biophysically demanding (see Koppl, 1997), but also has direct behavioral

relevance for sound localization and communication (Hafter and Trahiotis, 1997; Heffner and Heffner, 1992) and should be selected for.

We will discuss the appearance of similar physiological and morphological adaptations for encoding both time and level information in the auditory brainstem of birds and mammals, and draw two major conclusions. First, the similarities among the brainstem circuits that encode sound in birds and mammals appear to be the result of parallel evolution. Second, the existence of these similar circuits allows us to identify algorithms shared by the auditory system of birds and mammals, and to argue that these are suited to extracting the stimulus variables relevant for auditory coding; thus, studies of evolution can inform computational neurobiology. For further discussion of this topic, the reader is directed to recent reviews including Grothe *et al*. (2005) and Carr and Soares (2002).

Animals with tympanic ears should experience similar constraints in detecting sounds. The essential features of auditory coding are very similar in birds and mammals. Understanding the evolutionary and developmental events behind the similar form and function of temporal coding cells in birds and mammals will require a detailed knowledge of multiple species under study, and of their phylogenetic relationships. This requires a deliberate concentration upon comparative neurobiology, and the differences among animals (see Relevance of Understanding Brain Evolution). In this review we compare similar coding strategies in the auditory systems of a few species of birds and mammals in order to identify shared features (Section 2.21.2). We then examine the special case of temporal coding in birds and mammals, and use these comparative studies to argue that natural selection has produced suitable solutions to the problems of temporal coding (Sections 2.21.3 and 2.21.4).

2.21.2 Encoding Sound: Similar Strategies in Birds and Mammals

Both birds and mammals use similar strategies to encode various aspects of the auditory scene. In fact, all animals may use similar auditory codes (Fay, 1988; Manley, 2005). Vertebrate auditory systems exhibit a similar basic Bauplan, in which the auditory nerve enters the hindbrain and bifurcates to contact different subdivisions of the cochlear nucleus (Ryugo and Parks, 2003). The cochlear nuclei give rise to different connections within the lower auditory system, including projections to groups of neurons in the superior olive and the lateral lemniscus before these pathways reunite at the level of the auditory midbrain. Despite these large-scale similarities, the details of the connections and cell types show substantial diversity between the major vertebrate clades (reviews in Cant, 1992; Carr and Code, 2000; McCormick, 1999).

In both birds and mammals, the cochlear nuclei encode parallel ascending streams of auditory information. In birds, the auditory nerve projects to nucleus magnocellularis and nucleus angularis in the pattern described for the bird and reptile morphotype. The nucleus magnocellularis is the origin of a neural pathway that encodes timing information, while a parallel pathway for encoding sound level originates with nucleus angularis (Takahashi and Konishi, 1988a). In mammals, auditory nerve afferents send an ascending branch to the anterior ventral cochlear nucleus and a descending branch to both the posterior ventral cochlear nucleus and the new dorsal cochlear nucleus. This division may also support parallel encoding of sound level and time, although evidence for this simple division is not strong. Instead, it appears that the cells in the mammalian cochlear nucleus form many parallel ascending streams (Cant and Benson, 2003; see the discussion below). In both birds and mammals, the auditory nerve forms different types of terminals onto different cell types in the cochlear nucleus (Ryugo and Parks, 2003). Endbulbs of Held terminals are formed on bushy cells (see Section 2.21.3), and bouton-like terminals on the other cell types in the cochlear nuclei. The auditory nerve appears to use glutamate as a transmitter, often with the postsynaptic cell expressing 'fast' AMPA type glutamate receptors that can mediate precise temporal coding (Parks, 2000).

The avian and mammalian cochlear nuclei both contain heterogeneous cell populations, and exhibit similar responses to sound (Koppl and Carr, 2003; Soares *et al*., 2002; for review of the mammalian cochlear nuclei, see Romand and Avan, 1997; Cant and Benson, 2003). We do not know if these similar features evolved as a response to selective pressures to encode airborne sound. There are two reasons why similarities might be due to parallel evolution. First, true tympanic ears arose independently in birds and mammals (Clack, 1997). These peripheral changes would have had different reorganizing effects upon the ancestral population of brainstem auditory neurons. Second, the cell types of the avian and mammalian cochlear nuclei are similar but not identical. We describe the similarities and differences between birds and mammals in this section. A satisfactory study will, however, require detailed analyses of the development, morphology and

physiology of cell types in the cochlear nucleus in all amniote groups, including turtles, basal lizards, and crocodilians.

2.21.2.1 Organization of the Cochlear Nuclei in Mammals and Birds

It has been historically difficult to compare the mammalian and avian cochlear nuclei. To do so in this article, we will first describe the cochlear nuclei in mammals and birds, then discuss their similarities and differences. The mammalian cochlear nuclear complex is divided into dorsal and ventral nuclei that contain separate, well-defined populations of cells. The large projection cells are distinguished on the basis of morphology, projections, and physiological responses to sound (Cant and Benson, 2003; Rhode and Greenberg, 1992; Rouiller, 1997; Young, 1998). The anterior part of the ventral cochlear nucleus contains bushy cells that respond in a primary or auditory nerve-like fashion to the auditory stimulus. The posterior part of the ventral cochlear nucleus contains octopus cells that respond to onsets or stimulus transients and two classes of multipolar neurons that respond principally with 'chopper' firing patterns.

Bushy cells receive endbulb inputs from the auditory nerve and exhibit accurate temporal coding. There are two forms of bushy cells, spherical and globular. Spherical cells dominate the anterior ventral cochlear nucleus, respond to lower best frequencies and project to the medial superior olive, which is sensitive to interaural time differences (ITDs). Globular bushy cells by comparison sometimes chop or exhibit onset responses to the stimulus, respond to higher frequencies, and project to the lateral superior olive and the medial nucleus of the trapezoid body. These projections may mediate detection of interaural level differences. Octopus cells in the posterior ventral cochlear nucleus are multipolar, with thick dendrites that extend across the nerve root (Oertel *et al*., 2000). This morphology enables them to integrate auditory nerve inputs across a range of frequencies. Octopus cells encode the time structure of stimuli with great precision and exhibit onset responses to tonal stimuli. Onsets play an important role in theories of speech perception, and segregation and grouping of sound sources (Bregman, 1990). Cochlear root neurons send widespread projections to areas of the reticular formation involved in startle reflexes and autonomic functions. Type I multipolar cells may encode complex features of natural stimuli and send excitatory projections directly to the inferior colliculus. Type II multipolar cells send inhibitory projections to the contralateral cochlear nuclei (Cant and Benson, 2003).

The dorsal cochlear nucleus (DCN) appears for the first time in mammals, perhaps associated with the development of high-frequency hearing and motile external ears. DCN cells exhibit wide variety of response types, with one theory of function relating to echo suppression. The DCN is composed of a cerebellar-like circuit in the superficial layers, with projection cells in the deep layers that receive auditory nerve inputs (Young, 1998). The granule cells in the superficial layers receive sensory input that may convey information about head and ear position. The deep portion of the DCN contains fusiform and giant cells. Fusiform cells exhibit complex (Type IV) frequency-tuning curves, with small areas of excitation at best frequency and at the edges of the response curves. Type IV responses appear well suited to detecting the notches in sound level created by the pinna that provide cues for locating sound in elevation (May, 2000). Fusiform cell responses may mediate localization of sounds based on spectral cues and send direct excitatory projections to the inferior colliculus. Giant cells in the DCN also project directly to the inferior colliculus; some of them may convey inhibitory inputs to the contralateral cochlear nucleus as well (Cant and Benson, 2003).

In birds the auditory nerve projects to two cochlear nuclei, the nucleus magnocellularis and the nucleus angularis (Carr and Code, 2000). The nucleus magnocellularis principal cells dominate all but the low best-frequency region of the nucleus, and project to the nucleus laminaris, which is sensitive to ITDs. The nucleus angularis contains 4–5 cell types that project to the superior olive, to the lemniscal nuclei, and to the central nucleus of the auditory midbrain. The parallel ascending projections of angularis and laminaris may or may not overlap with one another, and probably do overlap in the primitive condition.

The mammalian bushy cells are very similar to the avian magnocellular neurons (see Sections 2.21.3 and 2.21.4) and originally nucleus angularis was thought to be similar to the mammalian DCN (Boord, 1969; Sachs and Sinnott, 1978; Sachs and Young, 1980). Closer examination has, however, shown that there are no deep morphological correspondences between nucleus angularis (NA) and the DCN (Soares and Carr, 2001) although there are physiological similarities. Both nuclei contain a cell type that exhibits type IV (complex nonmonotonic) physiological responses (Koppl and Carr, 2003; Sachs and Sinnott, 1978; for review of DCN see Young *et al*., 1988). Parsimony would suggest that

the type IV responses observed in the redwing blackbird by Sachs and Sinnot and in the barn owl by Koppl and Carr may have emerged in parallel with similar responses in mammalian DCN. The DCN appears to be a unique feature of the mammalian auditory system. Furthermore, unlike the case with the NA, the DCN shares many common features with the cerebellum, including unique cell types and cortical circuitry (Berrebi *et al*., 1990; Oertel and Young, 2004; Wright and Ryugo, 1996).

2.21.2.2 Morphology of Cell Types in Birds and Mammals

The morphological characteristics of neurons contribute to the input–output functions of neural circuits. We suggest that similar rules of dendritic organization apply to the cochlear nuclei of both mammals and birds. Furthermore, examination of similar cell types in birds and mammals may reveal shared computational strategies.

Neurons of the rat ventral cochlear nucleus that project to the DCN have been divided into two main groups: radiate and planar (Doucet and Ryugo, 1997). Radiate neurons have long dendrites perpendicular to isofrequency contours and are sensitive to a broad range of frequencies. Planar neurons, on the other hand, have dendrites that are confined to an isofrequency plane, therefore more sensitive to a narrow range of frequencies.

At a first approximation, the avian NA has an organization similar to that seen in the rat ventral cochlear nucleus. In both the barn owl (Soares and Carr, 2001) and the chicken (Fukui and Ohmori, 2003; Soares *et al*., 2002), NA contains several major morphological classes of neurons. In the barn owl, these are classified as planar, radiate, vertical, and stubby. Planar neurons are confined to an isofrequency band, whereas radiate neurons have dendrites that could extend across an isofrequency band. Vertical cells have long dendrites oriented perpendicularly to isofrequency bands. Stubby cells are confined to an isofrequency band because of their short dendrites. Representatives of all cell classes can be found throughout NA of the chicken (Fukui and Ohmori, 2003; Soares *et al*., 2002). Thus, a similar pattern of organization appears to have evolved in parallel in the cochlear nuclei of both birds and mammals, in which one population (planar, stubby, and bushy) remains within an isofrequency band, another (radiate) extends across the isofrequency axis, and a third (vertical, marginal, and octopus) has a dendritic orientation orthogonal to the isofrequency axis.

Although there are shared morphological characteristics among individual neurons of both clades, other organizational rules differ. First, there are many cell types within the mammalian ventral cochlear nucleus that are not included in Doucet and Ryugo's classification scheme, principally bushy cells, octopus cells, and small cell types (for reviews, see Cant and Benson, 2003). Bushy cells appear to be similar to nucleus magnocellularis (NM) neurons, but there are no obvious morphological counterparts to octopus cells in the avian cochlear nuclei. This is significant because both octopus cells and cells in NA respond to sound with onset responses (Sullivan, 1985; Warchol and Dallos, 1990). Nevertheless, Golgi analyses of barn owl NA neurons and intracellular labeling of cells in chicken NA have not revealed cells with the characteristic octopus cell morphology – thick dendrites that extend across the incoming auditory nerve inputs (Soares and Carr, 2001; Soares *et al*., 2002). Thus, it appears that the evolution may not necessarily have produced identical solutions for encoding onset of sounds. Second, the majority of NA cells are stubby neurons that have no obvious counterpart within the multipolar cell types of the mammalian ventral cochlear nucleus. Instead, they most closely resemble NM neurons and bushy cells. Third, small cells rarely appear in NA (Soares and Carr, 2001), and it seems that they are not as various or numerous in NA as in the mammalian cochlear nucleus. Finally, the avian cochlear nuclei have neither the granule cell layer that characterizes mammalian cochlear nucleus, nor a DCN.

2.21.2.3 Intracellular Physiological Responses of Cochlear Nucleus Neurons in Birds and Mammals

Descriptions of neural circuitry are based on both dendritic morphology and physiological characteristics. Descriptions of both *in vivo* and *in vitro* responses complement the morphological studies, and responses of cochlear nucleus neurons in brain slices from both birds (chicken) and mammals (rat, mouse) can be compared.

There appear to be no direct one-to-one physiological correspondences between neurons in birds and mammals, with the exception of neurons that are specialized for temporal coding, such as mammalian bushy cells and avian magnocellular neurons. Even these very similar responses may have evolved in parallel: the suite of features that distinguish temporal coding neurons in auditory nuclei, including the nuclei of the lateral lemniscus (Wu, 1999a), is also found in temporal coding

neurons in electric fish (Carr, 1986; Rashid *et al.*, 2001). One cannot therefore use shared features to argue for homology among cochlear nucleus neurons in birds and mammals. Instead, shared features may be used to identify common computational strategies.

Avian NM neurons and NA stubby neurons respond with only one spike when depolarized. The responses of NA stubby neurons are similar to those of both bushy and octopus cells in the mammalian VCN (Golding *et al.*, 1999; Manis and Marx, 1991; Wu and Oertel, 1984). These mammalian cell types exhibit the depolarization-activated, dendrotoxin-sensitive, low-threshold K^+ conductance that is activated at rest (see Section 2.21.2; Manis and Marx, 1991; Bal and Oertel, 2000; Brew and Forsythe, 1995). A similar dendrotoxin-sensitive conductance underlies the responses of NA one spike neurons (Fukui and Ohmori, 2003), NM and nucleus laminaris (NL) neurons (Rathouz and Trussell, 1998; Reyes *et al.*, 1994, 1996) and the irregularly firing principal cells of the tangential nucleus (Gamkrelidze *et al.*, 1998, 2000). These biophysical similarities suggest that stubby neurons, like bushy, octopus, NM and NL neurons, may mediate accurate transmission of temporal information.

There are additional shared physiological characteristics between the cochlear nuclei of both groups. Despite the multiplicity of cochlear nucleus cell types, auditory nerve synapse kinetics are similar in all. In avian NA, spontaneous excitatory post synaptic current (EPSC) receptor kinetics are the same for all cell types (MacLeod and Carr, 2005). This is also the case for bushy, T-stellate, tuberculoventral and octopus cells in the mammalian VCN (Gardner *et al.*, 1999). Other NA cell types share biophysical features with mammalian VCN neurons (Soares and Carr, 2001).

2.21.2.4 Ascending Lemniscal Projections

Avian and mammalian cochlear nuclei both share ascending lemniscal projections, but these differ in many respects (Figure 1). The greatest difference may be the comparative lack of descending projections in birds when compared with mammals (Carr and Code, 2000).

In birds and crocodilians, the NA projects to the superior olive, the contralateral posterior portion of the dorsal nucleus of the lateral lemniscus (Figure 1a; Takahashi and Konishi, 1988a; Wild *et al.*, 2001) and the inferior colliculus (Conlee and Parks, 1986; Yang *et al.*, 1999). The posterior division of the dorsal nucleus of the lateral lemniscus is the first site of binaural interactions in the intensity pathway of the barn owl and is where sensitivity to interaural level differences first appears (Manley *et al.*, 1988; Moiseff and Konishi, 1983). The pathways encoding ITDs and interaural level differences ultimately converge in the external nucleus of the inferior colliculus, where neurons are selective for combination of interaural time and level differences (Figure 1a, for review, see Konishi, 2000). The projections of the mammalian cochlear nucleus are more elaborate than those in birds (Figure 1b; for reviews see Cant and Benson, 2003; Cant and Hyson, 1992; Romand and Avan, 1997).

2.21.3 Encoding Temporal Information

Auditory nerve fibers encode temporal information by phase-locking to the waveform of the acoustic stimulus, and preserve this temporal information in projections to NM and NA. Three lines of evidence show that accurate temporal coding is important for sound localization. First, measurements of the vector strength of the auditory nerve signal, calculated from the variability in the timing of spikes with respect to the phase of the acoustic stimulus, show an improvement in high frequency phase-locking in the owl as compared to other animals by an octave or more (Koppl, 1997). Second, models of coincidence detection perform better when the vector strength of the inputs improves (Colburn *et al.*, 1990; Simon *et al.*, 1999). Third, inactivation of NM neurons with lidocaine removes sensitivity to ITDs from the responses of midbrain space-mapped neurons (Takahashi *et al.*, 1984).

We will review the features associated with preserving temporal cues up to the point where ITDs are detected. There are several shared features of temporal coding circuits in the auditory systems of birds and mammals. These include high-quality inputs, presynaptic specializations to make neurotransmitter release precise, and postsynaptic specializations, including specific glutamate receptors, potassium conductances, and characteristic neuronal morphology. These features have been reviewed in Oertel (1999), Trussell (1999), and Carr and Friedman (1999).

2.21.3.1 Precise Synaptic Transmission

The task of accurately representing the stimulus phase becomes more difficult with increasing stimulus frequency (Hill *et al.*, 1989). This is because the absolute temporal precision required for phase-locking to high frequencies is greater than that needed for low frequencies, that is, the same

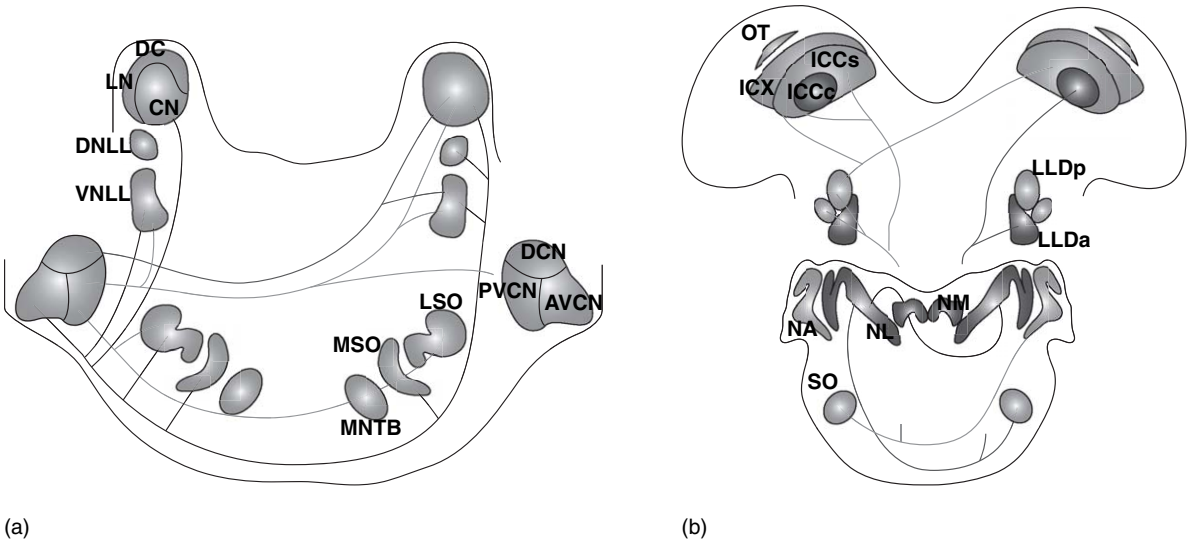

Figure 1 Schematic showing the connections in the mammalian and avian auditory brainstem. a, Mammals: the auditory nerve bifurcates to give rise to an ascending and a descending branch. The ascending branch innervates the AVCN, and the descending branch innervates first the PVCN and then the DCN. The projections of the cochlear nuclei are denoted as different lines (AVCN – dark lines, PVCN – light lines, and DCN – dotted lines). The cochlear nuclei send ascending projections to the olivary and periolivary nuclei, which include the MNTB, MSO, and LSO. The IC in mammals is subdivided into a central nucleus, an external cortex, and a dorsal cortex. Stellate cells from VCN and fusiform and giant cells from DCN project to the contralateral central nucleus of the IC giving rise to banded inputs. The central nucleus receives bilateral input from LSO and a mostly ispilateral input from MSO, also forming banded, tonotopically organized projections. It also receives projections from the nuclei of the lateral lemniscus (DNLL and VNLL). b, Birds: in barn owls, separation into time and sound level pathways (dark lines and light lines, respectively) begins with the cochlear nuclei. Eighth nerve afferents divide to innervate both the level-coding NA and the time-coding NM. NM projects bilaterally to NL, which in turn projects to superior olive, nucleus of the lateral lemniscus, and to the central nucleus of the inferior colliculus. The superior olive projects back to NA, NM, and NL (projections are not drawn). In birds, the IC is subdivided into two principal subnuclei, the external nucleus (ICX) and the more medially located central nucleus (ICC). a, Reproduced from Sound Source Localization, 2005. Development of sound localization, Kubke, M. F. and Carr, C. E. (*eds.* A. N. Popper and R. R. Fay), Springer. With kind permission of Springer Science and Business Media. b, Reproduced from Kubke, M. F., Gauger, B., Basu, L., Wagner, H., and Carr, C. E. 1999. Development of calretinin immunoreactivity in the brainstem auditory nuclei of the barn owl (*Tyto alba*). *J. Comp. Neurol.* 415, 189–203.

variation in temporal jitter of spikes translates to greater variation in phase for high frequencies. Hill *et al*. (1989) estimated phase-locking in the auditory fibers of the pigeon in terms of the commonly used synchronicity index (vector strength) as well as by measuring temporal dispersion. Vector strength of phase-locking decreases for frequencies above 1 kHz. Temporal dispersion, however, also decreases with frequency, indicating enhanced temporal synchrony as frequency increased (Koppl, 1997). The upper frequency limit of phase-locking therefore appears to depend upon the ability of hair cells to phase-lock and upon an irreducible jitter in the timing of spikes. It is about 8–9 kHz for barn owls, and between 4 and 6 kHz for most other birds and mammals studied (Koppl, 1997).

Endbulb or caliciform synapses mediate the transmission of phase-locked spikes. These synapses form a fenestrated cup that envelopes most or part of the cell body, and contains large numbers of active zones (Nicol and Walmsley, 2002). The invasion of the presynaptic spike into the calyx leads to the synchronous release of quanta at these sites, endowing the synapse with a high safety factor (Sabatini and Regehr, 1999). Studies of endbulbs in both the avian NM and the medial nucleus of the trapezoid body (MNTB) have shown that the invading presynaptic spike is extremely narrow (Turecek and Trussell, 2001), probably due to rapid repolarization mediated by specific potassium conductances. Calcium influx into the presynaptic terminal is also brief and occurs only during the falling phase of the presynaptic spike (Turecek and Trussell, 2001). Because the spike is narrow, its downstroke occurs quickly, as does calcium influx, reducing the synaptic delay. The brief period of calcium influx produces a confined and phasic period of neurotransmitter release, increasing the temporal precision of transmission across the synapse (Brenowitz and Trussell, 2001b).

Endbulb terminals may have emerged as an adaptation for accurate transmission of phase

information for frequencies above ~500 Hz, perhaps also associated with the development of hearing in land vertebrates. Large endbulb-like terminals have been found in all amniote groups examined. There are no data on turtles, but in the alligator lizard the auditory nerve forms both large somatic terminals and smaller boutons in NM (Szpir *et al*., 1990). In crocodilian NM, the rostral high best frequency NM neurons receive endbulb-like projections, while lower best frequency NM neurons receive bouton terminals (Soares, unpublished). Large endbulb terminals are found in both birds and mammals (Ryugo and Sento, 1991), and may be developed in parallel in archosaurs and mammals to mediate accurate transmission of temporal information at higher sound frequencies (Carr and Soares, 2002). Evidence for the parallel evolution of endbulbs in birds and mammals comes from studies of their development, which follows different trajectories (Rubel and Fritzsch, 2002), and from studies of transmitter release modulation (Trussell, 2002).

Support for the hypothesis that endbulbs evolved to facilitate transmission of high best frequency phase-locking comes from comparisons of low and high best frequency regions of NM. Endbulb terminals do not appear to be essential for transmission of phase-locked spikes at low frequencies, because they are not found in low best frequency regions. The very low best frequency cells of the NM receive large bouton terminals from the auditory nerve and phase-lock to frequencies below ~1 kHz (Koppl, 1997), while in crocodilian NM, only the rostral high best frequency NM neurons receive endbulb-like projections (Soares, unpublished).

2.21.3.2 Glutamate Receptors

Activation of AMPA type glutamate receptors at endbulb synapses generates brief, large synaptic currents that are suited to the transfer of temporally precise information from pre- to postsynaptic cell (Raman and Trussell, 1995; Zhang and Trussell, 1994). The brevity of EPSCs in these neurons depends not only on the time course of release but also on the specific properties of the postsynaptic receptors. AMPA receptors are made up of glutamate receptor subunit (GluR) splice variants, and the GluR3 and 4_{flop} isoforms found in auditory neurons have fast kinetics and rapid desensitization rates, such that the duration of miniature EPSCs in auditory neurons are among the shortest recorded for any neuron (Gardner *et al*., 1999; Trussell, 1999). These rapid kinetics are due to a characteristic 'auditory' pattern of expression (Parks, 2000). In the chicken NM, where a homogeneous population of neurons makes mRNA analysis possible, the relative abundance of the four AMPA receptor subunits reveal very low levels of GluR2, and higher levels of the 'fast' flop splice variants of GluR3 and 4 (Parks, 2000; Ravindranathan *et al*., 1996a). Similar splice variants characterize mammalian bushy cells (Gardner *et al*., 2001). Further support for the role of GluR4 has emerged from developmental studies. AMPA receptor-mediated EPSCs in NM and MNTB become faster in decay time as animals mature (Brenowitz and Trussell, 2001a; Joshi *et al*., 2004; Koike-Tani *et al*., 2005). In parallel with the increase in kinetics, GluR4 flop increases from P7 to P14 and changes little thereafter (Koike-Tani *et al*., 2005).

2.21.3.3 Potassium Conductances

Although brief EPSCs underlie the precisely timed responses of neurons that phase-lock to the auditory stimulus, the intrinsic electrical properties of these neurons also shape the synaptic response as well as the temporal firing pattern. Two K^+ conductances are important for phase-locked responses in auditory neurons: a low-threshold conductance and a high-threshold conductance (Brew and Forsythe, 1995; Manis and Marx, 1991; Rathouz and Trussell, 1998; Reyes *et al*., 1994; Wang and Kaczmarek, 1998).

The low-threshold conductance activates at potentials near rest and is largely responsible for the outward rectification and nonlinear current–voltage relationship around the resting potential seen in a number of auditory neurons (for review, see Oertel, 1999). Activation of the low-threshold conductance leads to a short active time constant so that the effects of excitation are brief and do not summate in time (Wu and Oertel, 1984). Only large excitatory post synaptic potentials (EPSPs) reaching threshold before significant activation of the low-threshold conductance would produce spikes with short latencies, whereas small EPSPs that depolarize the membrane more slowly would allow time for low threshold conductance activation to shunt the synaptic current and prevent spike generation and thus long latency spikes. Blocking the low-threshold conductance elicits multiple spiking in response to depolarizing current injection (Manis and Marx, 1991; Rathouz and Trussell, 1998) or synaptic activation (Brew and Forsythe, 1995). The K^+ channels underlying this conductance appear to be composed of Kv1.1 and Kv1.2 subunits. Both subunits are

expressed in auditory neurons, although the subcellular distribution is unknown (Grigg *et al*., 2000). In NM, neurons express Kv1.1 potassium channel mRNA and protein, in a gradient that is highest in the high-BF region of NM (Fukui and Ohmori, 2004; Lu *et al*., 2004).

The high-threshold conductance is characterized by an activation threshold around –20 mV and by fast kinetics (Brew and Forsythe, 1995; Rathouz and Trussell, 1998; Wang and Kaczmarek, 1998). These features of the high-threshold conductance result in fast spike repolarization and a large but brief afterhyperpolarization without influencing input resistance, threshold, or spike rise time. Thus, the high-threshold conductance can keep spikes brief without affecting spike generation. In addition, the high-threshold conductance minimizes Na^+ channel inactivation, allowing cells to reach firing threshold sooner and thereby facilitating high-frequency firing. In the MNTB, blockade of this conductance diminishes the ability to follow high-frequency stimuli in the range of 300–400 Hz, but has little effect on responses to low-frequency (< 200 Hz) stimulation (Wang and Kaczmarek, 1998). Also in MNTB, elimination of the Kv3.1 gene in mice results in the loss of a high-threshold component of potassium current and failure of the neurons to follow high-frequency stimulation (Macica *et al*., 2003).

Similar potassium conductances characterize other time-coding cells. There are numerous examples, many discussed in Oertel's (1999) review. In addition to the NM and mammalian MNTB neurons discussed above, the coincidence detectors in the avian NL and mammalian medial superior olive also express similar conductances and respond with temporal precision to the auditory stimulus (see the discussion below). The reasons for temporal precision are clear for the circuit that detects ITDs. There are also other aspects of the auditory stimulus that require temporal precision. In particular, the mammalian cochlear nucleus octopus cells form the origin of a circuit that encode timing of events, especially broadband transients. Octopus cells produce the briefest, most sharply timed synaptic responses in mouse cochlear nucleus (Golding *et al*., 1995). Octopus cells are characterized by both a large low-threshold conductance and a high-threshold conductance (Bal and Oertel, 2000). Type II cells in the ventral nucleus of the lateral lemniscus produce sharply timed responses and receive endbulb input from octopus cells (Wu, 1999b). Thus, selection for temporal accuracy may in each case drive expression of conductances that improve neuronal performance and behavioral accuracy.

2.21.3.4 Large Neurons

Selective pressure for temporal accuracy at the synapse, particularly for high-frequency inputs, may have driven the evolution of larger cell size. Larger somata and axons are less vulnerable to noise caused by stray currents, since their low input resistance keeps the influence of voltage fluctuations to a minimum. Many of the known time-coding pathways include large cells (Carr and Amagai, 1996). Enlarged size must be accompanied by an increase in synaptic current. Fast, large synaptic currents minimize the influence of ambient voltage fluctuations on the timing of spikes. Reducing the electrotonic distance between the synapse and the site of integration can enhance these effects. This occurs in electric fish neurons that encode the phase of the electric organ signal and in the cells of the nucleus magnocellularis and the nucleus laminaris in birds (Jhaveri and Morest, 1982; Smith and Rubel, 1979; Carr and Boudreau, 1993) and in electric fish (Kawasaki and Guo, 1996). In the mammalian auditory system, both bushy and MNTB neurons are large, and characterized by calciform synapses and large brief synaptic currents (see Cant and Benson, 2003).

2.21.4 Coincidence Detection and Coding of ITDs

Behavioral experiments have shown that most animals use ITDs to localize sound (Fay, 1988). These time differences depend upon head size, and in some cases also upon an interaural canal. In general, animals with large heads have larger time differences available to them. Thus, animals with smaller heads have to achieve much greater resolution of binaural time differences than a large animal in order to obtain the same degree of accuracy. For example, barn owls and humans have very similar abilities to localize sound, since psychophysical studies have shown that human subjects are able to localize a frontal tone with an accuracy of about 2°, while owls have a best accuracy of 3° (Bala and Takahashi, 2000; Middlebrooks and Green, 1991). The human discrimination task is easier than the barn owl's because the human head is larger, but both humans and barn owls are extremely accurate. Heffner and Heffner (1992) have suggested that a major selective pressure on localization comes when animals with narrow fields of best visual acuity such as a fovea use accurate sound localization to direct their gaze. Animals with smaller heads or animals that do not look directly at a sound source tend to have poor localization ability.

How good is an ability to discriminate targets 3° apart? Given the barn owl's head size, 1.5° separation is equivalent to an ITD of about 3 μs (Moiseff and Konishi, 1981). Discrimination of these microsecond ITDs requires accurate transduction and processing of the original stimulus, followed by detection of ITDs. The preferred model for detecting temporal disparities was first proposed by Jeffress with his place theory (Figure 2; Jeffress, 1948). The model circuit is composed of two elements, delay lines, and coincidence detectors. The delay lines are created by varying axonal path lengths, and the coincidence detectors are neurons that respond maximally when they receive simultaneous inputs, that is, when the time difference is exactly compensated for by the delay introduced by the inputs. The Jeffress model explains not only how ITDs are measured but also how they are encoded. The circuit contains an array of coincidence detectors receiving input from afferent axons serving as delay lines. Because of its position in the array, each neuron responds only to sound coming from a particular direction, and thus the anatomical place of the neuron encodes the location of the sound. These neurons compute a new variable, time difference, and in the process, transform the time code into a place code. The selectivity of all higher-order auditory neurons to time difference derives from the 'labeled-line' output of the place map (Carr, 1993; Joris *et al*., 1998; Konishi, 2003).

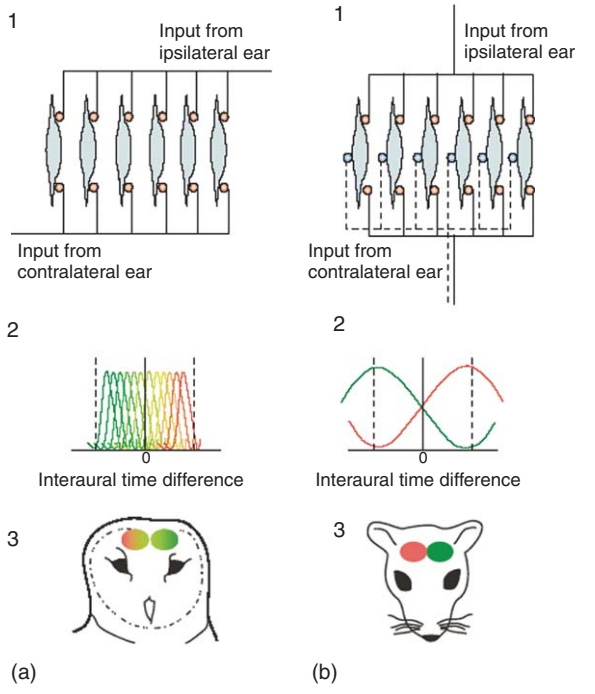

Figure 2 ITD coding strategies in birds and mammals. a, In birds, ITD detection depends upon a circuit composed of coincidence detectors and delay lines. (1) Each neuron in NL acts as a coincidence detector and fires maximally when inputs from the two sides arrive simultaneously. This occurs when the interaural phase differences are compensated for by an equal and opposite delay in the delay line inputs. (2) In the barn owl coincidence detector neurons are sharply tuned for ITDs relative to the width of the head (dotted lines). The lateral position of a sound source is read out as the position within the array that is maximally active. The different ITD tuning of single neurons is indicated by different colors. (3) Neurons in each brain hemisphere are tuned to different lateral positions in contralateral space. b, In mammals, the model of ITD-sensitive neurons does not depend on a map of ITDs created by delay line inputs. (1) In the gerbil, Grothe (2003) proposed that without inhibitory inputs, axonal conduction delays are distributed around zero ITD. The addition of glycinergic input from the contralateral ear (dotted lines) would shift the peaks of ITD functions toward longer ITDs. (2) The distribution of peak responses is positioned beyond the physiological range (dotted lines), centered on 45° interaural phase difference with respect to neural tuning for sound frequency. The sensitive slope of the broadly tuned functions is positioned within the physiological range. (3) The relative activation of the two brain hemispheres could provide a code of lateral position (McAlpine *et al*., 2001). Reproduced from McAlpine, D. and Grothe, B. 2003. Sound localization and delay lines – do mammals fit the model? *Trends Neurosci.* 26, 347–350, with permission from Elsevier.

2.21.4.1 Delay Line – Coincidence Detection Circuits in Birds

The avian circuit conforms to the requirements of the Jeffress model, in that the axons from NM act as delay lines to create maps of ITD, and NL neurons act as coincidence detectors (see discussion below; Figure 2). Recent evidence from small mammals, however, suggests that the Jeffress model does not completely explain how ITDs are encoded in the mammalian auditory brainstem (McAlpine and Grothe, 2003). There are many shared features of ITD coding circuits in birds and mammals; principally, both encode the phase of the auditory stimulus, and both encode ITD, but there are significant differences between the two clades. We will therefore discuss birds, then mammals, and finally compare the coding strategies employed in each group.

In birds, the projections from NM to NL act as delay lines to create maps of ITDs, and NL neurons act as coincidence detectors (Figure 2a). NL neurons phase-lock to both monaural and binaural stimuli but respond maximally when phase-locked spikes from each side arrive simultaneously, that is, when the difference in the conduction delays compensates for the ITD (Carr and Konishi, 1990; Overholt *et al*., 1992; Pena *et al*., 1996). Within the birds, the details of delay line circuit organization vary. In the chicken, NL is composed of a monolayer of

bipolar neurons that receive input from ipsi- and contralateral cochlear nucleus onto their dorsal and ventral dendrites, respectively (Rubel and Parks, 1975). These dendrites increase in length with decreasing best frequency. Evidence from brain slices suggests that only the projection from the contralateral cochlear nucleus acts as a delay line, while inputs from the ipsilateral cochlear nucleus arrive simultaneously at all neurons (Overholt *et al*., 1992). This pattern of inputs creates a single map of ITD in any tonotopic band in the mediolateral dimension of NL (Figure 2a; Overholt *et al*., 1992).

The barn owl is capable of great accuracy in detecting time differences, and its auditory system is hypertrophied in comparison to the chicken (Kubke *et al*., 2004). The nucleus laminaris is both much larger and reorganized when compared to the plesiomorphic condition exemplified by the chicken (Kubke *et al*., 2002, 2004). Magnocellular axons from both cochlear nuclei act as delay lines (Carr and Konishi, 1988). They convey the phase of the auditory stimulus to NL such that axons from the ipsilateral NM enter NL from the dorsal side, while axons from the contralateral NM enter from the ventral side. Recordings from these interdigitating ipsilateral and contralateral axons show regular changes in delay with depth in NL (Carr and Konishi, 1990). Thus, these afferents interdigitate to innervate dorsoventral arrays of neurons in NL in a sequential fashion, and produce multiple representations of ITD within the nucleus. Despite the differences in organization of NL in owls and chickens, ITDs are detected by neurons that act as coincidence detectors in both species (Joseph and Hyson, 1993; Kubke *et al*., 2002; Pena *et al*., 1996; Sullivan and Konishi, 1984).

A consistent feature of both avian and mammalian coincidence detectors is that they share physiological features with NM neurons and mammalian bushy cells (see Section 2.21.03.3). Coincidence detectors exhibit specific K^+ conductances that lead to a single or few well-timed spikes in response to a depolarizing stimulus *in vitro* (Kuba *et al*., 2002; Reyes *et al*., 1996; Smith, 1995). The low-threshold conductance channels should decrease the effective membrane time constant, that is, the average membrane time constant for a cell receiving and processing *in vivo* rates of EPSPs, which will be much shorter than the passive membrane time constant (Gerstner *et al*., 1996; Grau-Serrat *et al*., 2003; Softky, 1994). These fast conductances may be critical to coincidence detection, and current models suggest that fast membrane time constants are instrumental in keeping the firing rate near zero when the inputs are completely out of phase, and in allowing nonzero firing rate when the inputs are monaural.

2.21.4.2 ITD Detection Circuits in Mammals

The mammalian superior olive (MSO) contains neurons that receive excitatory input from the cochlear nucleus, and act as coincidence detectors to encode ITD (Goldberg and Brown, 1969; Yin and Chan, 1990). Despite this similarity with NL, the two structures may not be homologous, and their similarities may have emerged from the constraints of encoding ITD (Grothe, 2003). The reasons for assuming that the two nuclei are not homologous are both anatomical and physiological. First, the MSO is located in the ventral brainstem and forms part of the mammalian superior olivary complex, while the NL is dorsal and closely associated with NM. Thus, the embryological origins of the two structures may not be the same. Second, although MSO neurons act as coincidence detectors, it is not clear if their cochlear nucleus inputs act as delay lines to form maps of ITD. Anatomical data from the cat suggest that contralateral, but not ipsilateral inputs, could act as delay lines (Beckius *et al*., 1999), but there is as yet no unambiguous physiological evidence for a map formed by the incoming axons. Finally, the MSO receives fast, well-timed inhibitory inputs from the medial and lateral nucleus of the trapezoid body (Cant and Hyson, 1992; Grothe, 2003). These inhibitory inputs may enhance coincidence detection in several ways. Inhibition may produce a somatic shunt during coincidence detection to decrease the membrane time constant (Brughera *et al*., 1996). Inhibition may also modify the neural code for ITD. In the Mongolian gerbil, a small mammal with low-frequency hearing, precisely timed glycine-controlled inhibition in the MSO shifts the ITD curve so that the greatest change in firing rate falls within the physiologically relevant range of ITDs (Brand *et al*., 2002).

Inhibitory inputs are also found in the avian NL, but they are more diffuse, and appear to decrease excitability through a gain-control mechanism, rather than being phase-locked (Funabiki *et al*., 1998; Monsivais *et al*., 2000; Pena *et al*., 1996; Yang *et al*., 1999). Avian superior olivary neurons receive projections from the NA and NL and provide a GABAergic feedback projection to NM, NA, and NL (Lachica *et al*., 1994; Takahashi and Konishi, 1988b). This olivary input appears to provide tonic inhibition (Monsivais *et al*., 2000; Yang

et al., 1999), in contrast to the inhibitory projections in the mammalian MSO. It appears that the GABAergic input in birds maintains the coincidence detector in the optimal range of operation (Funabiki *et al.*, 1998). *In vivo* recordings from the barn owl support this interpretation (Pena *et al.*, 1996; Takahashi and Konishi, 2002).

The differences in how birds and mammals encode ITDs goes beyond differences in the neural circuit for ITD detection to the deeper issue of how ITDs are coded in the brain. Recent evidence from gerbils indicates that ITDs are not represented by maximal discharges of a few neurons, but rather by the relative activity in both MSOs. It has been proposed that this activity is regulated by inhibition, not delay-lines, and that there is no requirement for a map of azimuthal space in the MSO (Figure 2b; reviews in Grothe, 2003; McAlpine and Grothe, 2003). These observations, together with the lack of evidence for maps of ITD in the mammalian inferior colliculus, have been used to support the hypothesis that the neuronal representation of auditory space differs in birds and mammals, indicating again a parallel evolution of spatial hearing.

2.21.4.3 Models of Coincidence Detection and Dendritic Structure

In both birds and mammals, coincidence detectors are bitufted neurons with inputs from each ear segregated on separate sets of dendrites (Figure 3). Modeling studies suggest this dendritic separation improves coincidence detection (Agmon-Snir *et al.*, 1998; Grau-Serrat *et al.*, 2003). The dendritic separation may allow each dendrite to act as a current sink for inputs on the other dendrite, thus decreasing the amount of current to the soma when inputs arrive only on one side. This effect might be boosted by the presence of a low-threshold K^+ conductance similar to that found in NM and bushy neurons so that out-of-phase inputs are subtractively inhibited (Grau-Serrat *et al.*, 2003). With only monaural input, the low-threshold K^+ conductance in the opposite dendrite is somewhat activated, producing a mild current sink. When, however, there are recent EPSPs in the opposite dendrite due to out-of-phase inputs, the low-threshold K^+ conductance is strongly activated and acts as a large current sink suppressing spike initiation. Thus, the model predicts the experimental finding (Carr and Konishi, 1990; Goldberg and Brown, 1969; Yin and Chan, 1990) that the monaural firing rate, while lower that the binaural in-phase rate, is higher than the binaural out-of-phase rate. The benefits conveyed by the neuronal structure of the coincidence detectors further supports the idea that the evolution of coincidence detectors in the bird NL and mammalian MSO may have occurred in parallel (Carr and Soares, 2002).

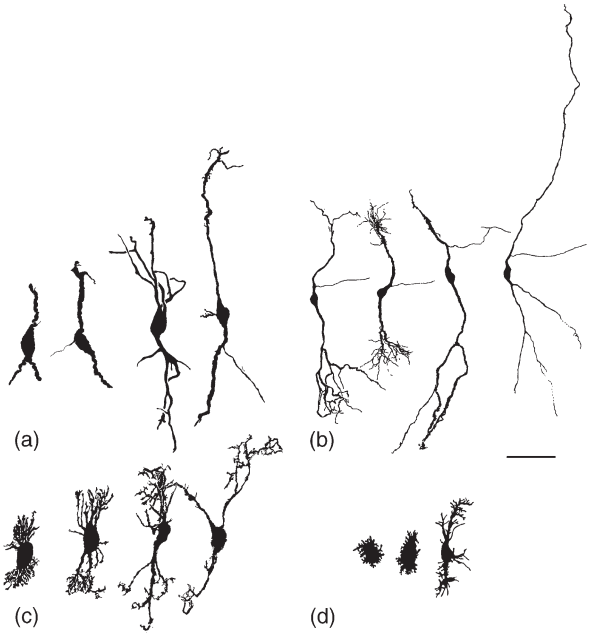

Figure 3 Coincidence detectors share bitufted morphology. a, Alligator NL neurons labeled with Golgi technique, from presumed high to low best frequency regions of NL (left to right; Soares, unpublished). b, Chicken NL neurons labeled with Golgi technique, from high to low best frequency regions of NL (Jhaveri and Morest, 1982). c, Guinea pig MSO neurons (Smith, 1995). d, Barn owl NL labeled with Golgi technique (Carr and Boudreau, 1993). Dendritic length increases from left to right except in the principal cells of the medial superior olive from the guinea pig, where a frequency gradient is not apparent (Smith, 1995). The bipolar architecture and the segregation of the inputs arriving from both ears are common to both mammalian and avian coincidence detectors. In the barn owl, coincidence detectors have lost this bipolar organization, except in low best frequency regions where their short dendrites radiate around the cell body. Reproduced from Carr, C. E. and Soares, D. 2002. Evolutionary convergence and shared computational principles in the auditory system. *Brain Behav. Evol.* 59, 294–311, with permission from Karger, Basel.

2.21.5 Summary and Conclusions

The cochlear nuclei of birds and mammals share similar features, including heterogeneous cell populations, and similar responses to sound. These shared characteristics may represent similar responses to selective pressures to encode the features of airborne sound. The principal reason for arguing that the similarities in the cochlear nuclei of birds and mammals may be due to similar

selective pressures, and not homology, is that the ancestors of birds and mammals separately developed true tympanic ears (Clack, 1997). A second reason is that close comparisons of bird and mammal cochlear nuclei reveal many differences. A third is that the observed similarity in the morphology and physiology of cochlear neurons is a plausible outcome of parallel evolution, because neurons in both birds and mammals experience similar constraints in detecting sound. Thus, although a common population of brainstem auditory neurons existed in the tetrapod ancestor, distinct evolutionary forces may have acted on these two groups allowing for the emergence of different ears and in turn, dissimilar organization in the brainstem.

Comparisons of temporal coding reveal shared computational principles. When compared with a simple integrate-and-fire unit, the auditory neurons that phase-lock, detect coincidences, and encode temporal patterns all exhibit a suite of physiological and morphological adaptations that suit them for their task. The core features of auditory coding are very similar in birds and mammals (and probably in other animals as well). Comparative studies of temporal coding can therefore add to the discussion of whether neuronal function follows form. A case can be made for this in time-coding neurons of the auditory brainstem of birds and mammals, and for phase-coding neurons in weakly electric fish (Kawasaki and Guo, 1998).

If there are computational advantages to particular neuronal architectures, similar forms should be expected. For example, we argue that the bitufted structure of coincidence detector neurons in birds and mammals is computationally advantageous. Therefore, morphological similarities might not support homology, but rather similar computational demands, and we can argue that the neurons of nucleus laminaris and MSO may have converged upon their similar form (Carr and Soares, 2002). In another example, it appears that large somatic terminals on NM or bushy cells are an ancestral feature of amniote auditory nerve. A shared pressure to encode higher-frequency sounds may have driven the parallel appearance of complex endbulbs in archosaurs and mammals.

Finally, phenotypically different neurons can produce similar computations. Neurons may differ in the expression and/or distribution of their ionic channels and still behave similarly. Thus, there may be numerous acceptable ways to carry out a particular computation. These may be revealed by comparative studies.

Acknowledgments

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2.22 Evolution of the Visual Tectogeniculate and Pretectogeniculate Pathways in the Brain of Amniote Vertebrates

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Glossary

accessory optic system Group of nuclei in the ventral tegmentum that receive retinal projections through the basal optic root and are involved in the regulation of eye movements.

advanced More recently derived from ancestral stock.

amniotes Turtles, diapsid reptiles (lizards, snakes, crocodiles) and birds, mammals. They are characterized by an amnion, a membrane surrounding the embryo, and an amniotic fluid.

anterior dorsal ventricular ridge Part of the pallium in reptiles and birds that primarily receives sensory projections from dorsal thalamic sensory nuclei, noticeably from the relay (rotundal nucleus) in the tectofugal visual subsystem.

AOS Accessory optic system.

dendritic field Area occupied by all branches of neuronal dendrites.

derived characters Certain newly acquired features that can be found in a particular taxon as a result of adaptive specialization, and not traced in ancestral forms.

DLA Anterior dorsolateral complex, the group of retinorecipient nuclei in the dorsal thalamus of birds (the relay in the thalamofugal visual subsystem), comparable to mammalian GLd.

DLL DLA lateral subdivision.

GABA γ-aminobutyric acid, an inhibitory neurotransmitter.

GLd Dorsal lateral geniculate nucleus, the retinorecipient nucleus in the dorsal thalamus of reptiles and mammals (the relay in the thalamofugal visual subsystem).

GLv Ventral lateral geniculate nucleus, the retinorecipient nucleus in the ventral thalamus of amniotes.

Gt Nucleus griseus tectalis, the retinorecipient nucleus in the pretectum of reptiles and birds.

homology Common character observed in a monophyletic taxon; this character can be traced back to a common ancestor.

IGL	Intergeniculate leaflet, the retinorecipient nucleus in the ventral thalamus of amniotes.
immunoreactivity	Visualization of neuroactive substances using the antigen–antibody reaction.
interneuron	Local-circuit neuron intercalated between a primary sensory neuron and a projection neuron.
Lm	Nucleus lentiformis mesencephali, the retinorecipient nucleus in the pretectum of reptiles and birds.
Lp/Pulv	Lateral posterior nucleus/pulvinar, the nuclei in the dorsal thalamus of mammals (the relay in the tectofugal visual subsystem).
nBOR	Nucleus of the basal optic root, the retinorecipient nucleus in the ventral tegmentum of reptiles and birds belonging to the AOS.
neuropil	Region devoid of projection neuron cell bodies but containing dendrites and nerve fibers, in which most synaptic contacts occur.
NOL	Nucleus olivaris, the retinorecipient nucleus in the pretectum of mammals.
NOT	The nucleus of the optic tract, the retinorecipient nucleus in the pretectum of mammals.
NPP	Nucleus pretectalis posterior, the retinorecipient nucleus in the pretectum of mammals.
phylogeny	Evolutionary development of a taxon or taxa.
plesiomorphy (plesiomorphic characters)	Primitive characters inherited from ancestral stock and remaining unchanged during phylogeny.
primitive characters	Any feature that can be traced early in the phylogeny and conserved in advanced (or recent) lineage.
Ptv	Nucleus pretectalis ventralis, the nonretinorecipient nucleus in the pretectum of reptiles.
retinotopic organization	Representation of the retinal spatial organization in the visual structures of the brain.
Rot	Nucleus rotundus, the nonretinorecipient nucleus in the dorsal thalamus of reptiles and birds (the relay in the tectofugal visual subsystem).
saccades	Rapid eye movements that allow repositioning of the eyes to fix a new object entering the visual field.
SC	Superior colliculus, the retinorecipient mesencephalic roof of mammals (equivalent to the optic tectum of reptiles and birds).
SGC	Stratum griseum centrale, the central cellular layer of the optic tectum and SC.
SGFS	Stratum griseum et fibrosum superficiale, the superficial cellular and fiber layer of reptilian and avian optic tectum.
SGP	Stratum griseum periventriculare, the periventricular cellular layer of the optic tectum and SC.
SGS	Stratum griseum superficiale, the superficial cellular layer of the mammalian SC.
SO	Stratum opticum, the optic fiber layer of the optic tectum and SC.
visuomotor behavior	Motor reaction elicited by the movements of images across the retina of an animal.
X, Y, and W Cells	Morphologically and functionally different retinal ganglion cells of mammals.

2.22.1 Introduction

Visual information reaches the cortex of the brain of amniote vertebrates by way of several pathways. The retina projects directly to a thalamic nucleus (the GLd of reptiles and mammals, or the anterior dorsolateral complex (DLA) of birds), from which projections arise to the reptilian lateral dorsal cortex, the avian Wulst, or the mammalian striate cortex. This retinothalamotelencephalic pathway is generally referred to as the thalamofugal visual subsystem. In addition, the retina projects to the optic tectum (or its mammalian homologue, the SC), from which projections arise to the thalamic nucleus rotundus (Rot) of reptiles and birds, or to the mammalian complex formed by the lateral posterior nucleus and the pulvinar (Lp/Pulv), these in turn projecting to the anterior dorsal ventricular ridge of reptiles, the ectostriatum of birds, and the extrastriate cortex of mammals, to form a tectofugal visual subsystem.

These two pathways are not, however, independent. Projections from the optic tectum or the mammalian SC to the GLd have been described in all amniote groups (see below); this tectogeniculate pathway is complemented by a second, pretectofugal series of projections arising in some of the pretectal nuclei and also terminating in the GLd.

In the pages that follow, we propose analyzing in detail the anatomical organization of the tectogeniculate and pretectogeniculate pathways in different groups of amniotes, and attempting to specify their main evolutionary tendencies during the phylogeny of this vertebrate group (see The Evolution of Visual Cortex and Visual Systems, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?, Visual Cortex of Turtles, Evolution of Color Vision and Visual Pigments in Invertebrates).

2.22.2 The Tectogeniculate Pathway

The optic tectum (or mammalian SC) is one of the most phylogenetically conservative structures of the vertebrate visual system. Its projection to the GLd has been described in many reptilian species (Ulinski *et al.*, 1992; Reiner, 1994; Butler and Hodos, 1996; ten Donkelaar, 1997; Martínez-Marcos *et al.*, 1998; Belekhova *et al.*, 2003; Kenigfest *et al.*, 2004). In birds, the principal optic nucleus of the dorsal thalamus, the DLA, is generally considered as the homologue of the GLd (Watanabe, 1987; Miceli *et al.*, 1990; Wu and Karten, 1998), and receives projections from the optic tectum (Watanabe, 1987; Wild, 1989; Sugita *et al.*, 1996). In mammals, a colliculogeniculate projection has been described in many species (for review, see Harting *et al.*, 1991).

In all amniotes, the tecto/collicular projection to the GLd is ipsilateral and is considerably sparser than that to the dorsal thalamic relay of the tectofugal visual subsystem (the Rot or the mammalian Lp/Pulv complex), and to the ventral lateral geniculate nucleus (GLv) and intergeniculate leaflet (IGL).

2.22.2.1 Location of the Tectogeniculate Projection Neurons

The optic tectum/SC of amniotes varies considerably in its degree of differentiation, although three main cellular layers can be consistently distinguished: the periventricular stratum griseum periventriculare (SGP), the stratum griseum centrale (SGC), and the stratum griseum et fibrosum superficiale (SGFS) of reptiles and birds or the mammalian stratum griseum superficiale (SGS).

Among the reptiles, the highest degree of tectal differentiation is observed in the dracomorph lizards, in which the tectogeniculate projection neurons are located almost exclusively in the retinorecipient SGFS, those neurons projecting to the Rot lying in the nonretinorecipient SGC. In contrast, in lacertomorph lizards, turtles, and snakes, the tectal layers are less well differentiated, and tectogeniculate projection neurons are found in all three layers but preferentially in the SGFS; the neurons projecting to the Rot are found in the SGC and to some extent in the SGP.

In birds, which have a highly differentiated optic tectum, the tectogeniculate neurons are located, as in dracomorph lizards, exclusively in the SGFS (Wild, 1989), while the tectorotundal neurons are virtually exclusively observed in the SGC, with an insignificant proportion being located in the SGFS (Karten *et al.*, 1997; Hellmann and Güntürkün, 2001).

The colliculogeniculate projection of all mammalian species arises from neurons situated in the most dorsal sublayer of the superficial collicular layer (Harting *et al.*, 1991), well separated from those neurons of the ventral sublayer of the SGS and upper layer of the stratum opticum (SO) which project to the Lp/Pulv complex (Major *et al.*, 2000). In the tree shrew and bush baby, different tectal cells project to separate layers of the GLd (Diamond *et al.*, 1991; Lachica and Casagrande, 1993).

Since the phylogeny of the amniote visual system shows a progressive migration of neurons from deep to superficial tectal layers, Northcutt (1984) and Reiner (1994) have speculated that the location of the projection neurons in different tectal layers may reflect the pattern of neuronal migration from the periventricular to the superficial regions of the tectum. Turtles and lacertomorph lizards on the one hand, and birds on the other, may be considered as the two extremes of organization of tectothalamic projections in nonmammalian amniotes, with a completion of the outward migration of projection neurons being observed in mammals.

2.22.2.2 Morphology of the Tectogeniculate Projection Neurons

Information concerning the morphology of the tectogeniculate projection neurons in reptiles is somewhat scarce. In turtles, most of these neurons, located in the SGFS, are small, fusiform, and vertically oriented; their apical dendrites extend into the optic fiber layer (SO) and have relatively narrow fields of terminal arborization. The tectogeniculate projection neurons in the other tectal layers (SGC and SGP) share these morphological features; their long ascending dendrites also give rise to narrow fields of arborization in the most superficial retinorecipient layer of the tectum. Another population of projection neurons, situated in the deep nonretinorecipient sublayer of the SGFS, has been described in turtles; these give rise to moderately wide dendritic fields in the most superficial layers of the SGFS.

In lacertilian lizards (Martínez-Marcos *et al.*, 1998), two types of tectogeniculate projection

neurons have been described: (1) small, radially oriented neurons located in the SGFS and SGP, similar to those described in turtles, and which project to the neuropil of the GLd; and (2) large, multipolar neurons located in the SGC which project to the GLd cell plate.

In birds, also, the tectogeniculate projection arises from neurons morphologically similar to those described in turtles, with narrow fields of terminal arborization. Reiner (1994) considers the projection cells of turtles and birds as a homologous population of neurons which only differ by their location in a radial gradient of migration during development.

The vast majority of the colliculogeniculate projection neurons of mammals resemble those of reptiles and birds; they are small, fusiform, radially oriented cells with small, well-defined receptive fields. In rodents, these neurons receive an input from the W ganglion cells of the contralateral retina, or, in the case of primates and cats, an additional input from the Y cells of the ipsilateral retina. In the cat, two additional populations of polygonal projection neurons have been described; these receive a predominantly corticofugal afferent supply with little or no retinal input (Harting *et al.*, 1991).

2.22.2.3 Location of the Tectal Projections in the GLd: Light and Electron Microscopic Data

In reptiles, several retinorecipient nuclei, with a variety of morphological features, comprise the complex of the GLd (Rainey and Ulinski, 1986; Repérant *et al.*, 1992; Butler and Hodos, 1996). The retinal projections to these nuclei have been described in considerable detail, but the extraretinal afferent supply is less well documented. In turtles, lacertilian lizards, and some snakes, tectofugal terminals have been described in both the cell plate and neuropil of the nucleus (Reiner, 1994; Martínez-Marcos *et al.*, 1998; Belekhova *et al.*, 2003). In turtles, the tectofugal projections to the GLd are denser in the inner neuropil and cell plate; in these regions, the type I and III retinal terminals are also located. In the outer neuropil of the GLd, in which type II retinal terminals are mainly located, tectal projections are virtually absent (for a discussion of the three types of retinal terminals, see Sjöström and Ulinski, 1985). The tectal projections to the turtle GLd overlap the corticogeniculate projections (Kenigfest *et al.*, 1998, 2004).

In birds, several subnuclei of the DLA receive tectal projections; these predominate in the lateral subdivision of the complex (DLL; Watanabe, 1987; Wild, 1989; Sugita *et al.*, 1996) which also receives projections from the Wulst (Watanabe, 1987). The available data are too scanty to support any speculations as to possible homologies between the subcomponents of the avian DLA and the different layers of the mammalian GLd.

In the majority of mammalian species, the tectogeniculate projection is visuotopically organized, and innervates the small-celled regions of the GLd that contain neurons receiving input from W retinal ganglion cells and projecting to layers 1–3 of the striate cortex. In some species (Diamond *et al.*, 1991; Lachica and Casagrande, 1993), these may be resolved into two distinct pathways arising from distinct populations of collicular neurons which project to different small-celled layers of the GLd that have different targets in the striate cortex. This degree of specification of the colliculogeniculate projections is higher than that of the tectogeniculate projections in nonmammalian amniotes.

Few ultrastructural studies of the tectogeniculate projections have been carried out, and are limited to turtles (Kenigfest *et al.*, 2004) and mammals (Feig and Harting, 1994). In both groups, they comprise γ-aminobutyric acid (GABA)-immunonegative, small-axon terminals, containing rounded synaptic vesicles, which establish asymmetrical synaptic contacts. In turtles, these contacts are made with dendrites of relay cells, mainly in the cell plate and inner neuropil, regions which are also the main targets of cortical terminals. In monkeys, the colliculogeniculate terminals generally form synapses with small dendrites of W-type relay cells in the koniocellular layers of the GLd.

2.22.3 The Pretectogeniculate Pathway

The pretectal region of the amniote brain is generally a highly conservative region of the diencephalomesencephalic junction containing several retinorecipient and nonretinorecipient nuclei with ill-defined contours. Almost all pretectal nuclei have tight reciprocal connections with the optic tectum and the nuclei of the accessory optic system (AOS). The pretectal projection to the GLd has been demonstrated in all amniotes, but only recently in turtles (Kenigfest *et al.*, 2000, 2004). In reptiles, birds, and mammals, the pretectogeniculate projection is ipsilateral and considerably weaker than the pretectogeniculate projection to the Rot or the mammalian Lp/Pulv complex, or to the ventral thalamic GLv and IGL.

2.22.3.1 Location of the Pretectogeniculate Projection Neurons

Among reptiles, the pretectogeniculate pathway has only been described in turtles (Kenigfest *et al.*, 2000, 2004). The chelonian GLd receives a weak ipsilateral

pretectal projection arising from neurons of the retinorecipient nucleus lentiformis mesencephali (Lm) and nucleus griseus tectalis (Gt), as well as cells of the nonretinorecipient nucleus pretectalis ventralis (Ptv), which is a source of a major input to the Rot (Kenigfest *et al.*, 2000). Those pretectal nuclei projecting to the GLd also have reciprocal projections with the optic tectum, GLv, and the nucleus of the basal optic root (nBOR) of the AOS (Fite, 1985; Fan *et al.*, 1995; Weber *et al.*, 2003) and with visual telencephalic areas (Kenigfest *et al.*, 2000).

In birds, the pretectogeniculate pathway also arises from the retinorecipient Lm and Gt (Wild, 1989; Wylie *et al.*, 1998); as in turtles, this pathway is weaker than the pretectorotundal pathway arising from the complex of nonretinorecipient pretectal nuclei (Kenigfest *et al.*, 2000). The avian Lm also receives afferents from the visual Wulst, and has reciprocal connections with the optic tectum and nBOR (Wylie *et al.*, 1998). The main difference between reptiles and birds is that the pretectal nuclei of birds are considerably better differentiated than in turtles, indicating that the pretectogeniculate and pretectorotundal pathways are less well separated in turtles than in birds.

In mammals, the pretectal region has undergone a remarkable reorganization during the course of evolution. As a result, the mammalian pretectum shows considerable interspecific variation; it consists of numerous retinorecipient and nonretinorecipient nuclei, whose homology with avian or reptilian pretectal nuclei is not always obvious. The pretectal projection to the mammalian GLd arises mainly from three retinorecipient nuclei: (1) the nucleus of the optic tract (NOT); (2) the nucleus pretectalis posterior (NPP); and, to a lesser extent, (3) from the nucleus olivaris (NOL); the mammalian NOT/NPP may be considered as possible homologues of the reptilian and avian Lm. The same three mammalian pretectal nuclei give rise to projections to the Lp/Pulv complex, which arise from different cell populations than those to the GLd. As in reptiles and birds, the retinorecipient nuclei of the mammalian pretectum have reciprocal connections with the SC, the terminal nuclei of the AOS, and the visual cortical areas (see Simpson *et al.*, 1988, for review).

2.22.3.2 Morphology and Neurochemical Properties of the Pretectogeniculate Projection Neurons

In turtles, the neurons of the Lm and Gt which project to the GLd are relatively small, round or fusiform in shape, with long, profusely branching dendrites. The neurons of the Ptv, which vary in both shape and size, are larger than the projection neurons of the Lm and Gt. The extensive dendritic branches of the Ptv projection neurons remain confined within the boundaries of the nucleus. The vast majority of neurons in all three pretectal nuclei are GABA-immunoreactive, thus giving rise to GABAergic projections to the GLd and Rot (Kenigfest *et al.*, 2000).

In birds, as in turtles, the neurons of the Lm that project to the DLA are small in size, whereas those pretectal neurons projecting to the Rot are larger. The pretectothalamic projection neurons are GABA-immunoreactive and thus have an inhibitory influence on the DLA and Rot (Kenigfest *et al.*, 2000).

In mammals, both the pretectogeniculate and pretectopulvinar projection neurons vary in size and shape. All the pretectal nuclei are well endowed with GABA-immunoreactive cells that project in different proportions to the GLd and Lp/Pulv complex (Cucchiaro *et al.*, 1991, 1993; Reimann and Schmidt, 1996). Because of the heterogeneity of the size and shape of pretectal projection neurons in mammals, it is difficult to compare the morphologies of cells projecting to the GLd and Lp/Pulv across mammalian species. However, it is apparently the case that the neurons of the NPP and NOT that project to the Lp/Pulv complex appear to be generally small in size, whereas those projecting to the GLd appear to be larger (Kubota *et al.*, 1988; Cucchiaro *et al.*, 1991; Reimann and Schmidt, 1996); the contrary is true for reptiles and birds.

2.22.3.3 Location of the Pretectal Projections in the GLd: Light and Electron Microscopic Findings

The location of pretectal terminals in the reptilian GLd and avian DLA is similar to that of the tectogeniculate projections. Both pathways terminate in zones that receive predominantly corticofugal projections and considerably fewer retinal projections (Wild, 1989; Wylie *et al.*, 1998; Kenigfest *et al.*, 2000, 2004).

In turtles, some recent findings (Kenigfest *et al.*, 2004) have shown that, under the electron microscope, the pretectal terminals are more numerous in those regions of the GLd (cell plate and inner neuropil) that receive tectal and cortical afferents than in the outer neuropil, which is the major target of retinal projections. The pretectal terminals contain pleomorphic synaptic vesicles, are GABA-immunoreactive and establish both symmetric and asymmetric synaptic contacts, almost exclusively with the dendrites of geniculocortical projection neurons, and to a very small extent with the dendrites of GABAergic interneurons. The principal influence of the GABAergic pretectogeniculate

projections upon the geniculocortical neurons of the turtle is thus inhibitory.

In birds, no ultrastructural studies of the pretectogeniculate projections appear to have been carried out.

In mammals, the pretectogeniculate projection arising from the retinorecipient NOT is retinotopically organized, while the less dense projections from the NPP and NOL are not (Kubota *et al.*, 1988). While a GABAergic, inhibitory pretectogeniculate projection exists in all studied mammalian species, and their location within the GLd differs from that of the corticogeniculate terminals, the laminar location of the former shows remarkable interspecific variation. In the majority of mammalian species, these terminals are located in the parvo- and/or magnocellular layers of the GLd which contain X- and Y-type cells involved respectively in detailed object vision or motion detection and which project to layer 4 of the striate cortex; they are much less evident in the small-celled layers containing W-type neurons (Cucchiaro *et al.*, 1993; Feig and Harting, 1994; Uhlrich and Manning, 1995; Büttner-Ennever *et al.*, 1996). The pretectogeniculate terminals contain flattened or pleomorphic synaptic vesicles, and make symmetrical synaptic contacts (Cucchiaro *et al.*, 1993; Feig and Harting, 1994; Wang *et al.*, 2002). In the monkey, the majority of the pretectogeniculate GABAergic terminals establish contacts with the geniculocortical projection neurons, in both the retinorecipient and corticorecipient zones (Feig and Harting, 1994). On the other hand, in the cat (Cucchiaro *et al.*, 1993; Wang *et al.*, 2002), the postsynaptic target of the pretectogeniculate terminals is exclusively the dendrites of GABAergic interneurons. Thus, the main influence of the pretectal input to the GLd is, in the cat, the disinhibition of relay neurons by means of inhibition of interneurons, while in the monkey the greater part of the pretectal input directly inhibits the relay cells.

In general, in mammals, the pretectogeniculate pathway is both more heterogeneous and more widely distributed within the GLd than the colliculogeniculate pathway.

2.22.4 Functional Considerations

In reptiles, birds, and mammals, the tectum and pretectum, together with the AOS, are involved in the organization of visuomotor behavior. These structures contain directionally sensitive neurons, and have multiple descending projections to premotor and motor centers of the hindbrain responsible for eye and head movements (Jassik-Gerschenfeld and Hardy, 1984; Fite, 1985; McKenna and Wallman, 1985; Fan *et al.*, 1995). Although little functional information is available concerning the tecto- and pretectogeniculate pathways in non-mammalian amniotes, the purely morphological and neurochemical data indicate strongly that they may well be involved in the modulation of the sensitivity of geniculocortical projection neurons during visuomotor behavior. Wylie *et al.* (1998) have suggested that, in birds, the pretectal and AOS input to the visual thalamofugal subsystem may be involved in perception of the three-dimensional layout of the environment, distinguishing object motion from self-motion, and in spatial cognition.

In mammals, the function of the two pathways and their role in visuomotor activity are more obvious. Neurophysiological data show that the stimulation or suppression of activity in the SC modifies visually driven activity in both the GLd and visual cortex (Molotchnikoff *et al.*, 1986; Xue *et al.*, 1994).

The colliculogeniculate pathway, which arises in the superficial collicular layer, interacts with direct projections from the W-type retinal ganglion cells which provide information about slowly moving objects in the visual field. The integrated information is then passed to the striate cortex and combined with information from the magno- (Y-type) and parvo- (X-type) geniculocortical projection neurons. Some authors have suggested that the colliculogeniculocortical pathway is involved in selective visual attention (Harting *et al.*, 1991; Lachica and Casagrande, 1993).

Another pathway from the superficial collicular layers to the intermediate and deep premotor layers has been suggested in the involvement of visually guided orienting movements (Helms *et al.*, 2004). During saccadic eye movements, for which the SC is responsible, a suppression of activity in the magnocellular pathway, and to a lesser extent in the parvocellular pathway, of the GLd occurs; this is followed by a facilitation of activity in the GLd after the fixation of a new visual image upon the retina (Ramcharan *et al.*, 2001; Reppas *et al.*, 2002). The modulatory effect of saccades on the activity of the geniculocortical projection neurons can be exercised by both excitatory and inhibitory inputs from both cortical areas and subcortical or brainstem tectorecipient centers.

In contrast to the collicular neurons, the mammalian pretectal neurons involved in saccadic eye movements project directly to the GLd and modify the activity of the geniculocortical projection

neurons after a saccade. The most extensive studies of this process have been carried out in the cat (Cucchiaro *et al.*, 1991, 1993; Uhlrich and Manning, 1995; Schmidt, 1996; Wang *et al.*, 2002); the activity of geniculocortical relay cells, blocked by a saccade, is restored by inhibition of the inhibitory interneurons of the GLd by the GABAergic pretectal afferents. In primates, the influence of the pretectum on the activity of the GLd is somewhat more complicated, involving as it does both the direct inhibition and indirect disinhibition of the geniculate relay cells (Feig and Harting, 1994).

2.22.5 Conclusions

While tectal and pretectal inputs to the GLd appear to exist in all amniote groups, it would be somewhat premature to suggest that they exercise an identical function in each; nevertheless, the similarities in the morphological and neurochemical features of these pathways suggest that they exert a similar function in the processing of visual information by the thalamofugal subsystem. In all amniotes, the two pathways provide the opportunity for the integration of visual signals and visuomotor activity at an early stage of visual information processing. Both pathways are less elaborate than the massive tectal and pretectal inputs to the dorsal thalamic relay of the tectofugal visual subsystem and to other thalamic centers directly related to visuomotor activity.

The fundamental plan of morphological and neurochemical organization of these pathways, shared by all amniotes, indicates strongly that they may have a common origin in ancestral forms of this group.

During the course of evolution of the amniotes, the progressive segregation and specialization both between and within the tecto- and pretectogeniculate pathways have proceeded in parallel with the laminar and nuclear differentiation both of their sources in the tectum and pretectum, and of their targets in the GLd. Recent mammals, especially those with well-developed binocular vision, show a clear heterogeneous composition of both pathways that is only indicated in reptiles and some birds.

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2.23 Evolution of Myelinated Nervous Systems

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Glossary

desmosome	Specialized adherens junction between neighboring cells.
myelin internode	A myelin sheath that defines the region between two nodes of Ranvier or a node of Ranvier and the initial segment or distal region where myelin sheaths terminate.
myelin sheath	Found in both vertebrate and invertebrate nervous systems, they are multilayered membranes that surround large-caliber axons and facilitate saltatory conduction.
nodes of Ranvier	Axonal membrane where sodium channels are concentrated. These are seen as segmented interruptions in the myelin sheaths.
oligodendrocyte (OL)	Central nervous system glial cell that produces myelin sheaths around one or more (upwards of 40) internodes.
Schwann cell	Derived from the neural crest, this cell either produces single myelinated nerves in the peripheral nervous system or establishes nonmyelinating relationships with one to multiple small-caliber peripheral nerve axons. The cells bordering the squid giant axon are often referred to as Schwann cells. As cells in the peripheral nervous system that associate with axons, this definition may apply.

2.23.1 Introduction

Associations between glial cells and large-caliber axons are common features among invertebrate and vertebrate nervous systems examined (Schweigreiter *et al.*, 2006). At one extreme are squid giant axons, which reach 1 mm or more in diameter and 10–20 cm in length in widely dispersed species. Tens of thousands of glial cells associate with each giant axon (Villegas and Villegas, 1984; Brown and Abbott, 1993). Another well-studied animal model, the sea lamprey, has many, if not all, of its larger-caliber axons surrounded by glial cells (Bertolini, 1964; Merrick *et al.*, 1995). The reasons why glial cells first appeared in ancient nervous systems, why they began to associate selectively with larger axons, remain a mystery. Clearly, they not only contribute to efficiency of nerve impulse propagation by preventing/reducing cross-talk, they also provide structural support and nutrition for the axons and other larger neurites with which they associate.

The largeness of squid giant axons allows investigators to separate axoplasm from the axolemmal membrane and surrounding glial cells by simple extrusion (Brown and Lasek, 1990). This feature led investigators to discover that glial cells transfer a subset of newly synthesized proteins to giant axons (Gainer *et al.*, 1977; Lasek *et al.*, 1977; Sheller *et al.*, 1995). It seems reasonable that similar mechanisms exist in other large-caliber axons for, although protein transfer was not measured directly, a transfer of glial-derived proteins and other molecules is thought to underlie the long-term survival of these axons following their separation from neuronal soma (Hoy *et al.*, 1967; Bittner, 1991). Although efforts to understand better the multifaceted nature of interactions between neurons and glial cells are ongoing (Kretzschmar and Pflugfelder, 2002; Oland and Tolbert, 2003; Edenfeld *et al.*, 2005; Sattelle and Buckingham, 2006), our focus is on the specific interactions that result in the formation of myelinated axons.

High-resistance myelin sheaths covering nearly the entire surface of large-caliber vertebrate axons are crucial to saltatory conduction, a process that brings both unprecedented efficiency and speed to axon signaling and allows the unmatched

complexity of modern-day vertebrate nervous systems. Whereas many invertebrate and all vertebrate nervous systems (except cyclostomes) evolved the ability to form myelinated axons, both structural and biochemical evidence (see below) suggests that myelination originated independently several times in invertebrates and only once in vertebrates (Waehneldt, 1990). Furthermore, whereas the strategies used to make the vertebrate myelinated nervous system are being ever more clearly understood, strategies underlying invertebrate glial cell myelination are totally unknown and form the variety of structural features expressed (see below), probably quite varied (see Compensatory Innervation in Development and Evolution, Basic Nervous System Types: One or Many?, Origin and Evolution of the First Nervous System, Adult Neurogenesis and Neuronal Regeneration in the Teleost Fish Brain: Implications for the Evolution of a Primitive Vertebrate Trait).

2.23.2 Myelin Sheaths in Invertebrates

Despite a long history, of well over a century, that includes many descriptions of multilayered myelin-like sheaths that surround invertebrate axons/neurites, current focus on invertebrate myelination is sparse (Bullock, 2004; Pan *et al*., 2006). In contrast to prominence of myelinated axons in vertebrate nervous systems, generally the numbers of axons covered with multi-layered membranes in annelid and crustacean nervous systems are small. In striking contrast to an invariant nature of vertebrate myelinated axons, the nature of invertebrate myelin sheaths is highly variable. Some myelin sheaths, as in the crayfish, *Procambarus clarkii* (Cardone and Roots, 1991, 1996), are loosely wound, whereas others, e.g., the eyestalk of the crab, *Cancer irroratus* (McAlear *et al*., 1958) and earthworm, *Lumbricus terrestris* (Günther, 1973, 1976; Roots and Lane, 1983) are highly compacted, more like vertebrate myelin. Among the annelids, myelin-like wrappings have been reported in members of three oligochaete families, i.e., two species of Lumbricidae, *Eisenia foetida* (Hama, 1959) and *L. terrestris* (Coggeshall, 1965; Günther, 1973, 1976; Roots and Lane, 1983), one species of Lumbriculidae, *Lumbriculus variegatus* (Drewes and Brinkhurst, 1990), and one Tubificid, *Branchiura sowerbyi* (Zoran *et al*., 1988). Three families of polychaetes, Capitellidae, Spionidae, and Maldanidae (Nicol, 1958), have large axons covered with multilayered sheaths. Among crustacea, Malacostracan crustacea, several shrimps, prawns, crayfish, and crabs (Holmes, 1942; McAlear *et al*., 1958; Hama, 1966; Heuser and Doggenweiler, 1966; Kusano, 1966; Govind and Pearce, 1988; Cardone and Roots, 1991; Xu and Terakawa, 1999; Lenz *et al*., 2000; Weatherby *et al*., 2000) have axons covered with myelin-like sheaths.

Resemblances between oligochaete and vertebrate sheaths include spiral winding and sheath thicknesses that are correlated with axon caliber. In *E. foetida* sheath thickness ranges from two to 30 lamellae, whereas in *L. terrestris*, sheaths contain 60–200 lamellae. Also, interlamellar spacing can be highly variable. The sheath of the *L. terrestris* median giant fiber, in particular, contains a mix of tightly compacted membranes that resemble vertebrate myelin, and redundant loops formed where the sheath buckles and retains substantial quantities of cytoplasm between membranes. Stacks of desmosome-like structures run serially in register across some sheaths, attaching lamellae to each other (Figure 1). Although these structures resemble vertebrate desmosomes in electron micrographs, they differ in both their intramembranous organization, as revealed by freeze-fracture (Roots and Lane, 1983), and in their protein composition (Pereyra and Roots, 1988).

The sheaths found in crustaceans differ from those of annelids and vertebrates; they are concentric rather than spiral. Moreover, they come in two patterns. In the prawn *Palaemonetes vulgaris* (Heuser and Doggenweiler, 1966) and in shrimps, genus *Penaeus* (Xu and Terakawa, 1999), the concentric laminae join at a short seam reminiscent of vertebrate sheath mesaxons. The arrangement of seams is very regular with those of alternate laminae located on opposite sides of the axon. In the

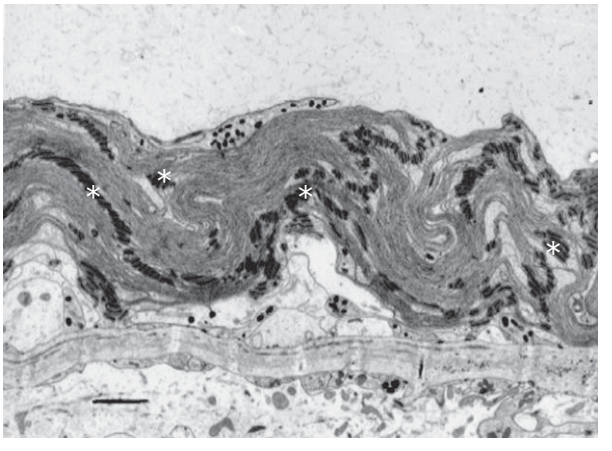

Figure 1 Myelin-like sheath in a longitudinal section of the median giant fiber of *Lumbricus terrestris* nerve cord. Note the desmosome-like structures (*) running in register across the sheath. Scale bar: 1 μm.

copepods *Undinula vulgaris*, *Neocalanus gracilis*, and *Euchaeta rimana*, the lamellae form complete circles and lack seams (Weatherby *et al.*, 2000). Another distinguishing feature of crustacean myelin is the location of glial cell nuclei, which are located in variable positions within the sheath (Heuser and Doggenweiler, 1966; Xu and Terakawa, 1999). The conditions whereby glial cell nuclei and perinuclear cytoplasm lie close to axons would tend to favor a potential metabolic transfer of glial proteins and other metabolites to axons.

The myelin-like sheath of the crab, *C. irroratus*, in particular, bears a striking resemblance to vertebrate myelin. Glial cell nuclei lie outside the sheaths, compaction is similar to that of vertebrate myelin, and structures resembling Schmidt–Lanterman incisures and nodes of Ranvier are present (McAlear *et al.*, 1958). At present it is unclear whether the sheath is spirally or concentrically wound.

As in annelids and vertebrates, in crustaceans myelin thickness correlates with axon caliber. Lamellar numbers vary from 1 or 2 up to 50. In copepods, interlamellar spacing varies between 3 and 30 nm while the compact intralamellar (fused membranes) space remains constant, around 18 nm in thickness (Weatherby *et al.*, 2000). In prawns, the interlamellar periodicity is more than 20 nm (Heuser and Doggenweiler, 1966), whereas in *Penaeus* shrimps it is 8 or 9 nm (Xu and Terakawa, 1999). Periodicity is species-dependent in both invertebrates and vertebrates (see following section; moreover, it is affected by tissue-processing methods (Kirschner and Blaurock, 1992; Roots, 1993). The periodicity of the fully compact myelin of *Penaeus setiferus*, determined by X-ray diffraction (probably the most accurate measurement), is 16 nm, a value similar to that in teleost peripheral myelin (Blaurock, 1986).

Interruptions, functionally comparable to vertebrate nodes of Ranvier, occur in both annelid and crustacean sheaths. In many decapod crustaceans (prawns, shrimps, and crabs), the nodes are strikingly similar to vertebrate nodes, both in terms of general morphology (Retzius, 1890; Holmes *et al.*, 1941; Holmes, 1942) and in the disposition of paranodal loops. As is the case in vertebrate loops (see below), these include structures that resemble septate desmosomes (McAlear *et al.*, 1958; Heuser and Doggenweiler, 1966). Internodal distances are generally shorter than in vertebrates (Holmes *et al.*, 1941). A more detailed comparison of crustacean and vertebrate nodes is available (Roots, 1984). Other shrimps, six species of the genus *Penaeus*, a number of copepods (Hsu and Terakawa, 1996; Xu and Terakawa, 1999; Weatherby *et al.*, 2000), and the earthworms *E. foetida* and *L. terrestris* (Hama, 1959; Günther, 1973, 1976) have completely different nodes. They have circular openings in the myelin sheath and are referred to as focal or fenestration nodes. In *L. terrestris*, there are two nodes of 10–15 μm diameter in each segment. In *Penaeus* shrimps, node diameter and internodal distance are both approximately proportional to fiber diameter. Node diameter varies between 5 and 50 μm and internodal distance from 3 to 12 mm (Xu and Terakawa, 1999). Another morphological feature, suggesting a novel mechanism of fast nerve conduction, occurs in shrimps of the genus *Penaeus*. A large gel-filled space is present between the axon and myelin sheath. This submyelinic space increases effective axon diameter and, as a consequence, conduction velocity. It is tightly sealed at the node regions, permitting saltatory conduction (Hsu and Terakawa, 1996; Xu and Terakawa, 1999).

Conduction velocity has been measured in only a few invertebrate nerves. In the median giant fiber of the earthworm *L. terrestris*, which is 90 μm in diameter, it is 30 m s^{-1} (Günther, 1976). In the shrimp, *Penaus japonicus*, it is 90–190 m s^{-1} in fibers 120 μm in diameter (Kusano, 1966; Kusano and LaVail, 1971). For comparison, a rat fiber of 4.5 μm diameter with a sheath of about 50% of its total diameter conducts at 59 m s^{-1}. Thus, vertebrate sheaths are far more effective in increasing conduction velocity. Reaction times for the escape responses of calanoid copepods have been measured (Lenz *et al.*, 2000). The fastest responses were recorded in myelinated species, the escape response being initiated 2–5 times more rapidly than in the nonmyelinated species. Myelin is found only in the more recently evolved copepod superfamilies, which also live in more diverse habitats. They not only live in neritic and deep-water environments but also in regions of the ocean where faster reaction times are essential for avoiding predators (Hayward and McGowan, 1979; Parks, 1986; Hays *et al.*, 1997; Lenz *et al.*, 2000). Thus, during the course of copepod evolution, the development of myelin has allowed them to occupy new habitats.

Very little is known about the distribution of sodium channels in invertebrate axons. Studies on the shrimp *P. japonicus* indicate that, as in vertebrates, sodium channels are concentrated at the nodes at a density of 530 channels μm^{-2} (Hsu and Terakawa, 1996; Xu and Terakawa, 1999). In the earthworm *L. terrestris*, sodium channels are also concentrated at the nodes (Günther, 1976; Roots, 1984, 1995b).

Information on the chemical compositions of different invertebrate myelin sheaths is limited.

Fortunately, techniques used to purify myelin membranes (based on size and density of the membrane) from vertebrates are adaptable to invertebrates (Pereyra and Roots, 1988; Waehneldt *et al.*, 1989). Analysis of lipid and protein compositions in annelid and crustacean myelin shows marked differences from vertebrate myelin (see below) and from one another. Birefringence studies of earthworm myelin showed the sheath to be qualitatively similar to that of frog sciatic nerve, with protein contributing 30–40% of the total birefringence and the rest attributed to lipids (Taylor, 1942). In the shrimp, *Penaus duorarum*, a strikingly high proportion of lipids is found in isolated myelin; the lipid-to-protein ratio is 15 : 1 (Okamura *et al.*, 1986b).

Galactolipids, major constituents of vertebrate myelin sheaths (Morell and Quarles, 1999) are not present in annelid or crustacean myelin. Instead, glucocerebroside in amounts equivalent to galactocerebroside in vertebrate myelin is present in crustacean, though not in earthworm, myelin. Sphingomyelin is absent from earthworm nerve cord and, although it is found in crayfish (*Cambarus clarki*) nerves, it is structurally quite different from vertebrate sphingomyelin (Komai *et al.*, 1973; Okamura *et al.*, 1986a). Thus, there is an evolutionary trend in which glucocerebrosides in protostomes are replaced by galactolipids in deuterostomes. Although deuterostomes also synthesize glucocerebroside (Tamai *et al.*, 1992), and do so under circumstances when galactolipid synthesis is blocked (Bosio *et al.*, 1998), evolutionary inclusion of galactosphingolipids in myelin (Roots, 1995a) may have occurred to foster lipid–lipid and lipid–protein interactions needed for node/paranode stabilization (Popko, 2000) and/or raft-dependent signaling involved in myelin assembly (Boggs and Wang, 2001).

The protein components of both annelid (earthworm *L. terrestris*) and crustacean (crayfish *P. clarkii* and pink shrimp *P. duorarum*) myelin are totally different from those of vertebrates. As in vertebrates, the protein pattern of earthworm myelin is relatively simple, with 80 and 42 kDa proteins predominating and 28–32 kDa proteins as minor components. The structure of these proteins is unknown and there is no cross-reactivity with antibodies to myelin proteins (see Section 2.23.4 for more information on these proteins), including myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated glycoprotein (MAG), and 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) (Pereyra and Roots, 1988; Cardone and Roots, 1990). In the pink shrimp, four major proteins, 21.5, 40, 78, and 85 kDa, and four minor proteins, 36, 41.5, 43, and 50 kDa, are found in purified sheath membranes. None of these proteins show cross-reactivity with antibodies that recognize mammalian MBP or PLP or trout MBP, 36 K or protein zero (P_0) (Okamura *et al.*, 1986b; Waehneldt *et al.*, 1989). A monoclonal antibody generated to earthworm myelin-like membranes and showing cross-reactivity with 30–32 and 40 kDa proteins cross-reacts with 60–65, 42, and 40 kDa proteins in crayfish (*P. clarkii*) axon-ensheathing membranes (Cardone and Roots, 1996). Thus, earthworm and crayfish myelin membrane proteins have some antigenic epitopes in common.

The presence of a myelin sheath confers several advantages in invertebrate survival. The startle reactions of earthworms, escape responses of crayfish, shrimp, and copepods, and retraction of eyestalks in crabs are behaviors in which speed is of paramount importance (Bullock, 1984). Alternatively, faster conduction may be achieved by simply increasing axon diameter, as occurs in squid giant axons (see above). However, this alternative is evolutionarily less favorable. A more efficient means of increasing conduction speed and, therefore, the rapidity with which escape mechanisms take place is the development of myelin sheaths. The nodes of invertebrate nerve fibers serve to allow saltatory conduction in a similar fashion to vertebrate nodes of Ranvier. It should be noted that the points of emergence of small collaterals serve as nodes in both vertebrates (Roots, 1984) and the shrimps *Penaeus chinensis* and *P. japonicus* (Xu and Terakawa, 1999).

2.23.3 Morphological Features of Vertebrate Myelin

Myelination arose in the common gnathostome ancestor as cyclostomes; lamprey and hagfish totally lack myelin (Bullock *et al.*, 1984) and all gnathostomes examined have myelinated nervous systems that are structurally rather invariant, as descriptions for mammalian myelin (Peters *et al.*, 1991; Hildebrand and Mohseni, 2005) readily apply to nonmammalian myelin/myelination and vice versa (see below). Furthermore, the growing understanding of vertebrate myelination is nurtured by continued cross-fertilization, as studies with mammals and nonmammals are often interpreted interchangeably (Kagawa *et al.*, 2001; Lobsiger *et al.*, 2002; Jessen and Mirsky, 2004, 2005; Miller and Reynolds, 2004; Le *et al.*, 2005; Richardson *et al.*, 2006). Several reasons why nonmammals have been chosen for study are: (1) quail-chick

chimeras allow ready oligodendrocyte (OL) lineage tracing (Cameron-Curry and Le Douarin, 1995; Pringle *et al.*, 1998); (2) fishes and some amphibians have the ability to regenerate their central nervous system (CNS) (Sivron *et al.*, 1990; Diekmann *et al.*, 2005); (3) a general interest in comparative myelination (Jeserich and Rauen, 1990; Jeserich *et al.*, 1990; Jeserich and Stratmann, 1992; Gould *et al.*, 1995; Nguyen and Jeserich, 1998; Park *et al.*, 2005); (4) a growing interest in comparative genomics (Aparicio *et al.*, 2002; Gilchrist *et al.*, 2004; Venkatesh and Yap, 2005); and (5) use of high-throughput genetic screens in zebra fish (Rossant and Hopkins, 1992; Sprague *et al.*, 2003), that adapt to identifying proteins important for myelination (Lyons *et al.*, 2005).

The first electron microscopic studies showing the spiraling nature of peripheral nerve myelin formation were conducted with nonmammals, i.e., chick (Geren, 1954) and chameleon (Robertson, 1955) nerves. Subsequent electron microscope observations made on frog (Maturana, 1960) and toad (Stensaas and Stensaas, 1968) CNS sections show similar spiral wrapping of axons with elaborations of OL plasma membrane. More recently, antibody studies of PLP expression showed that frog OLs displayed identical structural appearances during development to mammalian OLs (Yoshida, 1997).

We readily realize the morphological conservation of CNS and peripheral nervous system (PNS) myelination among gnathostomes with comparisons of images from developing and mature mammalian and nonmammalian species (Figures 2–4). Clearly, at both light and electron microscopic levels, multiple features, including selection of large-caliber axons, loose followed by tight wrapping, and development of nodal and paranodal specializations appear indistinguishable between these evolutionarily divergent species. At the time myelination begins in ventral spinal cord and medial longitudinal fascicle of elasmobranches (4–5 cm spiny dogfish fetuses) and mammals (newborn rats: Schwab and Schnell, 1989), one can clearly see that initial appearances of OLs and early myelin wrapping are quite similar (Figure 2). In both cases, early OLs hover around the edge of the tissue (Figures 2a and 2b, *). At the electron microscopic level, similar appearances of OL nuclei with chromatin clumped along the nuclear envelop and large regions of perinuclear cytoplasm filled with mitochondria, Golgi, rough and smooth endoplasmic reticulum, and numerous microtubules characteristic of myelinating mammalian OLs (Figure 2d) are also characteristic of myelinating spiny dogfish OLs. As development progresses in spiny dogfish ventral funiculi, the numbers of large

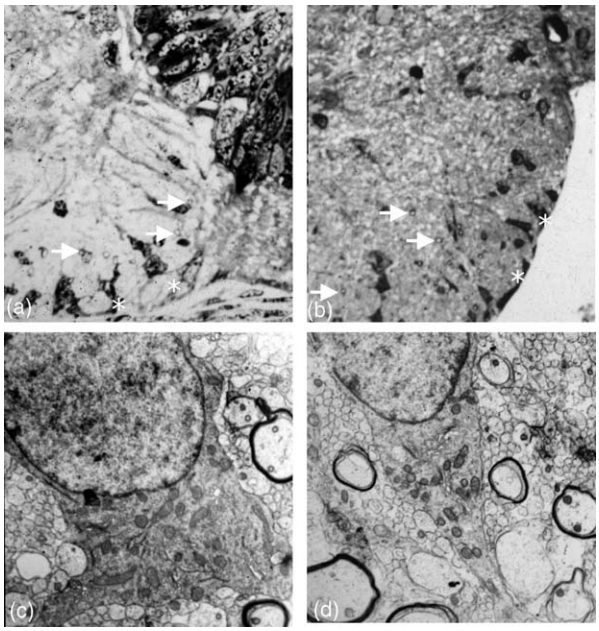

Figure 2 Transverse sections of developing ventral funiculi in spinal cords of spiny dogfish (left) and rat (right). a, From 4.5 cm spiny dogfish fetus showing the first appearances of OLs (small dark profiles lining the medial border) and initial ring-shaped myelin sheaths. b, From newborn rat pup showing similarly appearing early OLs (dark profiles) and beginning myelin sheaths. c, Electron micrograph from a 6 cm spiny dogfish fetus ventral spinal cord showing an OL (nucleus with clumped chromatin at upper left) and typical organelle-rich perinuclear cytoplasm with a few large-caliber axons in early stages of myelination. Many small unmyelinated axons also surround the OL. d, Electron micrograph from a P2 neonatal rat pup ventral spinal cord with a similar-appearing OL with surrounding large axons in early stages of myelination and clusters of many small-caliber unmyelinated axons. Arrows in (a) and (b) point to early myelin sheaths and asterisks (*) mark the tissue border. Adapted from Schweigreiter, R., Roots, B. I., Bandtlow, C. E., and Gould, R. M. 2006. Understanding myelination through studying its evolution. *Int. Rev. Neurobiol.* 73, 219–273, Elsevier.

myelinated fibers increase dramatically. When the fetus has more than doubled its size, large numbers of myelinated fibers and few OLs occupy a cross section of ventral cord (Figure 3a). Even though the ventricle seems filled with large-caliber myelinated fibers, there are still many very small fibers that are totally disregarded. At the electron microscopic level, these fibers are still bundled tightly together (Figure 3b). That these axons are eventually myelinated can be seen when a comparable region of ventral spinal cord is imaged in the adult spiny dogfish (Figure 3c). By this time, i.e., after several years, the entire funiculus is composed of myelinated fibers of widely different caliber. An evolutionary important lesson is that OLs show an absolute selectivity for those axons that are enlarging while

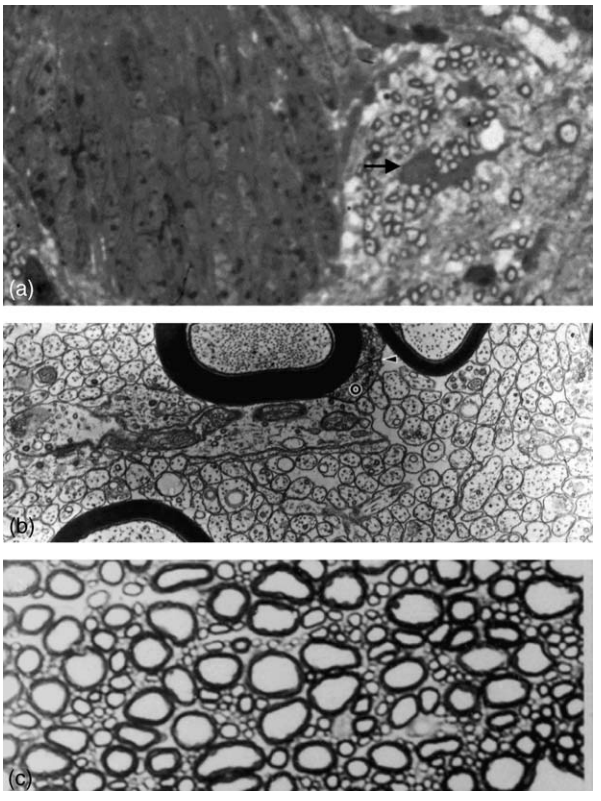

Figure 3 a, Transverse section from the ventral funiculus in the spinal cord of a 13 cm spiny dogfish, myelination. Now there are many myelinated fibers and a few OL profiles (arrow) per cross section. b, What is not appreciated is the very large number of small nonmyelinated fibers that are still present in the ventral spinal cord and can only be seen when viewed through the electron microscope. c, In the same region of the adult spiny dogfish spinal cord, axons of wide-ranging sizes are all myelinated.

disregarding small-caliber axons. Evidence is emerging as to signaling molecules important for this recognition (see following section).

As in the CNS, the Schwann cell-based PNS myelin is highly similar among vertebrates. Again, we will show examples from spiny dogfish to illustrate this point (Figure 4). We have followed the development of trigeminal nerve in spiny dogfish fetuses of 2–26 cm, the latter being the size at birth. In the smallest fetuses, bundles of axons are surrounded by an outer layer of immature Schwann cells, much like those seen in mice and rats (Figure 2; Jessen and Mirsky, 2005). When the fetuses reach 6 cm, nearly all the trigeminal axons are separated from all of their neighbors by Schwann cells (Figure 4A). A few have layers of noncompacted and compacted myelin (not shown). When the fetus reaches 13 cm in size, all of the axons are myelinated (Figure 4B). Myelin sheaths are related to Schwann cells in the same manner that has been described most frequently for the mammalian PNS (Peters *et al.*, 1991).

2.23.4 Biochemical and Molecular Features of Vertebrate Myelin Sheaths

Based on structural differences (high lipid content and large expanses of compact membrane layers), methods that separated mammalian brain and peripheral nerve myelin from other membranes were developed in the 1960s (Albers, 1981; Quarles *et al.*, 2005). Fortunately, these methods

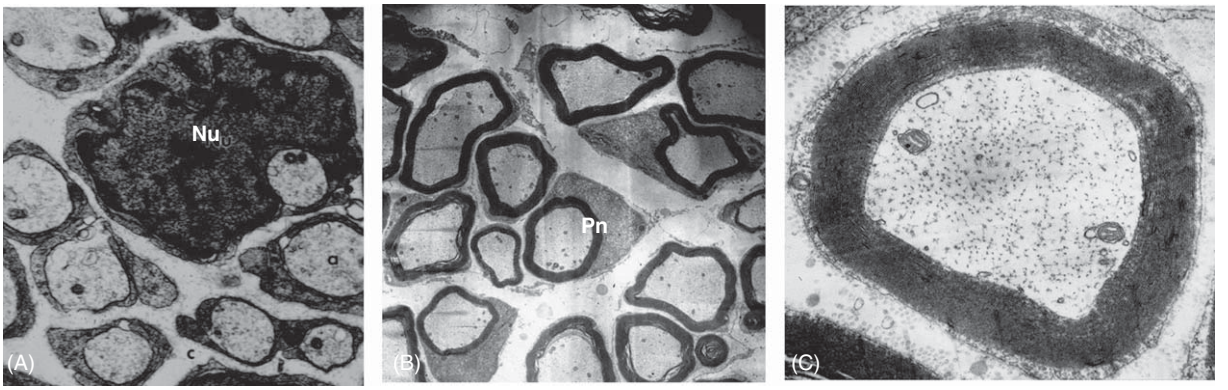

Figure 4 Transverse section of developing trigeminal nerve in spiny dogfish fetuses that were either 4.5 cm (A) or 22 cm (B and C). A, During early development, axons (a) reach a critical size (all axons in the field have reached this size) when they become separated from all neighboring axons and are covered by single Schwann cells segmentally arranged. A large nucleus (Nu) of one Schwann cell indicates that the section is taken at the point where the Schwann cell soma is found. Collagen-filled (c) extracellular space separates each individually ensheathed axon. B, At later developmental stages all larger trigeminal axons are myelinated with sheaths of thicknesses that relate to axon caliber. As at earlier times, axons sectioned near the center of the myelinating cell display nuclei and/or perinuclear (Pn) Schwann cell cytoplasm. C, At high magnification enrichment of neurofilaments and microtubules in the axon along with a few motochondria and smooth membranes are visible. A thin rim of organelle-filled Schwann cell cytoplasm surrounds compact myelin and a basal lamina covers the surface.

easily adapt to the isolation of myelin-like membranes from invertebrate neural tissues (see above) and from non-mammalian vertebrate neural tissues (Mehl and Halaris, 1970; Franz *et al.*, 1981; Waehneldt *et al.*, 1984). Analyses of myelin fractions from species representing each group of modern-day gnathostomes confirmed that, as in mammalian myelin preparations, they contain an abundance of lipids (70–80% of the isolated membrane fraction dry weight), including high portions of cholesterol, galactolipids, and ethanolamine plasmalogen (Bürgisser *et al.*, 1986; Kirschner and Blaurock, 1992), and a relatively simple protein profile (Waehneldt *et al.*, 1986a).

The major proteins in mammalian myelin, like those in invertebrate myelin, are of low molecular weight. In general antibodies recognizing mammalian myelin proteins recognize homologs in non-mammalian brain and nerve samples (Waehneldt *et al.*, 1986a). These studies revealed that all vertebrate myelin sheaths contain a dominant protein, either P_0 (one or two bands) or PLP (with a less abundant alternatively spliced variant, DM-20, absent only in amphibians; for review, see Waehneldt, 1990; Maisey, 1986). All peripheral nerve myelin, from elasmobranches to mammals, have P_0-based myelin sheaths. The difference between fish and tetrapods lies in CNS myelin. Cartilaginous and bony fishes express P_0, the same protein(s) they express in their PNS. In contrast, all tetrapod CNS myelin is PLP-based. This difference was first recognized through immunoblot comparisons of the proteins from fish and tetrapod myelin preparations (Franz *et al.*, 1981; Waehneldt *et al.*, 1985). This difference is apparent at the structural level as sheaths with P_0 have wider intraperiod lines, double in appearance. The difference is seen by both electron microscopy and X-ray diffraction (Kirschner *et al.*, 1984, 1989; Waehneldt *et al.*, 1984; Kirschner and Blaurock, 1992). Recent evidence that further supports the correlation of P_0 and wider intraperiod lines was obtained with transgenic mice with P_0 substituted for PLP (Yin *et al.*, 2006). A possible reason why the P_0-based CNS myelin of teleost and cartilaginous fishes evolved to PLP-based CNS myelin in amphibians and other tetrapods was recently suggested (Schweitzer *et al.*, 2006). Since the growth of the brain cases of tetrapods slows in adulthood, the tighter packing of a PLP-based CNS myelin would allow more myelin in a limited space.

Although PLP/DM-20 (fish do not have exon 3b, the PLP-specific exon; see below) and P_0 are co-expressed in amphibians (Takei and Uyemura, 1993; Yoshida and Colman, 1996) and teleost fishes (Schweitzer *et al.*, 2006), differences in intraperiod spacing (Kirschner and Blaurock, 1992) reflect the dominance of PLP in amphibian myelin and P_0 in fish myelin. P_0 is present at roughly 50% of total myelin protein, at least in mammalian PNS (Greenfield *et al.*, 1973), and additional information on properties of P_0 can be obtained in several reviews (Spiryda, 1998; Eichberg, 2002; Kirschner *et al.*, 2004). It is type I (single transmembrane) glycoprotein, a smallest member, which has a single immunoglobulin domain, of the immunoglobulin superfamily, and has HNK-1-reactive antigens in many species (Quarles, 2002). Other post-translational modifications include sulfation and fatty acylation. It is involved in adhesion at both the intraperiod and major dense lines (Kirschner *et al.*, 1996; Shapiro *et al.*, 1996). P_0 homologues were not detected in ascidians (Gould *et al.*, 2005), indicating that this protein likely evolved after the common gnathostome ancestor separated from the lineage leading to extant hemichordates.

PLP and DM-20 are tetraspan proteins of an ancient family, termed lipophilins (Gow, 1997; Hudson, 2004; Gould *et al.*, 2005; Schweitzer *et al.*, 2006), with both N- and C-terminal regions in the cytoplasm. Although PLP is restricted to tetrapod CNS myelin, DM-20-like proteins are present in teleost fish (Tohyama *et al.*, 1999, 2000; Brosamle and Halpern, 2002; Schweitzer *et al.*, 2006), elasmobranch (Kitagawa *et al.*, 1993; Sinoway *et al.*, 1994), and amphibians (Yoshida and Colman, 1996). The difference between PLP and DM-20 is a 35-amino-acid sequence (exon 3b), present in PLP, that lies in the cytoplasmic loop between the second and third transmembrane domain. There are two different proteins that resemble DM-20, called M6A and M6B. The latter proteins are expressed in neurons and probably to much lesser extents in glial cells (Yan *et al.*, 1996; Werner *et al.*, 2001). Homologues of these DM-20/M6a/M6b proteins have been identified in insects (Stecca *et al.*, 2000; Schweitzer *et al.*, 2006) and basal chordates (Gould *et al.*, 2005; Schweitzer *et al.*, 2006) based on database screening. High conservation of their structure and genomic organization indicates a widespread evolutionary importance of this protein family. The high number of PLP/DM-20 sequences has been used as one base for determining phylogenetic relationships among vertebrate groups (Tohyama *et al.*, 2000; Venkatesh *et al.*, 2001). Another less abundant tetraspan protein of mammalian PNS myelin is PMP22, a protein studied in part because mutations are related to peripheral nerve diseases (Suter and Scherer, 2003; Young and Suter, 2003; Suter, 2004). It is found in zebra fish (Wulf *et al.*, 1999)

and a homologue was identified in ascidian (Gould *et al.*, 2005), indicating that several tetraspan proteins, lipophilins, PMP22/claudins, and connexins may have been used for the original development of myelin sheaths.

MBP, a family of alternatively spliced members, is present in all CNS and PNS myelin sheaths (Campagnoni, 1988; Martenson and Uyemura, 1992) and, because of its recognized importance, has been termed the executive myelin protein (Moscarello, 1997). MBP is a member of the intrinsically disordered, highly adapted proteins (Harauz *et al.*, 2004). Perhaps with the openness of structure comes an attraction for post-translational modifications, which are numerous (Moscarello, 1997; Harauz *et al.*, 2004). The only study of post-translational modification of MBP in a nonmammal was conducted with MBP isolated from spiny dogfish brain and spinal cord. Modifications include phosphorylation, deamidation, and likely deimidation and praline hydroxylation. Clearly studies of MBP post-translational modification in other nonmammalian species will help clarify our emerging understanding of the roles these proteins play in glial cell development and myelination. Classic MBPs, the series of alternatively spliced proteins present in myelin, are members of a larger family of genes of OL lineage (GOLLI) that include four additional proteins transcribed from two additional upstream promoters (Campagnoni and Campagnoni, 2004). MBPs are needed for major dense line (extrusion of intervening myelin), as mutant mice (shiverer and mld) and rats (Long Evans shaker) that lack MBP have little myelin and, when it occurs, it is poorly compacted and contains an abundance of trapped cytoplasm (Privat *et al.*, 1979; Ganser and Kirschner, 1980; Kwiecien *et al.*, 1998). Furthermore, mammalian MBP isoforms that contain exon-2 are expressed developmentally early (Barbarese *et al.*, 1978), are enriched in radial component (Karthigasan *et al.*, 1996), a structure unique to CNS myelin (see below) and are targeted to nuclei where they may function in transcriptional regulation (Pedraza *et al.*, 1997). Interestingly, most likely the sequence for exon-2 does not occur in teleost fishes or amphibians based on analyses of the *Xenopus*, zebra fish and pufferfish databases (Gould, unpublished observations). Furthermore, only a single MBP homologue was identified in zebra fish (Brosamle and Halpern, 2002; Lyons *et al.*, 2005). Two alternative splice variants have been reported in elasmobranches, though both lack exon-2 (Saavedra *et al.*, 1989; Spivack *et al.*, 1993). cDNA sequences in the pufferfish database suggest some alternative splicing occurs there as well. Analysis of differences of MBP sequences can shed light on regions of the protein that might be used for signaling and for myelin compaction. A triproline region in mammalian and amphibian MBPs, thought to be structurally important in forming a hairpin loop, is absent in fish MBPs. No MBP homologues were detected in searches of the ascidian genome database (Gould *et al.*, 2005), indicating that, like P_0, MBP developed late in evolution.

In addition to P_0, MBP, and PLP/DM-20, there are a growing number of proteins associated with myelin sheaths proper and the process of myelination, based on comparison of earlier (Braun, 1984; Campagnoni, 1988) and later (Lazzarini *et al.*, 2004) reviews. Evolutionary studies of myelin proteins occurred at the time when the major structural proteins in isolated myelin were the focus (Waehneldt *et al.*, 1986b; Waehneldt, 1990; Jeserich and Waehneldt-Kreysing, 1992; Kirschner and Blaurock, 1992). Two minor-occurring proteins, CNP (Kurihara and Tsukada, 1967; Tsukada and Kurihara, 1992) and MAG (Everly *et al.*, 1973), have long histories as myelin-related proteins. Immunocytochemical studies have demonstrated that they are located near, but not within, compact myelin (Trapp *et al.*, 1988, 1989). CNP is present in bird and amphibian myelin (Kasama-Yoshida *et al.*, 1997), though not in fish myelin (Kasama-Yoshida *et al.*, 1997; Moll *et al.*, 2003). A related protein, goldfish regeneration-induced CNPase homologue (gRICH) that lacks CNPase activity, has been identified in goldfish (Ballestero *et al.*, 1995, 1997, 1999). A comprehensive review on CNP (Braun *et al.*, 2004) can be consulted for additional information. Furthermore, a recent study of transgenic mice lacking CNP expression suggests a crucial role for this protein in axon–glial cell communication (Lappe-Siefke *et al.*, 2003). Antibody-based evidence that MAG is ubiquitously expressed (Matthieu *et al.*, 1986) had been disputed (Zand *et al.*, 1991; Hammer *et al.*, 1993) and the issue was not resolved until recent screening of pufferfish and zebra fish genomes identified several MAG, also called siglec 4 (sialic acid-binding protein), isoforms in these fish (Lehmann *et al.*, 2004). Finding fish homologues of MAG, a protein associated with inhibition of myelin regeneration in the mammalian CNS (Filbin, 1995), will clearly impact efforts to understand the evolutionary loss of CNS regeneration. Another minor-occurring protein restricted to PNS myelin is PMP22, a protein involved in the process of myelin sheath formation (see above; Suter, 2004; Amici *et al.*, 2006), present in amphibians (*Xenopus* genome project) and fish (Wulf

et al., 1999), and which has a homologue in the ascidian, *Ciona intestinalis* (Gould *et al.*, 2005).

Within compact CNS myelin are radial bands, structures seen with electron microscopy (Peters, 1961). Although not studied widely, they are also found in amphibian CNS myelin (Schnapp and Mugnaini, 1976; Tabira *et al.*, 1978), though not any PNS myelin (Kosaras and Kirschner, 1990). Interestingly, the key structural proteins of radial bands, exon-2-containing MBP isoforms (Karthigasan *et al.*, 1996) and myelin-associated oligodendrocytic basic proteins (MOBP) (Yamamoto *et al.*, 1999), have not been identified in amphibians and other nonmammalian species.

Nodes of Ranvier, regions between internodal myelin and bracketed by paranodal loops, contain high concentrations of sodium channels and other proteins that are delivered to these sites via axonal transport (Poliak and Peles, 2003; Salzer, 2003). In the PNS, they are covered by microvillar extensions of adjacent Schwann cells. In the CNS, they are covered by a totally different cell, which is related to CNS astrocytes (ffrench-Constant *et al.*, 1986; Black *et al.*, 1989; Butt *et al.*, 1999) and expresses a proteoglycan, NG2 (Butt *et al.*, 1999; Martin *et al.*, 2001), that is well associated with the OL lineage (Dawson *et al.*, 2000). Almost nothing is known about the structural properties of nonmammalian proteins located in nodes of Ranvier and surrounding paranodes and juxtaparanodes. From comparative studies of proteins present in compact myelin, it is likely that antibodies to mammalian nodal, paranodal, and juxtanodal proteins will identify nonmammalian homologues and it would be rather straightforward to determine whether proteins involved in node and paranode formation and function behave the same in nonmammalian settings. Interestingly, some of the key components of paranodal junctions, contactin, and neurexin family proteins are used in glial ensheathment in insects (Bhat, 2003; Banerjee *et al.*, 2006).

A totally unrelated area that has not been considered from an evolutionary viewpoint is the origin of myelin-forming Schwann cells and OLs. The few studies that have been done with chickens and frogs are enmeshed in studies of mammals without due recognition of evolutionary significance (Richardson *et al.*, 2000, 2006; Qi *et al.*, 2002; Miller and Reynolds, 2004; Noble *et al.*, 2004; Rowitch, 2004; Jessen and Mirsky, 2005). A few studies have taken advantage of zebra fish with respect to understanding roles of olig family helix–loop–helix transcription factors (Park *et al.*, 2002; Shin *et al.*, 2003; Filippi *et al.*, 2005). Hopefully, the future will include a re-assessment of these studies in the light of evolution.

In addition a number of screens have emerged, including ones that identify proteins: (1) synthesized from mRNAs transported along with MBP mRNA to OL processes (Gould *et al.*, 2000); (2) essential for the development of myelin phenotypes in zebra fish, including transport of MBP mRNA to processes (Lyons *et al.*, 2005) and clustering of sodium channels at nodes (Talbot, personal communication); and (3) based on the presence of a signal sequence that targets Schwann cell proteins to plasma membranes, where they may be involved in interactions with axons and/or the basal lamina. The first screen has been applied to spiny dogfish and shows that some OL process mRNAs, though surprisingly, not MBP mRNA, are common to both fish and mammals (Gould, unpublished observations).

Studies of protein evolution are clearly impacting research on CNS myelin proteins with inhibitory influences on regeneration (Diekmann *et al.*, 2005; Schweigreiter *et al.*, 2006). Briefly, what these and others are doing is characterizing proteins, expressed in CNS myelin and/or OLs that inhibit regrowth of damaged CNS axons. It is well known that fish, some amphibians, chicks, and some mammals (Gaze, 1970; Stuermer *et al.*, 1992; Ferretti *et al.*, 2003) – the latter at premyelination stages only – are able to regenerate CNS axons following injury. Although it is not entirely clear why the ability to regenerate CNS axons is lost in birds and mammals following myelination, the presence of candidate proteins, including NOGO/RTN isoforms (Cadelli and Schwab, 1991; Chen *et al.*, 2000; GrandPré *et al.*, 2000; Prinjha *et al.*, 2000), NOGO/RTN receptor (Fournier *et al.*, 2001), MAG, and OL myelin glycoprotein (OMgp) (Spencer *et al.*, 2003; Woolf, 2003; Raisman, 2004; Sandvig *et al.*, 2004; Schwab, 2004; Domeniconi and Filbin, 2005; Schweigreiter *et al.*, 2006) suggests that there was a need to inhibit axon growth/elongation in regions (myelin-rich) where these proteins are located. With the identification of some of these molecules in fish (see above references), it will be possible to compare structural features of the proteins and expression patterns as a means to understand how they might selectively inhibit mammalian CNS regeneration.

Another important area that has not been considered from an evolutionary point of view is the axon–glial cell cross-talk involved in different stages of myelination. With rapid progress in mammalian systems, this is another area that should receive attention from evolutionary myelin biologists. Only when axons reach appropriate size, i.e., develop unique surface character, such as expressing sufficient amounts of neuregulin-1 type III on their surface, will OLs (Sherman and Brophy, 2005) and Schwann cells (Nave and Schwab, 2005; Sherman

and Brophy, 2005; Taveggia *et al.*, 2005) myelinate them. Furthermore, myelin thickness, at least in peripheral nerves, is dependent on the same neuregulin-erbB receptor signaling (Michailov *et al.*, 2004; Nave and Schwab, 2005). With knowledge of developmental time course of myelination in nonmammals, including birds (Ono *et al.*, 1995), reptiles (Nadon *et al.*, 1995), amphibians (Tabira *et al.*, 1978), teleost (Jeserich and Rauen, 1990), and cartilaginous fishes (Gould *et al.*, 1995), investigators will find ways of approaching evolutionary aspects of axon–glial cell interactions that are important for myelination.

2.23.5 Summary and Conclusions

One might wonder why, with the rapid progress in mammalian cell biology and molecular biology, the availability of many mammalian mutants with defects in myelination, and the knockout and knockin strategies to influence myelin-related proteins of interest, one should devote and, probably more importantly, fund efforts to study myelination in nonmammalian species. From the information already presented, it is clear that there has been an evolutionary driving force for generating myelinated axons that includes not only all gnathostomes, but also many invertebrates. Particularly in invertebrates, a variety of strategies have been developed and, clearly, knowledge of these strategies will provide a broader base for understanding how interactions between neurons, their large-caliber axons, and the glial cells with which they interact were focused on myelination. Since none of the invertebrates with myelinated axons have been or (to our knowledge) are planned to be subjects of whole-genome studies, the best alternative to obtain sequence information on myelin proteins and proteins important for myelination would be through proteomic methods. It is our hope that investigators with available knowledge and facilities will consider earthworms, shrimps, and/or other annelids/crustaceans, obtain sequences of proteins present in myelin-like membranes, and use this knowledge as a starting point for developing understanding of how the proteins participate in neuron/axon-generated myelin formation.

For those interested in using nonmammalian vertebrate models, tremendous advances in genomic research involving zebra fish (Key and Devine, 2003), medaka (Shima and Mitani, 2004), two species of pufferfish (Aparicio *et al.*, 2002; Jaillon *et al.*, 2004), amphibians (Hirsch *et al.*, 2002), chordates lacking myelin (amphioxus and sea lamprey) (Mulley and Holland, 2004), and urochordates (Dehal *et al.*, 2002; Meinertzhagen *et al.*, 2004) have been made. This is an exciting time. Obtaining protein sequences in published databases is relatively easy and straightforward (Brosamle and Halpern, 2002; Lehmann *et al.*, 2004). These groups identified zebra fish, and in some instances pufferfish, MBP, PLP-related DM-20, P_0, and MAG. Protein sequence information can be used in many ways to advance myelin research. Because of evolutionary pressures, domains in protein sequences important for function are most highly conserved and sequence alignments can be used to determine these domains and searches can be performed to see if these domains have orthologues and/or paralogues. Overall this information should help in supporting and/or negating structural models of known myelin proteins (see Shapiro *et al.*, 1996; Spiryda, 1998; Kirschner *et al.*, 2004; for discussions on P_0 structure and Martenson, 1992; for discussions on MBP structure). Clearly, the studies covered in this brief review are not comprehensive and we apologize to scientists whose research efforts and relevant studies were not cited.

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2.24 Adult Neurogenesis and Neuronal Regeneration in the Teleost Fish Brain: Implications for the Evolution of a Primitive Vertebrate Trait

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Glossary

adult neurogenesis	The generation of new neurons during adult stages of development.
hyperplasia	Growth of a tissue or an organ by an increase in the number of cells.
hypertrophy	Enlargement of a tissue or an organ as a result of an increase in size rather than the number of constituent cells.
neuronal regeneration	The replacement of neurons lost to injury or neurodegenerative diseases by newly generated ones.

2.24.1 Introduction

In mammals, the potential to generate new neurons in the intact brain, or to replace neurons lost to injury or neurodegenerative disease, is severely limited. The former phenomenon, called adult neurogenesis, is restricted to two brain regions: the anterior part of the subventricular zone of the lateral ventricle, from where the immature neurons migrate via the so-called rostral migratory stream into the olfactory bulb (Altman, 1969; Luskin, 1993; Lois and Alvarez-Buylla, 1994; Lois *et al.*, 1996; Pencea *et al.*, 2001) and the subgranular zone of the dentate gyrus, from where the new cells migrate a short distance into the granule cell layer of the hippocampus (Altman and Das, 1965; Kaplan and Bell, 1984; Eriksson *et al.*, 1998; Gould *et al.*, 1999; Kornack and Rakic, 1999; Seri *et al.*, 2001).

However, neurogenic potential also persists in several other regions of the brain beyond embryonic stages of development. From these regions, cells have been isolated that are quiescent *in vivo*, but

proliferate and develop into neurons after treatment with proper exogenous factors *in vitro*, or after transplantation into the olfactory bulb or the hippocampus (Reynolds and Weiss, 1992; Suhonen *et al.*, 1996; Weiss *et al.*, 1996; Palmer *et al.*, 1999; Kondo and Raff, 2000; Shihabuddin *et al.*, 2000; see The Evolution of Arthropod Nervous Systems: Insights from Neural Development in the Onychophora and Myriapoda).

This neurogenic potential in the intact brain is closely linked to the ability to replace damaged neurons by newly generated ones, a phenomenon commonly referred to as neuronal regeneration. Increased proliferation of progenitor cells in the subgranular and subventricular zones, and even of quiescent neural progenitor cells in regions where adult neurogenesis is absent in the intact brain, has been observed after brain injuries (Magavi *et al.*, 2000; Chen *et al.*, 2004). However, such attempts of the adult mammalian brain to regenerate fall short because of the failure of the new cells to develop further and/or to survive for sufficiently long periods of time. After experimentally induced stroke, immature neurons born in the subventricular zone migrate to the striatal area damaged through the ischemic insult. Although these cells start to express markers for striatal medium-sized spiny neurons – the phenotype most severely affected by the insult – the majority of them die within a few weeks after the stroke (Arvidsson *et al.*, 2002). Most significantly, the total number of new neuronal cells is far too low to replace the degenerated neurons. In the latter study, the number of new neurons totaled just 1600, a rather insignificant number compared to the estimated 80 000 degenerated cells.

By contrast, teleost fish exhibit an enormous potential both for adult neurogenesis and for neuronal regeneration (for reviews, see Zupanc, 1999a, 2001, 2006). New neurons are continuously produced in dozens of regions of the adult brain, and their total number is at least one, if not two orders of magnitude larger than in the adult mammalian brain. In addition, teleost fish are capable of regenerating neural tissue after injury rapidly and with high efficiency. This advantage over mammals makes it particularly interesting to study adult neurogenesis and neuronal regeneration in teleosts. Elucidation of the cellular mechanisms mediating these phenomena in fish will not only answer fundamental biological questions, but is also likely to provide important insights into the factors that limit the generation of new neurons in the adult mammalian brain. Such investigations could, therefore, form the basis to define novel strategies for wound healing and treatment of neurodegenerative diseases.

2.24.2 Approaches to Study Cell Proliferation in the Adult Brain

Studies of cell proliferation in the adult brain have been based on the incorporation of [^{3}H]thymidine or the thymidine analogue 5-bromo-2′-deoxyuridine (BrdU) into newly synthesized DNA during the S-phase of mitosis. Following a specific survival time after the systemic administration, cells that have taken up [^{3}H]thymidine or BrdU are detected through autoradiography or anti-BrdU immunohistochemistry, respectively (Figure 1). The length of the postadministration survival time is determined by the aims of the investigation. Short survival times – typically 1–2 h – are employed to provide information on the sites where cells proliferate. Longer survival times – typically days, weeks, or even

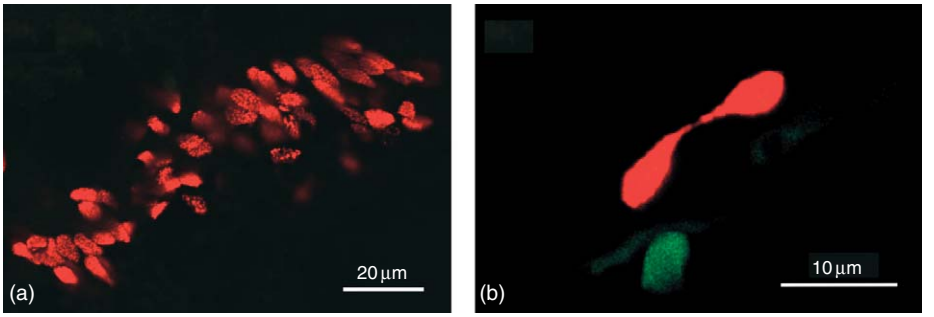


Figure 1 Confocal images of BrdU-labeled cells in the adult zebra fish brain at 2 days of survival after intraperitoneal injection of BrdU. a, Proliferation zone in periventricular gray zone of tectum opticum near its caudal end, revealed by labeling of mitotic cells with BrdU. In many of the labeled nuclei, a subdivision into labeled granules, probably corresponding to different domains of chromatin, is apparent. b, BrdU-labeled cell in ventral molecular layer of valvula cerebelli pars medialis, in the immediate vicinity of rhombencephalic ventricle. Evidently, the cell is in late telophase, as the two daughter nuclei are connected only by a thin string of labeled nuclear material. From Zupanc, G. K. H., Hinsch, K., and Gage, F. H. 2005. Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain. *J. Comp. Neurol.* 488, 290–319.

months – reveal details about the fate of the cells in S-phase at the time of the administration of [^{3}H]thymidine or BrdU. Comparison of the labeling pattern obtained after various survival times is used to reconstruct the path of migration of the young cells from their sites of proliferation to their final target site. Combination of [^{3}H]thymidine autoradiography or anti-BrdU immunohistochemistry with other methods, such as immunohistochemistry for neuron- or glia-specific markers, has proven a powerful approach to obtain information about the differentiation of the newly generated cells.

Quantitative analysis in the gymnotiform fish *Apteronotus leptorhynchus* – one of the major model systems in this area of research – has demonstrated that BrdU is metabolically available to label S-phase cells for approximately 4 h after a single intraperitoneal injection (Zupanc and Horschke, 1995). On the basis of this information, a survival time of 2 h was chosen for mapping of the proliferation zones and quantitative analysis of the rate of cell proliferation in the adult teleostean brain (Zupanc and Horschke, 1995; Zupanc *et al.*, 2005). Thus, BrdU was metabolically available for incorporation into newly synthesized DNA during the entire survival period.

2.24.3 Pattern of Cell Proliferation

Experiments employing post-BrdU-administration survival times of 2 h in *A. leptorhynchus* have shown that, on average, during any 2 h period, approximately 100 000 cells enter the S-phase of mitosis in the whole brain (Zupanc and Horschke, 1995). This corresponds to roughly 0.2% of the total number of cells in the adult brain of *A. leptorhynchus*. Approximately 25% of these new cells are generated in the telencephalon, diencephalon, mesencephalon, and rhombencephalon. The remaining 75% originate from the cerebellum.

In comparison to the enormous mitotic activity in the adult fish brain, the rate of cell proliferation in the adult mammalian brain is at least one, if not two, orders of magnitude lower. Louis and Alvarez-Buylla have estimated that, in adult mice, approximately 30 000 cells a day are formed in the subventricular zone, one of the two sites of ongoing neurogenesis in the adult mammalian brain (Louis and Alvarez-Buylla, 1994). This number corresponds to 0.03% of the estimated 110 million cells (Williams, 2000) in the whole brain of adult mice. In the dentate gyrus of the hippocampus – the second site where neurogenesis continues into adulthood in the mammalian brain – the production of only 9000 cells a day has been found in the adult rat (Cameron and McKay, 2001). The latter number corresponds to 0.003% of the approximately 330 million cells (Herculano-Houzel and Lent, 2005) in the whole brain of the adult rat.

2.24.4 Proliferation Zones in the Adult Fish Brain

Like in mammals, in the adult brain of *A. leptorhynchus* and other teleosts, the vast majority of the mitotic cells are found, at high concentrations, in small, well-defined areas of the brain (proliferation zones). Many of these zones are situated at or near the surfaces of ventricles or related systems. Although other zones, particularly in the cerebellum, are located in regions distant from any ventricle (see Section 2.24.4.4 below), many are derived from areas located at ventricular surfaces during embryonic stages of development. Then, as a result of the everted development of the fish brain during embryogenesis, the associated ventricular lumina are obliterated and translocated (Pouwels, 1978a, 1978b). In *A. leptorhynchus*, several dozen proliferation zones have been identified in the adult brain (Zupanc and Horschke, 1995; Figures 2 and 3). A similar large number has been found in two other teleostean species in which a detailed mapping of such zones has been performed – the three-spined stickleback (Ekström *et al.*, 2001) and the zebra fish (Zupanc *et al.*, 2005; Figures 4 and 5).

In the following, I will describe and discuss the pattern of cell proliferation in four regions of the teleostean brain that are of special interest from a comparative point of view: olfactory bulb, dorsal telencephalon, optic tectum, and cerebellum.

2.24.4.1 Olfactory Bulb

Quantitative analysis of BrdU labeling has shown that approximately 0.2% of all mitotic cells in the adult brain of *A. leptorhynchus* are found in the olfactory bulb. Most of these cells are spread over the external (glomerular) layer, whereas only a few mitotic cells are located in the internal cell layer (Zupanc and Horschke, 1995). Similar results have been obtained in other teleosts, including goldfish, Mediterranean barbel, carp, rainbow trout, and zebra fish (Alonso *et al.*, 1989; Byrd and Brunjes, 2001; Zupanc *et al.*, 2005). In the latter species, double-labeling experiments have revealed expression of the neuron-specific protein Hu by BrdU-labeled cells after longer survival times, indicating neuronal differentiation of the new cells (Byrd and Brunjes, 2001; Zupanc *et al.*, 2005).

Despite the low number of cells produced, neurogenesis in the olfactory bulb of teleosts is

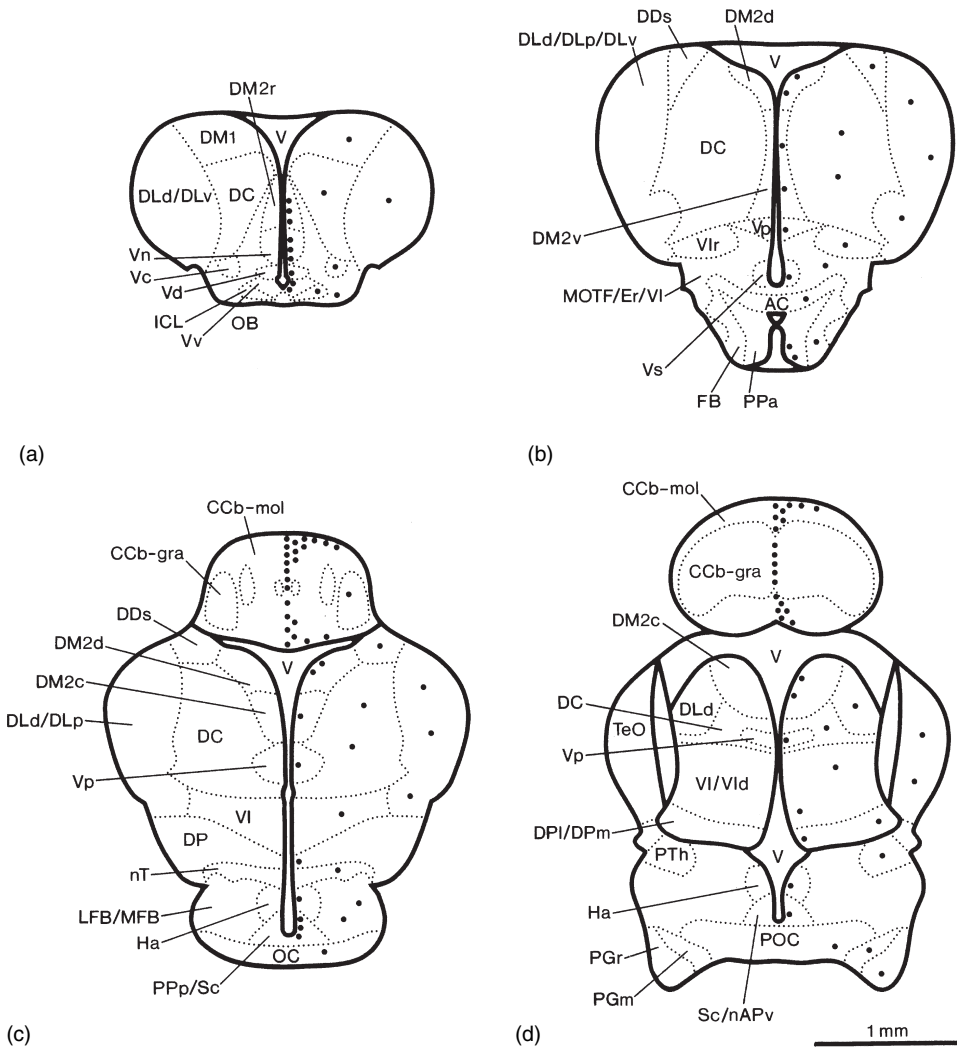


Figure 2 Proliferation zones in the telencephalon of adult *A. leptorhynchus*. The charts of transverse sections roughly correspond to levels 37 (a), 30 (b), 28 (c), and 26 (d) of the brain atlas of *A. leptorhynchus* (Maler *et al.*, 1991). Proliferation zones, as revealed by incorporation of BrdU into replicating DNA and subsequent anti-BrdU immunohistochemistry, are indicated by black dots. AC, anterior commissure; CCb, corpus cerebelli; DC, central division of the dorsal forebrain; DDs, superficial subdivision of dorsal division of the dorsal forebrain; DLd, dorsolateral telencephalon, dorsal subdivision; DLp, dorsolateral telencephalon, posterior subdivision; DLv, dorsolateral telencephalon, ventral subdivision; DM1, dorsomedial telencephalon, subdivision 1; DM2c, dorsomedial telencephalon, subdivision 2, caudal; DM2d, dorsomedial telencephalon, subdivision 2, dorsal; DM2r, dorsomedial telencephalon, subdivision 2, rostral; DM2v, dorsomedial telencephalon, subdivision 2, ventral; DP, dorsal posterior telencephalon; DPl, lateral subdivision of caudal dorsal posterior telencephalon; DPm, medial subdivision of caudal dorsal posterior telencephelon; Er, rostral entopeduncular nucleus; FB, forebrain bundle; gra, granule cell layer; Ha, hypothalamus anterioris; ICL, internal cell layer of olfactory bulb; LFB, lateral forebrain bundle; MFB, medial forebrain bundle; mol, molecular layer; MOTF, medial olfactory terminal field; nAPv, nucleus anterior periventricularis; nT, nucleus taenia; OB, olfactory bulb; OC, optic chiasma; PGm, preglomerular nucleus, medial subdivision; PGr, preglomerular nucleus, rostral subdivision; POC, postoptic commissure; PPa, nucleus preopticus periventricularis, anterior subdivision; PPp, nucleus preopticus periventricularis, posterior subdivision; PTh, nucleus prethalamicus; Sc, suprachiasmatic nucleus; TeO, optic tectum; V, ventricle; Vc, ventral telencephalon, central subdivision; Vd, ventral telencephalon, dorsal subdivision; Vl, ventral telencephalon, intermediate subdivision; Vld, ventral telencephalon, intermediate dorsal subdivision; Vlr, ventral telencephalon, intermediate rostral subdivision; Vl, ventral telencephalon, lateral subdivision; Vn, ventral telencephalon, nother subdivision; Vp, ventral telencephalon, posterior subdivision; Vs, ventral telencephalon, supracommissural subdivision; Vv, ventral telencephalon, ventral subdivision. From Zupanc, G. K. H. and Horschke, I. 1995. Proliferation zones in the brain of adult gymnotiform fish: A quantitative mapping study. *J. Comp. Neurol.* 353, 213–233.

interesting because the bulb is one of the two brain regions in which adult neurogenesis has been consistently demonstrated in mammals. Investigations in the latter taxonomic class have shown that progenitor cells of these neurons undergo cell division in the anterior part of the subventricular zone surrounding the lateral ventricles. From this proliferation zone, the neuroblasts

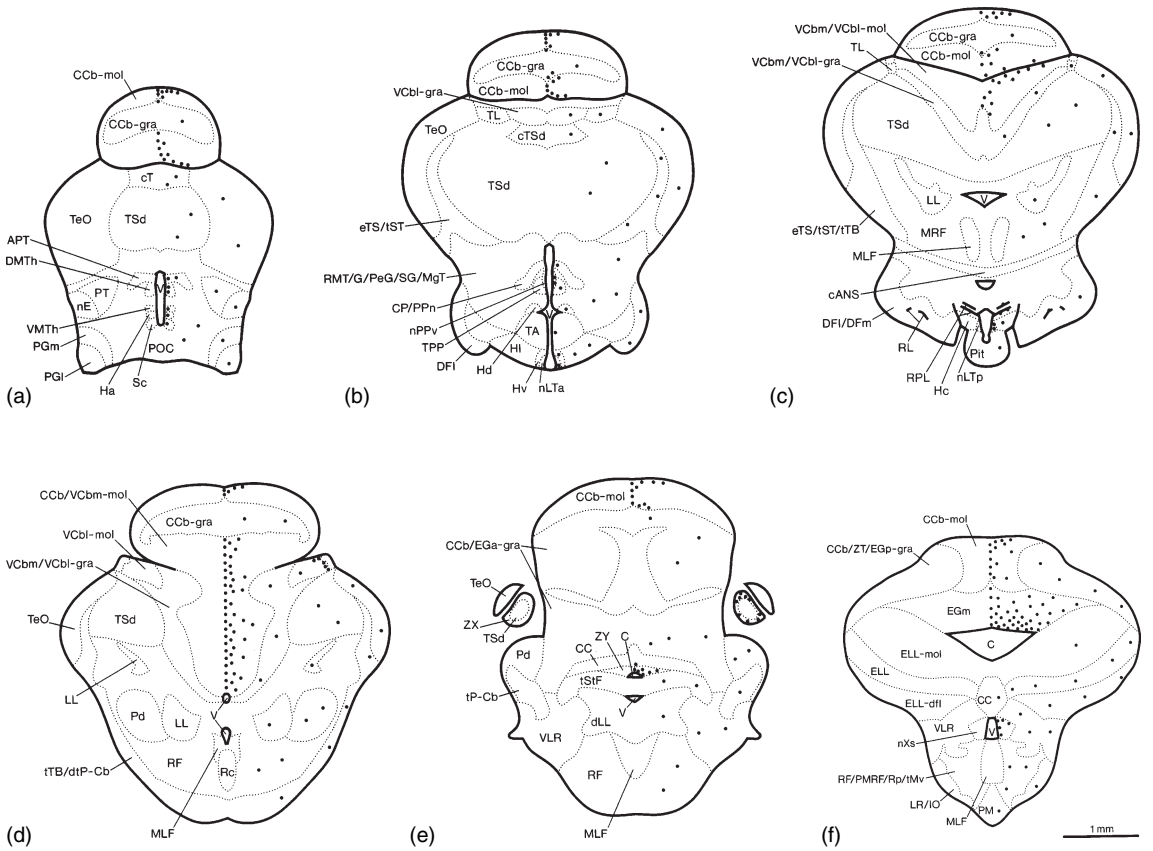
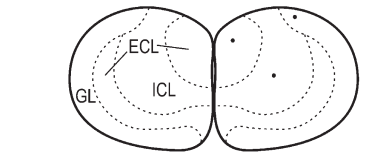


Figure 3 Proliferation zones in the diencephalon, mesencephalon, and rhombencephalon of adult *A. leptorhynchus*. The charts of transverse sections roughly correspond to levels 23 (a), 17 (b), 13 (c), 7 (d), 2 (e), and 8 (f) of the brain atlas of *A. leptorhynchus* (Maler *et al.*, 1991). Proliferation zones were revealed through labeling of mitotic cells with BrdU and are indicated by black dots. APT, area pretectalis; C, cerebellomedullary cistern; cANS, commissura ansulata; CC, crista cerebellaris; CCb, corpus cerebelli; CP, central posterior nucleus; cT, tectal commissure; cTSd, commissure of the torus semicircularis dorsalis; dfl, deep fiber layer of ELL; DFl, nucleus diffusus lateralis of the inferior lobe; DFm, nucleus diffusus medialis of the inferior lobe; dLL, decussation of the lateral lemniscus; DMTh, dorsomedial thalamus; dtP-Cb, decussation of tractus praeminentialis cerebellaris; EGa, eminentia granularis pars anterior; EGm, eminentia granularis pars medialis; EGp, eminentia granularis pars posterior; ELL, electrosensory lateral line lobe; eTS, torus semicircularis efferents; G, glomerular nucleus; gra, granule cell layer; Ha, hypothalamus anterioris; Hc, hypothalamus caudalis; Hd, hypothalamus dorsalis; Hl, hypothalamus lateralis; Hv, hypothalamus ventralis; IO, inferior ovary nucleus; LL, lateral lemniscus; LR, lateral reticular nucleus; MgT, magnocellular tegmental nucleus; MLF, medial longitudinal fasciculus; mol, molecular layer; MRF, mesencephalic reticular formation; nE, nucleus electrosensorius; nLTa, nucleus tuberis lateralis pars anterior; nLTp, nucleus tuberis lateralis pars posterior; nPPv, nucleus posterioris periventricularis; nXs, vagal sensory nucleus; Pd, nucleus praeminentialis dorsalis; PeG, periglomerular nucleus; PGl, preglomerular nucleus, lateral subdivision; PGm, preglomerular nucleus, medial subdivision; Pit, pituitary; PM, pacemaker nucleus; PMRF, paramedian reticular formation; POC, postoptic commissure; PPn, prepacemaker nucleus; PT, pretectal nucleus; Rc, nucleus raphé centralis; RF, reticular formation; RL, recessus lateralis; RMT, rostral mesencephalic tegmental nucleus; Rp, nucleus raphé posterioris; RPL, recessus posterioris pars lateralis; Sc, suprachiasmatic nucleus; SG, subglomerular nucleus; TA, nucleus tuberis anterior; TeO, optic tectum; TL, torus longitudinalis; tMv, ventral medullary tract; tP-Cb, tractus praeminentialis cerebellaris; TPP, periventricular nucleus of the posterior tuberculum; TSd, torus semicircularis, dorsal subdivision; tST, subtectal tract; tStF, tractus stratum fibrosum; tTB, tectobulbar tract; V, ventricle; VCbl, valvula cerebelli pars lateralis; VCbm, valvula cerebelli pars medialis; VLR, ventrolateral rhombencephalon; VMTh, ventromedial thalamus; ZT, transitional zone; ZX, zone X; ZY, zone Y. From Zupanc, G. K. H. and Horschke, I. 1995. Proliferation zones in the brain of adult gymnotiform fish: A quantitative mapping study. *J. Comp. Neurol.* 353, 213–233.

migrate, as chains of closely apposed cells, over several millimeters along a specific pathway – the rostral migratory stream – to the olfactory bulb, where they differentiate predominantly into granule neurons and, to a lesser extent, periglomerular interneurons (Altman, 1969; Luskin, 1993; Lois and Alvarez-Buylla, 1994; Lois *et al.*, 1996; Pencea *et al.*, 2001).

2.24.4.2 Dorsal Telencephalon

Among all telencephalic areas, the number of mitotic cells is largest in the dorsal, ventral, and posterior



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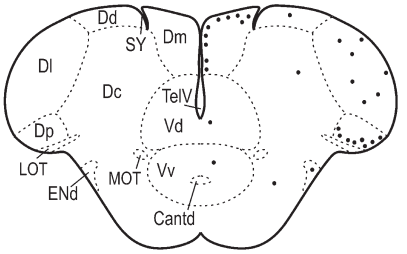

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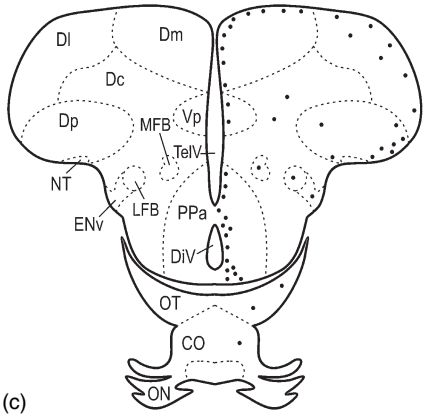

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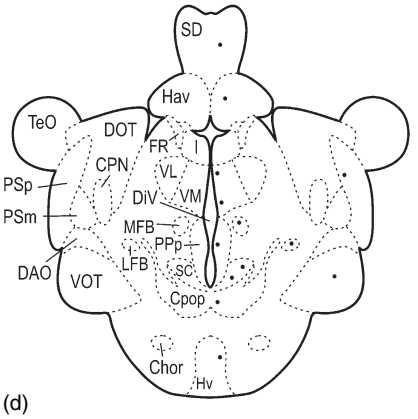

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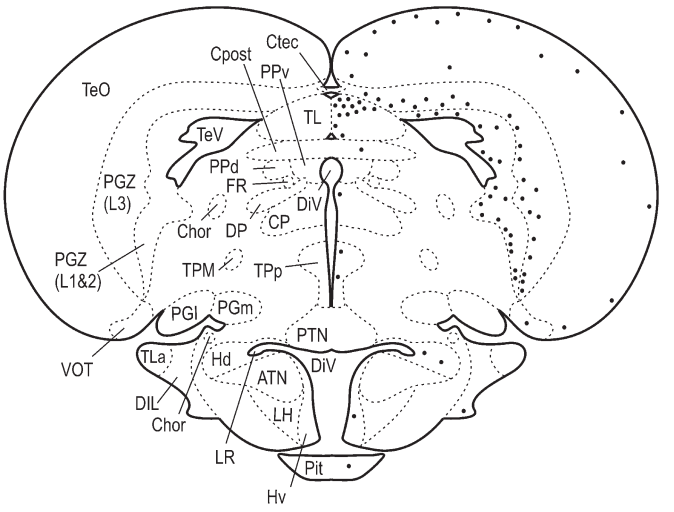

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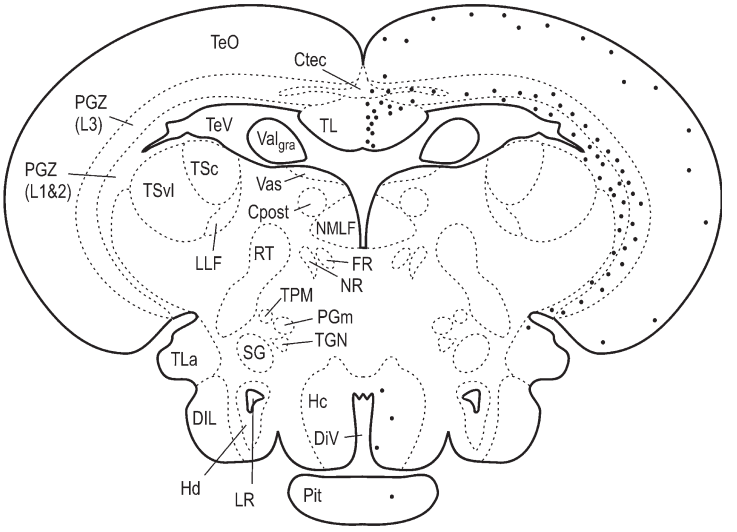

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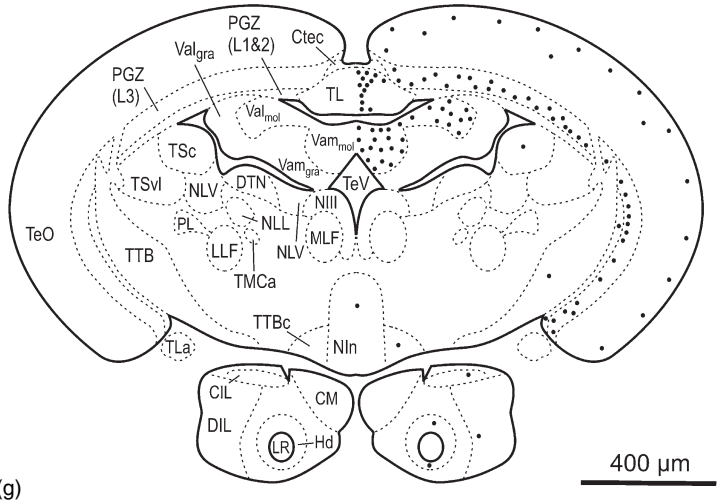

(g)

(*Continued*)

Figure 4 (Continued) Proliferation zones in rostral divisions of the brain of adult zebra fish. The presence of these zones has been revealed by analysis of BrdU labeling in transverse brain sections. These sections were obtained through intraperitoneal injection of BrdU, followed by a postadministration survival time of 2 h. Areas of mitotic activity are indicated by black dots. Their number correlates with the number of BrdU-labeled cells in a semiquantitative manner. The chartings of the transverse sections correspond roughly to levels 31 (a), 85 (b), 107 (c), 127 (d), 153 (e), 168 (f), and 185 (g) of the zebra fish brain atlas (Wullimann *et al.*, 1996). ATN, nucleus tuberis anterior (anterior tuberal nucleus); Cantd, commissura anterior, pars dorsalis (anterior commissure, dorsal part); Chor, commissura horizontalis (horizontal commissure); CIL, nucleus centralis lobi inferioris hypothalami (central nucleus of the inferior lobe); CM, corpus mamillare (mammillary body); CO, chiasma opticum (optic chiasm); CP, nucleus centralis posterior thalami (central posterior thalamic nucleus); CPN, nucleus praetectalis centralis (central pretectal nucleus); Cpop, commissura supraoptica = commissura postoptica (supraoptic commissure = postoptic commissure); Cpost, commissura posterior (posterior commissure); Ctec, commissura tecti = commissura intertectalis (tectal commissure = intertectal commissure); DAO, nucleus opticus accessorius dorsalis (dorsal accessory optic nucleus); Dc, area dorsalis telencephali, zona centralis (central zone of dorsal telencephalic area); Dd, area dorsalis telencephali, zona dorsalis (dorsal zone of dorsal telencephalic area); DIL, nucleus diffusus lobi inferioris hypothalami (diffuse nucleus of the inferior lobe); DiV, ventriculus diencephali (diencephalic ventricle); Dl, area dorsalis telencephali, zona lateralis (lateral zone of dorsal telencephalic area); Dm, area dorsalis telencephali, zona medialis (medial zone of dorsal telencephalic area); DOT, tractus opticus dorsomedialis (dorsomedial optic tract); Dp, area dorsalis telencephali, zona posterior (posterior zone of dorsal telencephalic area); DP, nucleus dorsalis posterior thalami (dorsal posterior thalamic nucleus); DTN, nucleus tegmentalis dorsalis (dorsal tegmental nucleus); ECL, stratum cellulare externum bulbi olfactorii (external cellular layer of olfactory bulb); ENd, nucleus entopeduncularis, pars dorsalis (entopeduncular nucleus, dorsal part); ENv, nucleus entopeduncularis, pars ventralis (entopeduncular nucleus, ventral part); FR, fasciculus retroflexus = tractus habenulointerpeduncularis (habenulointerpeduncular tract); GL, stratum glomerulosum bulbi olfactorii (glomerular layer of olfactory bulb); Hav, nucleus habenularis ventralis (ventral habenular nucleus); Hc, nucleus periventricularis hypothalami, zona caudalis (caudal zone of periventricular hypothalamus); Hd, nucleus periventricularis hypothalami, zona dorsalis (dorsal zone of periventricular hypothalamus); Hv, nucleus periventricularis hypothalami, zona ventralis (ventral zone of periventricular hypothalamus); I, nucleus intermedius thalami (intermediate thalamic nucleus); ICL, stratum cellulare internum bulbi olfactorii (internal cellular layer of olfactory bulb); LFB, fasciculus lateralis telencephali (lateral forebrain bundle); LH, nucleus lateralis hypothalami (lateral hypothalamic nucleus); LLF, fasciculus longitudinalis lateralis = lemniscus lateralis (lateral longitudinal fascicle = lateral lemniscus); LOT, tractus olfactorius lateralis (lateral olfactory tract); LR, recessus lateralis (lateral recess of diencephalic ventricle); MFB, fasciculus medialis telencephali (medial forebrain bundle); MLF, fasciculus longitudinalis medialis (medial longitudinal fascicle); MOT, tractus olfactorius medialis (medial olfactory tract); NIn, nucleus interpeduncularis (interpeduncular nucleus); NLL, nucleus lemnisci lateralis (nucleus of the lateral lemniscus); NLV, nucleus lateralis valvulae (nucleus of the lateral valvula); NMLF, nucleus fasciculi longitudinalis medialis (nucleus of the medial longitudinal fascicle); NR, nucleus ruber (red nucleus); NT, nucleus taeniae (nucleus taeniae); NIII, nucleus nervi oculomotorii (oculomotor nucleus); ON, nervus opticus (optic nerve); OT, tractus opticus (optic tract); PGl, nucleus praeglomerulosus lateralis (lateral preglomerular nucleus); PGm, nucleus praeglomerulosus medialis (medial preglomerular nucleus); PGZ (L1 and 2), stratum periventriculare tecti optici, laminae 1 and 2 (layers 1 and 2 of periventricular gray zone of optic tectum); PGZ (L3), stratum periventriculare tecti optici, lamina 3 (layer 3 of periventricular gray zone of optic tectum); Pit, hypophysis = glandula pituitaria (hypophysis = pituitary); PL, nucleus perilemniscularis (perilemniscal nucleus); PPa, nucleus praeopticus parvocellularis, pars anterior (parvocellular preoptic nucleus, anterior part); PPd, nucleus praetectalis periventricularis, pars dorsalis (periventricular pretectal nucleus, dorsal part); PPp, nucleus praeopticus parvocellularis, pars posterior (parvocellular preoptic nucleus, posterior part); PPv, nucleus praetectalis periventricularis, pars ventralis (periventricular pretectal nucleus, ventral part); PSm, nucleus praetectalis superficialis, pars magnocellularis (magnocellular superficial pretectal nucleus); PSp, nucleus praetectalis superficialis, pars parvocellularis (parvocellular superficial pretectal nucleus); PTN, nucleus tuberis posterior (posterior tuberal nucleus); RT, nucleus tegmentalis rostralis (rostral tegmental nucleus); SC, nucleus suprachiasmaticus (suprachiasmatic nucleus); SD, saccus dorsalis (dorsal sac); SG, nucleus subglomerulosus (subglomerular nucleus); SY, sulcus ypsiloniformis (ypsiloniform sulcus); TelV, ventriculi telencephali (telencephalic ventricles); TeO, tectum opticum (optic tectum); TeV, ventriculus mesencephali (tectal ventricle); TGN, nucleus gustatorius tertius (tertiary gustatory nucleus); TL, torus longitudinalis (longitudinal torus); TLa, torus lateralis (lateral torus); TMCa, tractus mesencephalocerebellaris anterior (anterior mesencephalocerebellar tract); TPM, tractus praetectomamillaris (pretecto-mammillary tract); TPp, nucleus periventricularis tuberculi posterioris (periventricular nucleus of posterior tuberculum); TSc, nucleus centralis tori semicircularis (central nucleus of semicircular torus); TSvl, nucleus ventrolateralis tori semicircularis (ventrolateral nucleus of semicircular torus); TTB, tractus tectobulbaris (tectobulbar tract); TTBc, tractus tectobulbaris cruciatus (crossed tectobulbar tract); Val_{gra}, valvula cerebelli, pars lateralis, stratum granulosum (granular layer of the lateral part of the valvula cerebelli); Val_{mol}, valvula cerebelli, pars medialis, stratum moleculare (molecular layer of the lateral part of the valvula cerebelli); Vam_{gra}, valvula cerebelli, pars medialis, stratum granulosum (granular layer of the medial part of the valvula cerebelli); Vam_{mol}, valvula cerebelli, pars medialis, stratum moleculare (molecular layer of the medial part of the valvula cerebelli); Vas, lacuna vasculosa areae postremae (vascular lacuna of area postrema); Vd, area ventralis telencephali, nucleus dorsalis (dorsal nucleus of ventral telencephalic area); VL, nucleus ventrolateralis thalami (ventrolateral thalamic nucleus); VM, nucleus ventromedialis thalami (ventromedial thalamic nucleus); VOT, tractus opticus ventrolateralis (ventrolateral optic tract); Vp, area ventralis telencephali, nucleus postcommissuralis (postcommissural nucleus of ventral telencephalic area); Vv, area ventralis telencephali, nucleus ventralis (ventral nucleus of ventral telencephalic area). From Zupanc, G. K. H., Hinsch, K., and Gage, F. H. 2005. Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain. *J. Comp. Neurol.* 488, 290–319.

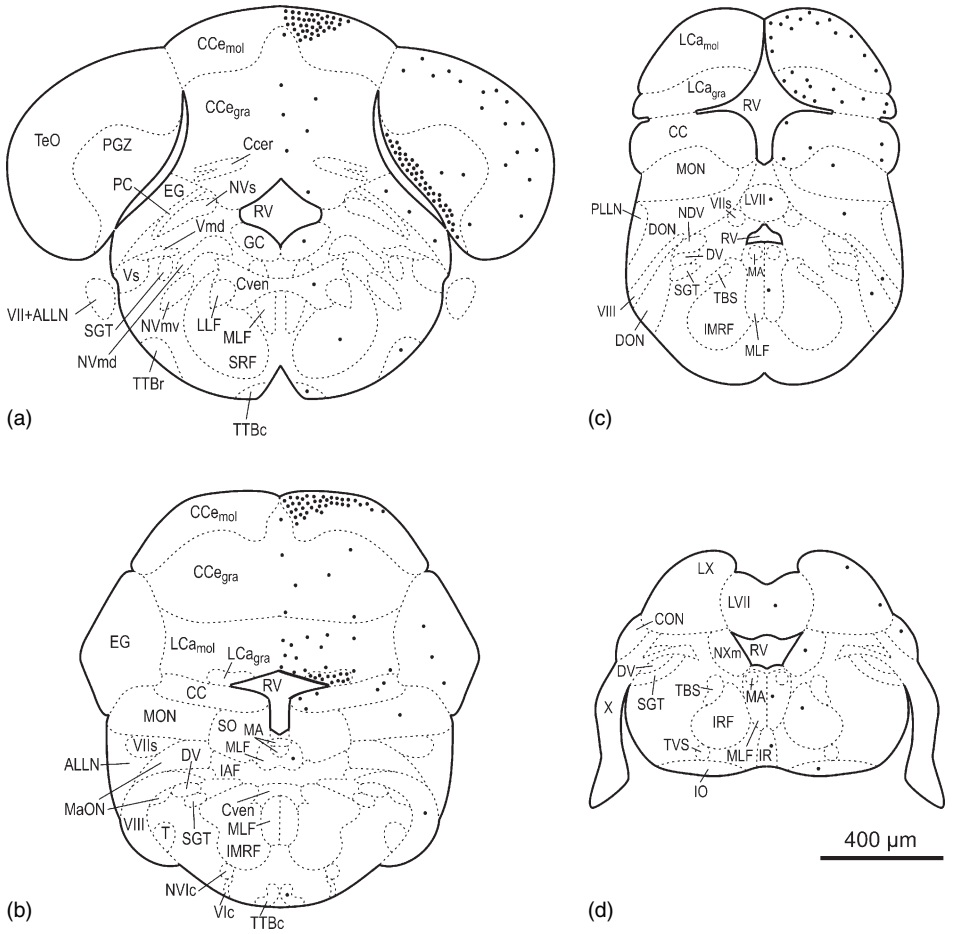

Figure 5 Proliferation zones in caudal divisions of the brain of adult zebra fish. The presence of these zones has been revealed by analysis of BrdU labeling in transverse brain sections at postadministration survival times of 2 h. Areas of mitotic activity are indicated by black dots. Their number correlates with the number of BrdU-labeled cells in a semiquantitative manner. The chartings of the transverse sections correspond roughly to levels 219 (a), 237 (b), 260 (c), and 283 (d) of the zebra fish brain atlas (Wullimann *et al.*, 1996). ALLN, nervi lineae lateralis anterioris (anterior lateral line nerves); CC, crista cerebellaris (cerebellar crest); CCe$_{gra}$, corpus cerebelli, stratum granulosum (granular layer of cerebellar corpus); CCe$_{mol}$, corpus cerebelli, stratum moleculare (molecular layer of cerebellar corpus); Ccer, commissura cerebelli (cerebellar commissure); CON, nucleus octavolateralis caudalis (caudal octavolateralis nucleus); Cven, commissura ventralis rhombencephali (ventral rhombencephalic commissure); DON, nucleus octavus descendens (descending octaval nucleus); DV, radix descendens nervi trigemini (descending trigeminal root); EG, eminentia granularis (granular eminence); GC, griseum centrale (central gray); IAF, fibrae arcuatae internae (inner arcuate fibers); IMRF, formatio reticularis, pars intermedia (intermediate reticular formation); IO, oliva inferior (inferior olive); IR, nucleus raphes inferior (inferior raphe); IRF, formatio reticularis, pars inferior (inferior reticular formation); LCa$_{gra}$, lobus caudalis cerebelli, stratum granulosum (granular layer of caudal lobe of cerebellum); LCa$_{mol}$, lobus caudalis cerebelli, stratum moleculare (molecular layer of caudal lobe of cerebellum); LLF, fasciculus longitudinalis lateralis = lemniscus lateralis (lateral longitudinal fascicle = lateral lemniscus); LVII, lobus facialis (facial lobe); LX, lobus vagus (vagal lobe); MA, fibra Mauthneri (Mauthner axon); MaON, nucleus octavus magnocellularis (magnocellular octaval nucleus); MLF, fasciculus longitudinalis medialis (medial longitudinal fascicle); MON, nucleus octavolateralis medialis (medial octavolateralis nucleus); NDV, nucleus descendens nervi trigemini (nucleus of the descending trigeminal root); NVmd, nucleus motorius nervi trigemini, pars dorsalis (trigeminal motor nucleus, dorsal part); NVmv, nucleus motorius nervi trigemini, pars ventralis (trigeminal motor nucleus, ventral part); NVs, nucleus sensorius principalis nervi trigemini (primary sensory trigeminal nucleus); NVlc, nucleus nervi abducentis, pars caudalis (abducens nucleus, caudal part); NXm, nucleus motorius nervi vagi (vagal motor nucleus); PC, tractus cerebellaris posterior (posterior cerebellar tract); PGZ, stratum periventriculare tecti optici (periventricular gray zone of optic tectum); PLLN, nervus lineae lateralis posterioris (posterior lateral line nerve) ; RV, ventriculus rhombencephali (rhombencephalic ventricle); SGT, tractus gustatorius secundarius (secondary gustatory tract); SO, populatio octavia secundaria (secondary octaval population); SRF, formatio reticularis, pars superior (superior reticular formation); T, nucleus tangentialis (tangential nucleus); TBS, tractus bulbospinalis (bulbospinal tract); TeO, tectum opticum (optic tectum); TTBc, tractus tectobulbaris cruciatus (crossed tectobulbar tract); TTBr, tractus tectobulbaris rectus (uncrossed tectobulbar tract); TVS, tractus vestibulospinalis (vestibulospinal tract); Vmd, radix motoria nervi trigemini, pars dorsalis (dorsal motor root of the trigeminal nerve); Vs, radix sensoria nervi trigemini (sensory root of the trigeminal nerve); VIc, radix caudalis nervi abducentis (caudal root of the abducens nerve); VII, nervus facialis (facial nerve); VIIs, radix sensoria nervi facialis (sensory root of the facial nerve); VIII, nervus octavus (octaval nerve); X, nervus vagus (vagal nerve). From Zupanc, G. K. H., Hinsch, K., and Gage, F. H. 2005. Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain. *J. Comp. Neurol.* 488, 290–319.

subdivisions of the dorsolateral telencephalon of *A. leptorhynchus* (Zupanc and Horschke, 1995). A similar concentration of proliferating cells has been reported for two other teleostean species, the three-spined stickleback (Ekström *et al.*, 2001) and the zebra fish (Zupanc *et al.*, 2005). Cells appear to proliferate in comparable areas of the dorsal telencephalon in the gilthead sea bream (Zikopoulos *et al.*, 2000), although comparison of the latter investigation with the results of the former studies is hampered by the rather coarse analysis of the distribution and the relative number of BrdU-labeled cells.

The high proliferative activity in the dorsolateral telencephalon is especially remarkable from a comparative point of view. Based both on neuroanatomical evidence (Northcutt and Braford, 1980; Nieuwenhuys and Meek, 1990; Braford, 1995; Northcutt, 1995; Butler, 2000; Vargas *et al.*, 2000) and on the results of functional studies (Rodríguez *et al.*, 2002; Portavella *et al.*, 2004), the lateral pallium of actinopterygian fishes (encompassing areas referred to in the zebra fish as the posterior zone of the dorsal telencephalic area and the ventral portion of the lateral zone of the dorsal telencephalic area) is thought to be homologous to the medial pallium or hippocampus of amniotes.

In mammals, the hippocampus is the second brain region in which adult neurogenesis continues into adult stages of development. New cells are formed in the subgranular zone of the dentate gyrus, from where the new cells migrate a short distance into the granule cell layer of the hippocampus and develop into mature granule neurons (Altman and Das, 1965; Kaplan and Bell, 1984; Eriksson *et al.*, 1998; Gould *et al.*, 1999; Kornack and Rakic, 1999; Seri *et al.*, 2001). Similarly, neurogenesis has been found in the adult hippocampus of reptiles (López-García *et al.*, 1988) and birds (Barnea and Nottebohm, 1994).

The subsequent development of the cells generated in the dorsal telencephalon has been examined in some detail in zebra fish by combining BrdU labeling with immunostaining against the neuron-specific marker protein Hu after post-BrdU administration survival times of up to 9 months (Zupanc *et al.*, 2005). These experiments demonstrated a large number of double-labeled cells in the medial, lateral, and posterior zones of the dorsal telencephalic areas, thus including the structure presumably homologous to the mammalian hippocampus. Most significantly, the number of these new neurons was several-fold larger than that in all remaining brain areas. This observation can be used as evidence for the importance of adult neurogenesis in the dorsal telencephalon of zebra fish. However, the function of this phenomenon remains elusive.

Some indication of the possible function of adult neurogenesis in the hippocampus has been obtained in mammals and birds. In the rat, more than an estimated 250 000 new cells are generated in the hippocampal dentate gyrus per month (Cameron and McKay, 2001). Roughly 50% of these cells acquire immunological properties of neurons within less than 2 weeks after entering the S-phase of mitosis. This number of new granule neurons generated each month corresponds to approximately 6% of the total size of the granule cell population, thus suggesting that adult neurogenesis plays an important role in the hippocampus. This notion is reinforced by the demonstration that the newly generated neurons in the rodent hippocampus exhibit physiological properties of mature functional hippocampal neurons (van Praag *et al.*, 2002). Furthermore, a number of functional studies suggests a close relationship between adult neurogenesis and certain types of learning mediated by the hippocampus. Such investigations have, for example, demonstrated that an enriched environment and learning enhance neurogenesis, especially through increasing the rate of survival of new neurons, whereas an experimentally induced reduction in neurogenesis results in an impaired performance in specific hippocampus-dependent learning tasks (Kempermann *et al.*, 1997; van Praag *et al.*, 1999; Shors *et al.*, 2001, 2002).

Evidence for an involvement of hippocampal adult neurogenesis in learning and memory processes has also been obtained through investigations in birds. In black-capped chickadees, it has been shown that newly formed neurons appear in the hippocampal complex during all times of the year, but with a marked peak in the fall (Barnea and Nottebohm, 1994). The new neurons live for a few months and then disappear. The peak in the formation of new neurons in the fall correlates with pronounced changes in the feeding behavior of chickadees. Then the diet shifts from insects to seeds. The birds hide a certain portion of these seeds and retrieve them after a period of hours or days. This requires enhanced spatial memory capabilities, which could be enabled by the recruitment of new neurons. In line with this hypothesis is the finding that there are more hippocampal neurons in juvenile chickadees than in adults (Barnea and Nottebohm, 1996). Similar to the situation in the fall, the excess production of neurons during juvenile stages is related to the need to learn a disproportionally huge amount of novel environmental information, compared to later stages when the individual is already largely familiar with its environment.

The discovery of adult neurogenesis in the teleostean homologue of the mammalian and avian

hippocampus has added an important piece of information to the biological understanding of this phenomenon. Comparison with other vertebrate taxa leads to the hypothesis that adult neurogenesis in the hippocampus is a primitive and highly conserved vertebrate trait. It will, therefore, be particularly exciting to identify the selection pressures that have led to the high degree of conservation of adult neurogenesis in the vertebrate hippocampus.

2.24.4.3 Optic Tectum

Analysis of cell proliferation has shown that in the optic tectum new cells are generated predominantly in a specific proliferation zone at the caudal pole. Such a pattern has been described for goldfish (Meyer, 1978; Raymond and Easter, 1983), *A. leptorhynchus* (Zupanc and Horschke, 1995), zebra fish (Marcus *et al.*, 1999; Zupanc *et al.*, 2005), gilthead sea bream (Zikopoulos *et al.*, 2000), and stickleback (Ekström *et al.*, 2001). The similarity in the pattern of cell proliferation among these rather distantly related species suggests that the proliferation zone in the caudal tectum is a widespread, and probably universal, source of germinal cells in the adult brain of teleosts.

In both zebra fish and *A. leptorhynchus* (Zupanc *et al.*, 2005; G. K. H. Zupanc, unpublished observations), examination of sections through the optic tectum taken after various post-BrdU survival times has indicated that the vast majority of the new cells continue to reside in the proliferation zone of the caudal tectum. Similar results have been obtained in goldfish by labeling of new cells with tritiated thymidine and examination of autoradiographs after various postadministration survival times (Meyer, 1978; Raymond and Easter, 1983). Taken together, these results suggest that the optic tectum grows primarily from its caudal end.

Interestingly, the presence of the proliferation zone in the caudal optic tectum appears to be independent of whether or not sensory input is dominated by the visual modality in the various species. Whereas in zebra fish, goldfish, guppy, stickleback, and gilthead sea bream visual cues play a predominant role in the context of many behaviors, *A. leptorhynchus* relies to a large extent on electric input for orientation and communication. It is unknown whether the differences in the amount of input received by the optic tectum from the eye lead to a quantitative difference in the number of new cells produced during adulthood. This possibility is worth examining because the optic tectum of *A. leptorhynchus*, when compared to the rest of the brain, is significantly smaller than in more visually guided species, such as goldfish and zebra fish.

2.24.4.4 Cerebellum

In *A. leptorhynchus*, approximately 75% of all new cells produced in the adult brain originate from proliferation zones within the various subdivisions of the cerebellum (Zupanc and Horschke, 1995). Qualitative analysis suggests a similar large number of proliferating cells in the cerebellum of other teleosts, including the guppy (Kranz and Richter, 1970), gilthead sea bream (Zikopoulos *et al.*, 2000), three-spined stickleback (Ekström *et al.*, 2001), and zebra fish (Zupanc *et al.*, 2005). In *A. leptorhynchus*, in two out of the three major cerebellar subdivisions – the corpus cerebelli and the valvula cerebelli – the vast majority of the cells are generated in the respective molecular layers. In the corpus cerebelli, these proliferation zones are largely restricted to areas at and near the midline in the dorsal and ventral molecular layers. In the valvula cerebelli pars medialis, these zones are found at the midline where they form distinct cell clusters exhibiting high mitotic activity. In the valvula cerebelli pars lateralis, the mitotic cells show a rather wide distribution within the molecular layer. In the third cerebellar subdivision of *A. leptorhynchus*, the eminentia granularis, the new cells are produced in a granule cell layer, the eminentia granularis pars medialis (Zupanc and Horschke, 1995; Zupanc *et al.*, 1996). Notably, mitotic cells are also found in the granule cell layer of the lobus caudalis of zebra fish (Zupanc *et al.*, 2005); this brain structure is thought to be homologous to the eminentia granularis pars medialis of gymnotiform fish (Bass, 1982). However, the number of labeled cells in the granule cell layer of the lobus caudalis of zebra fish is markedly lower than the number in the eminentia granularis pars medialis in *A. leptorhynchus*.

2.24.5 Development of the Young Cells in the Cerebellum

The subsequent development of the newly generated cells has been studied in detail in the cerebellum of two teleostean species: *A. leptorhynchus* and zebra fish. In the following, I will discuss several important aspects of this process.

2.24.5.1 Migration

In *A. leptorhynchus*, the new cells migrate from their sites of origin to specific target areas within the three cerebellar subdivisions (Zupanc *et al.*, 1996). These target areas are the granule cell layers

in both the corpus cerebelli and the valvula cerebelli partes lateralis and medialis (Figure 6). In the eminentia granularis, approximately 70% of the cells generated in the pars medialis migrate through the adjacent molecular layer to invade the second granule cell layer, the so-called eminentia granularis pars posterior (Figure 7). The remaining 30% of the cells continue to reside in the eminentia granularis pars medialis and the adjacent molecular layer. In all three cerebellar subdivisions, the majority of the young cells reach their target areas within 10 days after incorporation of BrdU.

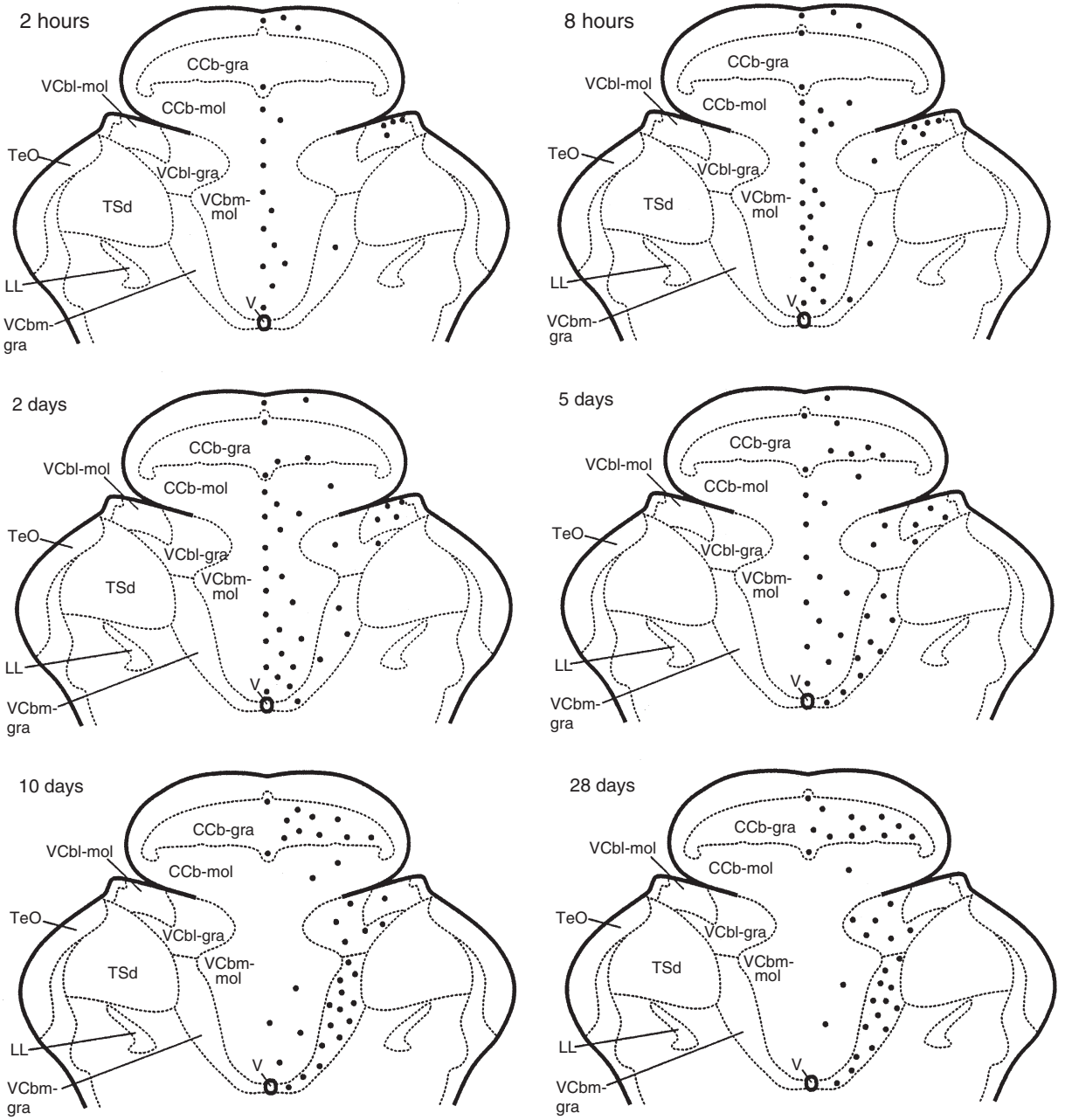

Figure 6 Development of cells generated during adulthood in the rostral cerebellum of *A. leptorhynchus*. The distribution of the young cells, identified by labeling with BrdU and indicated by black dots, is plotted after post-BrdU administration survival times ranging from 2 h to 28 days. The number of dots represents approximately the areal density of labeled cells. CCb-gra, granule cell layer of corpus cerebelli; CCb-mol, molecular layer of corpus cerebelli; LL, lateral lemniscus; TeO, optic tectum; TSd, dorsal subdivision of torus semicircularis; V, ventricle; VCbl-gra, granule cell layer of valvula cerebelli pars lateralis; VCbl-mol, molecular layer of valvula cerebelli pars lateralis; VCbm-gra, granule cell layer of valvula cerebelli pars medialis; VCbm-mol, molecular layer of valvula cerebelli pars medialis. After Zupanc, G. K. H., Horschke, I., Ott, R., and Rascher, G. B. 1996. Postembryonic development of the cerebellum in gymnotiform fish. *J. Comp. Neurol.* 370, 443–464.

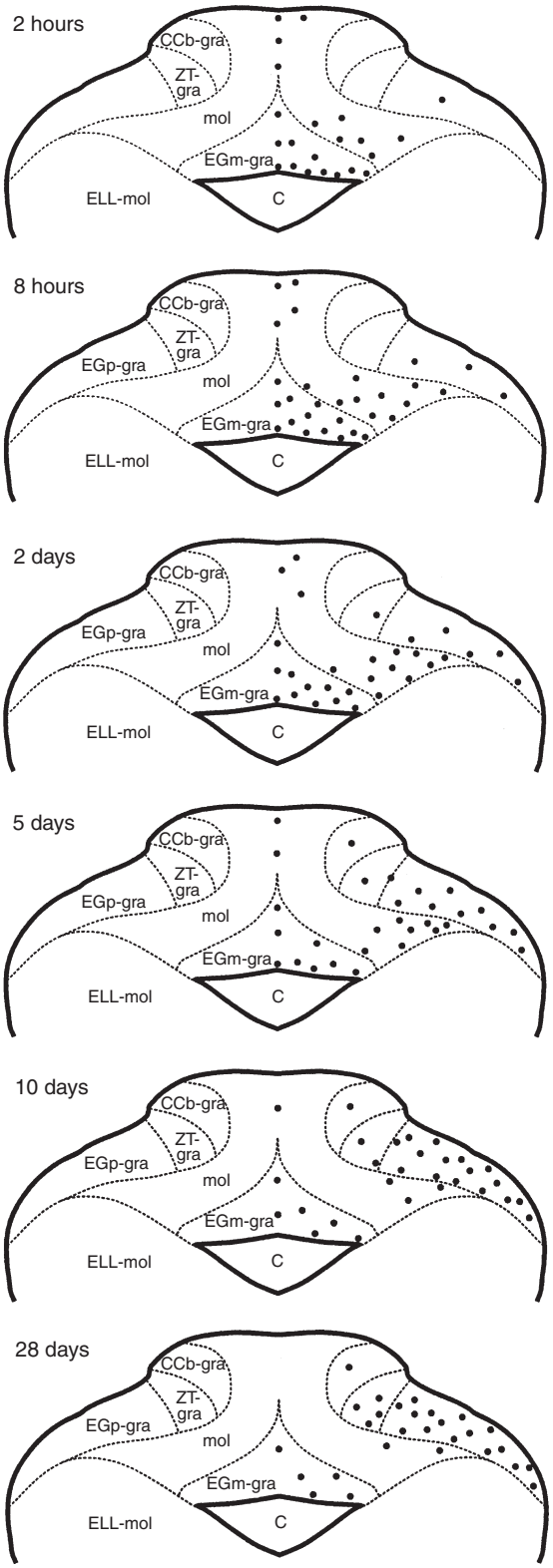

Figure 7 Development of cells born during adulthood in the caudal cerebellum of *A. leptorhynchus*. The principle of analysis and representation is identical to the one used in Figure 6. C, cerebellomedullary cistern; CCb-gra, granule cell layer of corpus cerebelli; EGm-gra, granule cell layer of eminentia granularis pars medialis; EGp-gra, granule cell layer of eminentia granularis pars posterior; ELL-mol, molecular layer of electrosensory lateral line lobe; mol, molecular layer of corpus cerebelli, eminentia granularis pars anterior, eminentia granularis pars medialis, and eminentia granularis pars posterior; ZT-gra, granule cell layer of transitional zone. After Zupanc, G. K. H., Horschke, I., Ott, R., and Rascher, G. B. 1996. Postembryonic development of the cerebellum in gymnotiform fish. *J. Comp. Neurol.* 370, 443–464.

Comparison of these patterns of migration in *A. leptorhynchus* with those found in zebra fish has revealed commonalities, but also interesting differences. As in *A. leptorhynchus*, in zebra fish the young cells generated in the corpus cerebelli and the valvula cerebelli migrate from their proliferation zones in the molecular layers to the corresponding granular layers (Zupanc *et al.*, 2005; Figure 8). In the caudal cerebellum, however, the migrational pattern differs between the two species. In contrast to *A. leptorhynchus*, there is no evidence in zebra fish that any significant portion of the cells that undergo mitosis in the granular layer of the lobus caudalis – the structure homologous to the eminentia granularis pars medialis of gymnotiform fish – migrate out of this structure (Figure 9). Thus, the new cells produced in the granule cell layer of the lobus caudalis of zebra fish show a similar absence of migratory behavior, as do the resident 30% of the cells generated in the eminentia granularis pars medialis of *A. leptorhynchus*. On the other hand, the approximately 70% of the new cells generated in the eminentia granularis pars medialis of *A. leptorhynchus* that migrate to the granule cell layer of the eminentia granularis pars posterior appear to lack a matching cellular population in zebra fish. This lack might be related to the fact that, in zebra fish, the number of mitotic cells in the granular layer of the lobus caudalis relative to other proliferation zones is significantly smaller than the relative number of S-phase cells in the eminentia granularis pars medialis in *A. leptorhynchus*.

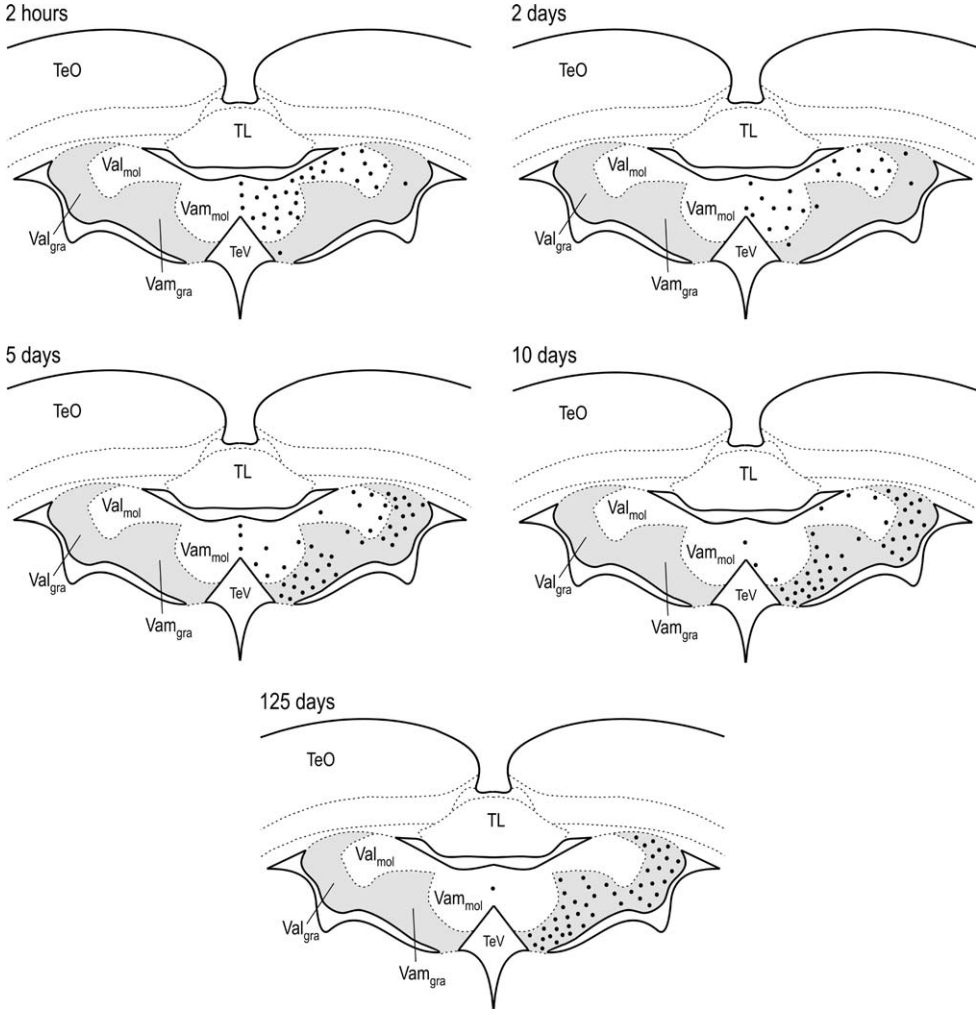

Figure 8 Schematic representation of the distribution of BrdU-labeled cells in the valvula cerebelli of adult zebra fish after various post-BrdU administration survival times. The location of labeled cells is indicated by black dots. The number of dots roughly represents the density of labeled cells in this brain region. TeO, tectum opticum; TeV, tectal ventricle; TL, torus longitudinalis; Val$_{gra}$, granule cell layer of the lateral division of the valvula cerebelli; Val$_{mol}$, molecular layer of the lateral division of the valvula cerebelli; Vam$_{gra}$, granule cell layer of the medial division of the valvula cerebelli; Vam$_{mol}$, molecular layer of the medial division of the valvula cerebelli. From Zupanc, G. K. H., Hinsch, K., and Gage, F. H. 2005. Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain. *J. Comp. Neurol.* 488, 290–319.

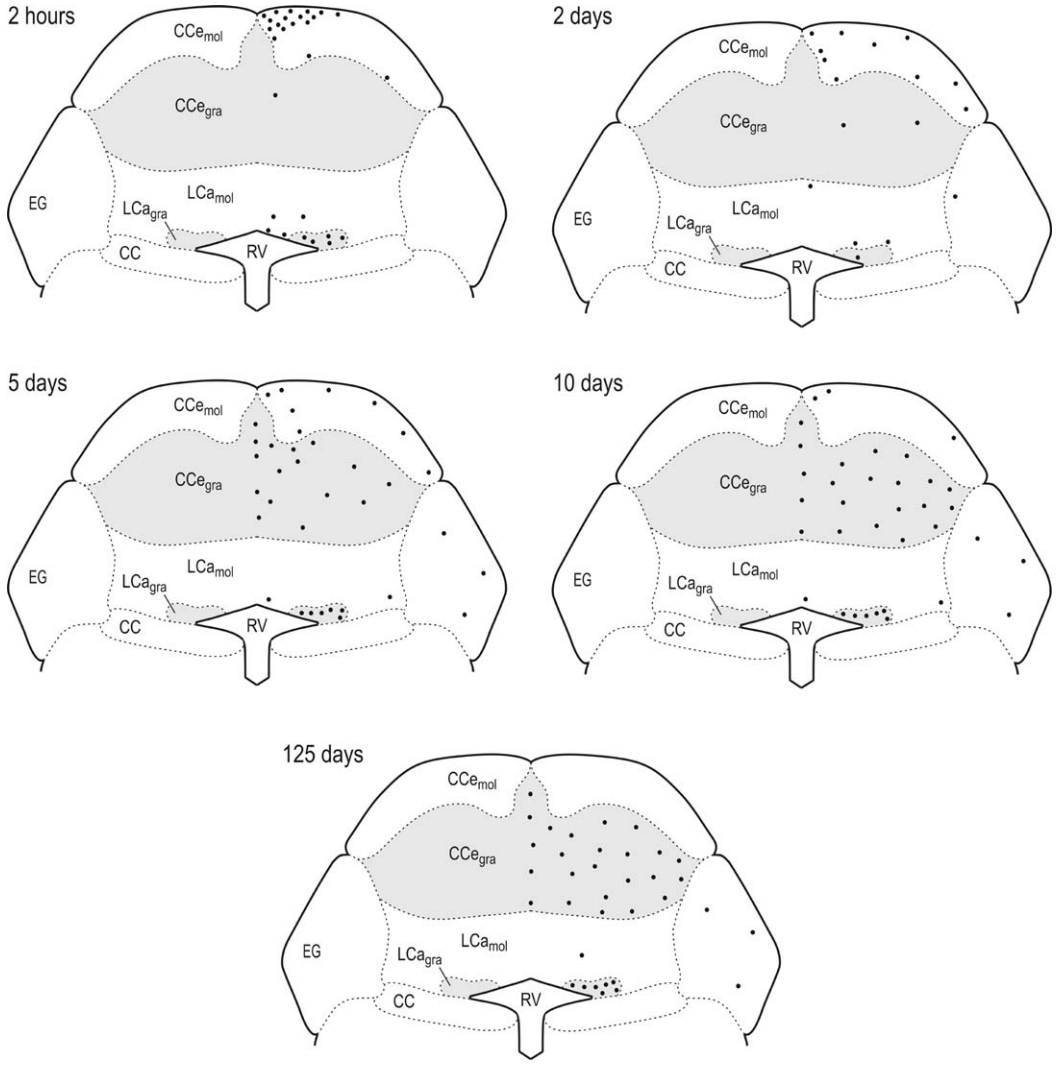

Figure 9 Schematic representation of the distribution of BrdU-labeled cells in the corpus cerebelli, eminentia granularis, and rostral part of lobus caudalis cerebelli of adult zebra fish after various post-BrdU administration survival times. The location of labeled cells is indicated by black dots. The number of dots roughly represents the density of labeled cells in this brain region. CC, crista cerebellaris; CCe$_{gra}$, granule cell layer of corpus cerebelli; CCe$_{mol}$, molecular layer of corpus cerebelli; EG, eminentia granularis; LCa$_{gra}$, granule cell layer of lobus caudalis cerebelli; LCa$_{mol}$, molecular layer of lobus caudalis cerebelli; RV, rhombencephalic ventricle. From Zupanc, G. K. H., Hinsch, K., and Gage, F. H. 2005. Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain. *J. Comp. Neurol.* 488, 290–319.

The difference in the pattern of cell proliferation between the two species might be causally linked to the massive electrosensory input received by the granule cell layer of the eminentia granularis pars posterior in gymnotiform fish (Sas and Maler, 1987), and the lack of such input in nonelectroreceptive fish. The caudal lobe and its homologous structure in gymnotiform fish may, therefore, be well suited to study, from a comparative point of view, the factors responsible for interspecies differences in proliferation and migration of new cells generated in the adult brain, as well as the functional consequences of these differences.

2.24.5.2 Guidance of the Migrating Young Cells

Several lines of evidence, obtained in *A. leptorhynchus*, suggest that, in the course of migration from their proliferation zones to the final target areas, the young cerebellar cells are guided by radial glial fibers (Zupanc and Clint, 2003). These fibers form two populations: one immunopositive for glial fibrillary acidic protein (GFAP), the other for vimentin. The morphology and distribution of these two fiber populations are similar but not identical, thus indicating a partial overlap. It is possible that in the course of their development, similar to the situation in mammals (Voigt, 1989), the fish radial glia first express vimentin, and then, while the

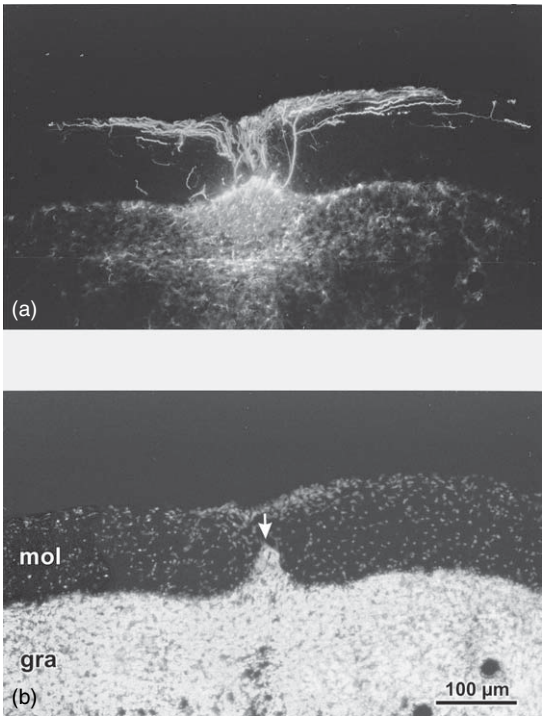

Figure 10 Radial glial fibers, identified by immunostaining against GFAP, in the dorsal portion of the corpus cerebelli, in *A. leptorhynchus* (a). At the midline of the dorsal granule cell layer (gra), the granule cell mass protrudes into the dorsal molecular layer (mol) by forming a tip (arrow), as is evident in the counterstain produced by applying the nuclear dye DAPI to the section (b). This tip is the origin of long fibers that initially take a trajectory parallel to the midline. As soon as they get close to the dorsal surface of the brain, these fibers split into two populations turning laterally to the left and right side, respectively. Following this turn, they continue to run parallel to the dorsal surface of the brain over a few hundred micrometers. As BrdU-labeling studies have shown, this fiber course exactly matches the route alongside which cells born in the molecular layer migrate into the granule cell layer. Reproduced from Zupanc, G. K. H. 2001. Adult neurogenesis and neuronal regeneration in the central nervous system of teleost fish. *Brain. Behav. Evol.* 58, 250–275, with permission from S. Karger AG, Basel.

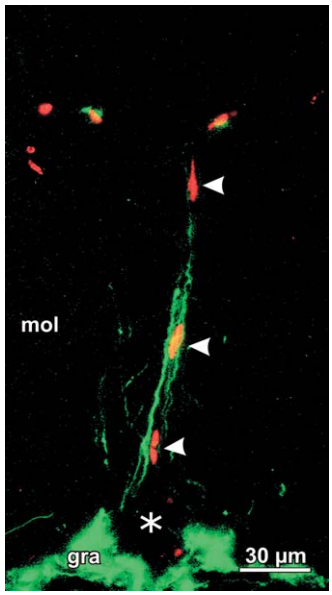

Figure 11 Guidance of young cells by radial glial fibers in the cerebellum of the *A. leptorhynchus*. Two days after the administration of the S-phase marker BrdU, the fish was killed, and the transverse section through the dorsal portion of the corpus cerebelli was immunostained against both BrdU (red) and GFAP (green). Long GFAP-positive radial glial fascicles run at the midline of the molecular layer (mol) through the so-called dorsal tip (asterisk) into the granule cell layer (gra). Several BrdU-labeled cells, indicated by arrowheads, are closely apposed to the radial glial fibers. Reproduced from Zupanc, G. K. H. 2001. Adult neurogenesis and neuronal regeneration in the central nervous system of teleost fish. *Brain Behav. Evol.* 58, 250–275, with permission from S. Karger AG, Basel.

vimentin expression is gradually reduced, an increasing amount of GFAP is produced. However, final verification of this hypothesis awaits further experimentation.

In all three cerebellar subdivisions, both the vimentin-positive fibers and the GFAP-expressing radial glial fibers delineate the path taken by the young cells in the course of their migration. For instance, in the corpus cerebelli such fibers originate from the tips formed at the midline by protrusion of granule cells from the granule cell layer into the dorsal and ventral molecular layers. Initially, the fibers run along the midline, but shortly before reaching the pial surface in the dorsal molecular layer, they make lateral turns and continue to run in lateral directions for up to several hundred micrometers (Figure 10). This fiber course exactly matches the route through which cells born in the molecular layer migrate into the granule cell layer. A similar matching between the distribution and orientation of GFAP- and vimentin-positive radial glial fibers on the one hand, and the migrational path taken by young cells on the other, is observed in the other two cerebellar subdivisions.

The hypothesis that radial glial fibers in the cerebellum provide a scaffolding for the migrating young cells receives further support by the results of double-labeling experiments. Intraperitoneal injection of BrdU, followed by sacrifice of the fish 2 days after administration (i.e., at a time when the young cells exhibit maximum migratory activity) reveals elongated BrdU-labeled cells in close apposition to GFAP-labeled radial glial fibers (Gheteu and Zupanc, 2001; Figure 11).

2.24.5.3 Regulation of the Number of New Cells by Apoptotic Cell Death

After arrival at their target sites within the cerebellum of *A. leptorhynchus*, the areal density of BrdU-labeled

cells drops by roughly 50% in the period 4–7 weeks after their generation (Zupanc *et al.*, 1996; Ott *et al.*, 1997). This decrease is thought to be due to apoptotic cell death. Such a mechanism of cell elimination has been suggested by experiments in which terminal deoxynucleotidyl-transferase-mediated dUTP-biotin nick end-labeling (TUNEL) of 3′-OH ends of DNA was employed (Soutschek and Zupanc, 1996). The latter approach is based on the identification of DNA fragmentation as a feature characteristic of apoptosis (Modak and Bollum, 1972; Gavrieli *et al.*, 1992; Surh and Sprent, 1994).

Studies applying TUNEL to brain sections of *A. leptorhynchus* have shown that a large number of cells continuously undergo apoptosis in the granular layers of the three subdivisions of the cerebellum (Soutschek and Zupanc, 1996). By contrast, the number of apoptotic cells is low in the molecular layers. This suggests that apoptosis is used as a mechanism to regulate the numbers of the young cells after they have reached their target areas.

A similar mechanism has been demonstrated in other brain systems, particularly those of mammals, during embryonic development. In these systems, apoptosis leads to the elimination of young cells which, after arrival at the target site, have failed to make proper connections with other neurons and to receive adequate amounts of specific survival factors produced by cells in the target area (Raff, 1992; Raff *et al.*, 1993).

2.24.5.4 Long-Term Survival of New Cells

In *A. leptorhynchus*, it is likely that most of the cells that have survived the massive wave of apoptotic cell death 4–7 weeks after their generation continue to exist for the rest of the fish's life. This is suggested by experiments in which post-BrdU-administration survival times of up to 440 days were employed (Zupanc *et al.*, 1996; Ott *et al.*, 1997). The latter period is equivalent to roughly half the adult life span of this species in captivity.

Long-term survival of new cells of at least 292 days has been demonstrated in a large number of brain regions of the zebra fish (Zupanc *et al.*, 2005). The entire life span of outbred zebra fish is roughly 3.5 years (Gerhard *et al.*, 2002), so that a survival time of 292 days covers approximately one-fourth of the entire life span and one-third of the remaining life expectancy after administration of BrdU (the fish used in the experiments received a single pulse of this thymidine analogue at an approximate age of 9–12 months).

A similar long-term persistence of new cells as found in *A. leptorhynchus* and zebra fish has been observed in the mammalian brain. In the granular layer of the hippocampus of adult mice, new neurons survive for at least 11 months (Kempermann *et al.*, 2003), which is equivalent to roughly half the life span of mice.

In *A. leptorhynchus*, long-term survival, together with the continuous production of new cells, leads to a permanent growth of the entire brain, except for very old fish in which the growth rate appears to reach a plateau. This parallels the continuous growth of the body in this species. A quantitative analysis has demonstrated that, while the body weight of the fish increases from 1 to 16 g, the total number of brain cells doubles from 5×10^7 to 1×10^8 (Zupanc and Horschke, 1995).

2.24.5.5 Neuronal Differentiation

A central question arising in the context of the generation of new cells in the adult fish brain concerns their cellular identity. This issue has been addressed in detail with regard to the zebra fish (Zupanc *et al.*, 2005). The approaches used include combination of anti-BrdU immunohistochemistry with immunostaining against Hu-C and Hu-D. The Hu family of RNA-binding proteins is neuron-specific and plays an important role in the development and maintenance of vertebrate neurons. These proteins have been shown to be expressed both by neurons generated during embryogenesis (Marusich *et al.*, 1994) and by neurons produced in the adult brain (Barami *et al.*, 1995).

Double-labeling experiments using this approach have revealed that, at post-BrdU administration survival times of 9 months, approximately 50% of the BrdU-labeled cells in the zebra fish brain express this neuron-specific protein. Such neurons are particularly abundant in the dorsal telencephalon, including the region presumably homologous to the mammalian hippocampus (see Section 2.24.4.2), but are also found in other areas of the brain.

Since after post-BrdU administration survival times of 7–10 months approximately 50% of all BrdU-labeled cells are situated in the granular layers of the corpus cerebelli and the valvula cerebelli partes lateralis and medialis, it is reasonable to assume that at least a part of the new cerebellar cells develop into granule cell neurons. Unfortunately, none of the anti-Hu antibodies tested thus far have failed to immunostain cerebellar granule cells. Therefore, using an alternative approach, the possible differentiation of young cerebellar cells into granule cell neurons has been examined by combination of BrdU labeling and neuronal tract tracing. Such experiments, conducted

in both *A. leptorhynchus* (Zupanc *et al.*, 1996) and zebra fish (Zupanc *et al.*, 2005), have indeed provided evidence in favor of the hypothesis that at least some of the cells generated in the adult cerebellum develop into granule cell neurons. Just as in these studies the BrdU-labeled granule cells were retrogradely traced by application of tracer substance to the molecular layers, the experiments also indicate that these cells have developed axons traveling from their somata in the granule cell layers to the corresponding molecular layers. Thus, it appears that they have integrated into the existing neural network of cerebellar neurons. It is unknown whether the axons of the newly generated granule cells make proper synaptic connections with other cells, such as Purkinje cells. It also remains to be examined whether the new granule cells are functional, and whether their physiological properties are similar to those of the older granular neurons.

2.24.6 Why Do Fish Produce New Neurons in the Adult Brain? The Numerical Matching Hypothesis

Why does the generation of new neurons cease in all but two regions of the mammalian brain during the early stages of development, but persist in many areas of the teleost fish brain throughout life? And why does adult neurogenesis specifically occur in the olfactory bulb and the hippocampus, but not in other areas of the adult mammalian brain?

A possible answer to these questions is suggested by the results of a comparative analysis of the differences in the development of motor structures and sensory organs between teleosts and mammals (Zupanc, 1999a, 2001, 2006). Although many species of teleosts and mammals grow during postembryonic stages of development and even throughout life, there is a distinct difference in the growth pattern between these two taxonomic classes. Whereas in mammals postembryonic growth is the result of an increase in size but not in number of individual muscle fibers (Rowe and Goldspink, 1969), in fish both the number of muscle fibers and the volume of individual fibers increase (Weatherley and Gill, 1985; Koumans and Akster, 1995; Zimmerman and Lowery, 1999; for review, see Rowlerson and Veggetti, 2001). It is, therefore, possible that the hyperplasia of peripheral motor elements prompts a concomitant increase in the number of central neurons involved in neural control of associated muscle activity.

Along similar lines, the number of sensory receptor cells, receptor organs, or receptor units in the periphery has been shown to increase with age in several species of fish. Such a formation of new sensory elements has been demonstrated for sensory hair cells in the inner ear of sharks (Corwin, 1981), for retinal cells in the eyes of goldfish (Johns and Easter, 1977), and for electrosensory receptor organs in the gymnotiform fish *Sternopygus darienses* (Zakon, 1984). Thus, like the formation of new motor elements, the continuous increase in the number of sensory elements in fish may lead to the generation of new neurons involved in the processing of sensory information, ensuring a numerical matching of central neurons and peripheral sensory elements.

New central neurons may also be generated if the sensory cells or muscle fibers in the periphery exhibit a turnover, instead of a net increase in total number. Then the production of a certain portion of sensory cells or motor fibers would lead to the generation of new central neurons (directly or indirectly) connected to them, whereas the degeneration of other sensory cells would be accompanied by a secondary degeneration of central neurons linked to this portion of peripheral elements. In such cases, the overall number of peripheral elements and central neurons would vary little, or not at all, over time, although changes in the number of sensory cells or muscle fibers still trigger changes in the number of central neurons so that a specific ratio between the two cellular populations is maintained.

In mammals, the production of sensory cells appears to cease by the end of gestation (e.g., Ruben, 1967), except for primary sensory neurons of the olfactory epithelium (Graziadei and Graziadei, 1979), which replace older olfactory neurons undergoing apoptosis (Magrassi and Graziadei, 1995). The axons of the primary sensory neurons make synaptic contacts with dendrites of mitral and tufted cells within the olfactory bulb. These cells project to the olfactory cortex, including the lateral entorhinal cortex. The latter structure, in turn, sends axons, via the lateral perforant path, to the hippocampus, which synapse on the distal dendrites of the dentate gyrus granule cells (Carlsen *et al.*, 1982; Insausti *et al.*, 2002; van Groen *et al.*, 2002, 2003). Thus, each of the two regions in which neurogenesis occurs within the adult mammalian brain – olfactory bulb and dentate gyrus – is part of the central olfactory pathway. Remarkably, also the entorhinal cortex, in which adult neurogenesis is absent, retains its neurogenic potential during adult life. Neurospheres from this region differentiate into neurons and glia *in vitro* and after transplantation to the adult dentate gyrus (Fontana *et al.*, 2006).

In fish, the matching hypothesis can explain the enormous mitotic activity in the cerebellum. Based

on anatomical, physiological, and behavioral experiments, the cerebellum, notably of weakly electric fish, has been proposed to play an important role not only in the well-established function of control and coordination of movements, but also in sensory processing. The latter has been demonstrated particularly in the context of the involvement of the cerebellum in tracking movements of objects around the animal, and in the generation and subtraction of sensory expectations (for reviews, see Paulin, 1993; Bell *et al.*, 1997). On the other hand, the lack of adult neurogenesis in the mammalian cerebellum could be causally linked to the absence of changes in the number of muscle fibers and sensory elements during periods of growth in the periphery.

At the central level, numerical matching has been demonstrated between the presynaptic granule cell population and its postsynaptic target neurons, the Purkinje cells, by making use of the neurologically mutant mouse strain lurcher. The cerebella of lurcher heterozygotes are characterized by early postnatal degeneration of several cell types, beginning with the loss of Purkinje cells during the second week of life. This stage is followed by degeneration of granule and olivary neurons and Bergmann glia (Swisher and Wilson, 1977; Caddy and Biscoe, 1976). In wild-type mice, there is a constant ratio of about 175 granule cells for each Purkinje cell. In lurcher chimeric mice, variation in the number of Purkinje cells is genetically caused, whereas the loss of granule cells appears to be a secondary, phenotypic consequence due to Purkinje cell loss (Wetts and Herrup, 1983).

In fish, there is some experimental evidence supporting the hypothesis that changes in the number of peripheral sensory elements lead to changes in the production of the corresponding central elements. Raymond *et al.* (1983) found that in goldfish permanent removal of the optic input by enucleation of the eye results in sustained depression of mitotic activity in the tectal proliferation zone on the denervated side compared to the intact one. Temporary denervation by optic nerve crush initially has a similar effect, but upon re-innervation of the tectum by the regenerating optic fibers, proliferation is enhanced on the experimental side compared to the control side.

2.24.7 Neuronal Regeneration

The enormous potential of teleost fish to continuously produce new neurons in the intact CNS during adulthood is closely related to a second phenomenon by which fish and other anamniotes are distinguished from amniote vertebrates: the ability to replace neurons lost to injury by newly generated ones. This so-called neuronal regeneration has been particularly well studied in three neuronal systems. The retina and its primary projection target, the optic tectum (for review, see Otteson and Hitchcock, 2003); the spinal cord (for review, see Waxman and Anderson, 1986); and the cerebellum (for reviews, see Zupanc, 1999a, 2001, 2006; Zupanc and Clint, 2003; Zupanc and Zupanc, 2006a). In the following, I will focus on the latter system, which is based on a series of investigations carried out in *A. leptorhynchus*.

2.24.7.1 The Lesion Paradigm

Investigations of the phenomenon of neuronal regeneration in the cerebellum of *A. leptorhynchus* have been greatly facilitated by the availability of a well-established lesion paradigm. This paradigm takes advantage of the prominent position of the corpus cerebelli, which forms a roof on top of the brain, except over the telencephalon (Figure 12a).

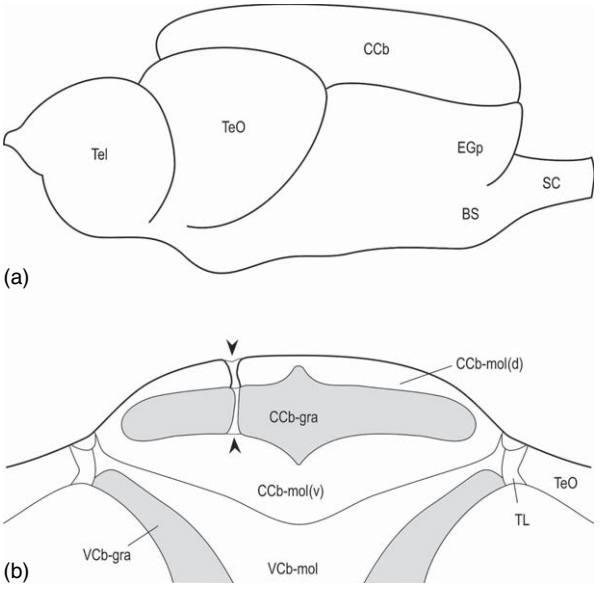

Figure 12 a, Side view of the brain of the brown ghost knifefish. One subdivision of the cerebellum, the corpus cerebelli (CCb), forms a roof on top of the brain, except over the telencephalon (Tel). Cerebellar lesions can, therefore, be made in a defined way by puncturing the skull of the fish with a surgical blade, without damaging other parts of the brain. b, Transverse section through the corpus cerebelli. The lesion path (arrowheads) travels through the dorsal molecular layer (CCb-mol(d)) and the granular layer (CCb-gra) roughly halfway between the midline of the brain and the lateral edge of the granular layer. BS, brainstem; CCb-mol(v), ventral molecular layer of corpus cerebelli; EGp, eminentia granularis pars posterior; SC, spinal cord; TeO, optic tectum; TL, torus longitudinalis; VCb-gra, granule cell layer of valvula cerebelli; VCb-mol, molecular layer of valvula cerebelli. Reproduced from Zupanc, G. K. H. and Zupanc, M. M. 2006a. New neurons for the injured brain: Mechanisms of neuronal regeneration in adult teleost fish. *Regen. Med.* 1, 207–216, with permission from Future Science Group.

Cerebellar lesions can, therefore, be made by puncturing the skull of the anesthetized fish with a sterile surgical blade, without damaging other parts of the brain (Figure 12b). Guided by landmarks on the fish's head, lesions are applied in a defined way, resulting in an approximately 1-mm-deep cut traveling in parasagittal direction and encompassing both the dorsal molecular layer and the granular layer of the corpus cerebelli.

Nissl stains of sections taken through the corpus cerebelli after various survival times have shown that the initially clearly visible path caused by the stab wound gradually becomes reduced with longer postlesioning survival times, until it disappears after a couple of weeks (Zupanc *et al.*, 1998). This indicates rapid and efficient neuronal wound healing.

2.24.7.2 Elimination and Removal of Damaged Cells

Detailed analysis has shown that the tissue repair is accomplished by a number of well-orchestrated processes. The first process involves the rapid elimination of damaged cells through apoptosis (Zupanc *et al.*, 1998), followed by removal of the resulting cellular debris by the action of microglia/macrophages (Zupanc *et al.*, 2003). Apoptosis was originally shown to regulate cell numbers in the mammalian brain during embryonic development (Raff *et al.*, 1993). It is believed that this type of cell death exerts a similar function in the intact fish brain during postnatal development (Soutschek and Zupanc, 1995, 1996; see Section 2.24.5.3). The involvement of apoptosis in the process of elimination of injured cells is remarkable because this contrasts with the situation in mammals. In the latter taxon, besides apoptosis (for reviews, see Beattie *et al.*, 2000; Vajda, 2002), necrosis occurs and appears to be the predominant type of cell death following lesions (Kerr *et al.*, 1987). Necrosis commonly leads to inflammation at the site of the injury (for review, see Kerr *et al.*, 1995). This inflammatory response triggers further necrotic events, thus gradually transforming the site of injury into large cavities devoid of cells (Zhang *et al.*, 1997). These cavities are typically bordered by scars that act as mechanical and biochemical barriers preventing the ingrowth of nerve fibers and the migration of cells into the lesion site (for review, see Reier *et al.*, 1983).

In *A. leptorhynchus*, the first apoptotic cells are detectable as early as 5 min after application of a lesion to the corpus cerebelli (Zupanc *et al.*, 1998). Thirty minutes after the lesion, the number of cells undergoing apoptosis reaches maximum levels. At 2 days postlesioning survival time, the number of apoptotic events, as indicated by TUNEL, starts to gradually decline until background levels are reached at approximately 20 days after the lesion (Figure 13).

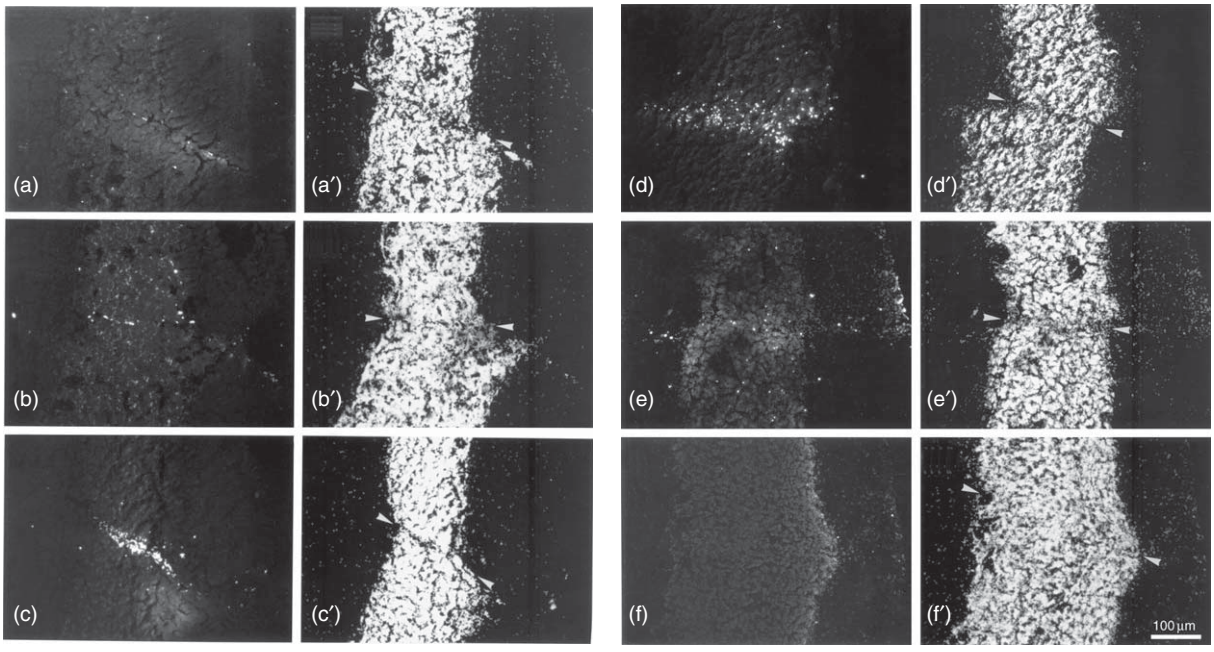

Figure 13 TUNEL-positive cells at the site of the lesion in the corpus cerebelli after survival times of 5 min (a), 15 min (b), 30 min (c), 1 day (d), 5 days (e), and 20 days (f). The location of the lesion is indicated by arrowheads in the DAPI counterstaining (a′–f′). The number of TUNEL-positive cells dramatically increases within the first 30 min, stays at high levels for another day, and then gradually declines to background levels. Reproduced from Zupanc, G. K. H., Kompass, K. S., Horschke, I., Ott, R., and Schwarz, H. 1998. Apoptosis after injuries in the cerebellum of adult teleost fish. *Exp. Neurol.* 152, 221–230, with permission from Elsevier.

Apoptosis is characterized by cell shrinkage, nuclear condensation, and production of membrane-enclosed particles that are digested by other cells. Most significantly, the side effects that accompany necrosis, such as inflammation of the surrounding tissue, are typically absent in apoptosis. This suggests that the use of this so-called clean type of cell death for the elimination of damaged cells is an essential component of the enormous regenerative capability of *A. leptorhynchus*.

Approximately 3 days after application of the lesion, the areal density of microglia/macrophages, identified by tomato lectin binding, starts to increase at and near the lesion site (Zupanc *et al.*, 2003; Figure 14). A similar increase, although less pronounced, is also seen in the contralateral hemisphere. The areal density of microglia/macrophages reaches maximum levels at approximately 10 days after the lesion and returns to background levels approximately 1 month after the lesion. The emergence of microglia/macrophages shortly after the number of apoptotic cells has reached maximum levels supports the notion that the former cells are involved in the removal of cellular debris caused by apoptotic cell death.

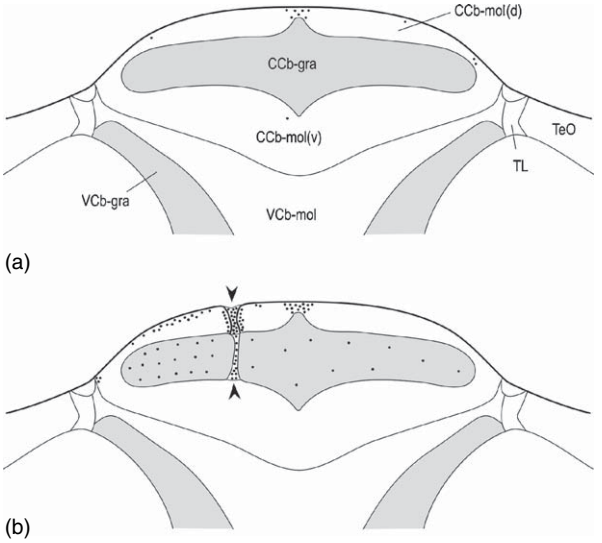

Figure 14 Distribution and relative density of lectin-labeled cells in the intact (a) and injured corpus cerebelli 10 days after setting the lesion (b). The schematic sketches are based on the analysis of camera lucida drawings of one complete series of lectin-labeled sections through the corpus cerebelli in one fish each. The number of black dots reflects the density of labeled cells in the various areas of the corpus cerebelli. The lesion (indicated by arrowheads) runs, in the case shown, from the brain surface through the dorsal molecular layer and the granule cell layer, without, however, entering the ventral molecular layer. CCb-gra, granule cell layer of corpus cerebelli; CCb-mol(d), dorsal molecular layer of corpus cerebelli; CCb-mol(v), ventral molecular layer of corpus cerebelli; TeO, optic tectum; TL, torus longitudinalis; VCb-gra, granule cell layer of valvula cerebelli; VCb-mol, molecular layer of valvula cerebelli. Reproduced from Zupanc, G. K. H., Clint, S. C., Takimoto, N., Hughes, A. T. L., Wellbrock, U. M. and Meissner, D. 2003. Spatio-temporal distribution of microglia/macrophages during regeneration in the cerebellum of adult teleost fish, *Apteronotus leptorhynchus* : A quantitative analysis. *Brain Behav. Evol.* 62, 31–42, with permission from S. Karger AG, Basel.

2.24.7.3 Generation of New Cells

The second major process mediating brain repair in *A. leptorhynchus* encompasses replacement of the neurons that are eliminated through apoptosis with newly generated ones. These neurons are recruited from two sources (Zupanc and Ott, 1999). First, new cells are produced in response to the injury in proliferation zones near the lesion; these areas of mitotic activity are detectable only after injury. Second, new cells are recruited from the pool of continuously generated undifferentiated cells.

The vast majority of cells of either of the two populations are produced 1–10 days following the lesion, with the maximum proliferative activity occurring at 5 days after injury. This upregulation of proliferative activity is thought to be due to an inductive effect exerted by the injury. In addition, cells that are born before the lesion also contribute to the restoration of damaged tissue. BrdU-labeling experiments have shown that cells generated as early as 2 days prior to lesioning of the cerebellum still participate in the process of regeneration (Zupanc and Ott, 1999).

The observation that cells generated before the injury can replace damaged neurons suggests a direct relationship between the continuous cell proliferation in the intact brain and the process of neuronal regeneration. The continuous provision of a pool of undifferentiated cells in the intact brain appears to enable fish to recruit new cells much more rapidly in the event of injuries than would be possible by recruiting only cells that are generated in response to such events. However, it is unclear whether this trait has, indeed, conferred a significant advantage in survival in the course of evolution. Individuals with brain injuries bear typically a dramatically increased risk of predation, particularly in the first few weeks before the wound has completely healed. It is, therefore, possible that the major driving force for the phylogenetic development of the continuous generation of new neurons in the adult brain has been the need to match the number of continuously produced new motor and sensory elements in the periphery of the intact organism (see Section 2.24.6), rather than the ability to regenerate brain tissue. Following this line of reasoning, the availability of a permanent pool of undifferentiated cells that can be rapidly accessed in case of an injury would be an epiphenomenon.

2.24.7.4 Migration and Differentiation of New Cells

As an important step in the further process of recruitment of the new cells for repair of the lesioned tissue, these cells have to migrate from the site of their production to the site of injury. Several lines of evidence suggest that this is achieved through guidance by radial glial fibers. Approximately 8 days following the lesion, the density of radial glial fibers, identified by morphological criteria and immunohistochemical staining against GFAP, increases greatly compared to the levels found in the intact cerebellum (Clint and Zupanc, 2001; Figure 15). This effect is restricted to the site ipsilateral to the lesion, and occurs at particularly high levels at and near the site of injury. The increase in radial fiber density is followed by a rise in the density of young cells, in the same cerebellar areas, approximately 2 days later. This arrival of young cells after the appearance of radial glial fibers, as well as the frequently observed close apposition of young cells to radial glial fibers, support the notion that radial glial fibers provide a scaffold to guide the young cells in the course of their migration from the site of origin to the site of injury.

Like the increase in areal density of GFAP-expressing radial glial fibers, the expression of vimentin in fibers at the site of the lesion and in the remaining ipsilateral molecular layer is upregulated in a specific spatiotemporal fashion (Clint and Zupanc, 2002). The areal density of vimentin-positive fibers increases significantly 15 days after application of a mechanical lesion to the corpus cerebelli and remains elevated throughout the time period of up to 100 days examined. It is possible that the vimentin-expressing fibers also represent a subpopulation of radial glia-like cells. Although there appears to be a certain degree of overlap between the two fiber populations, GFAP-positive fibers are typically found at greater distances from the lesion site in the ipsilateral hemisphere and exhibit a more elongated morphology than vimentin-positive fibers. Since the majority of the new cells arrive at the lesion site before the upregulation of vimentin, it is unlikely that this intermediate filament protein is involved in guidance of the migrating young neurons. Instead, vimentin may play a role in later developmental functions, such as promotion of cellular survival, differentiation, and/or outgrowth of dendrites.

Combination of BrdU labeling with neuronal tract tracing has shown that at least some of the new cells that replace damaged neurons are cerebellar granule cells (Zupanc and Ott, 1999). Because in

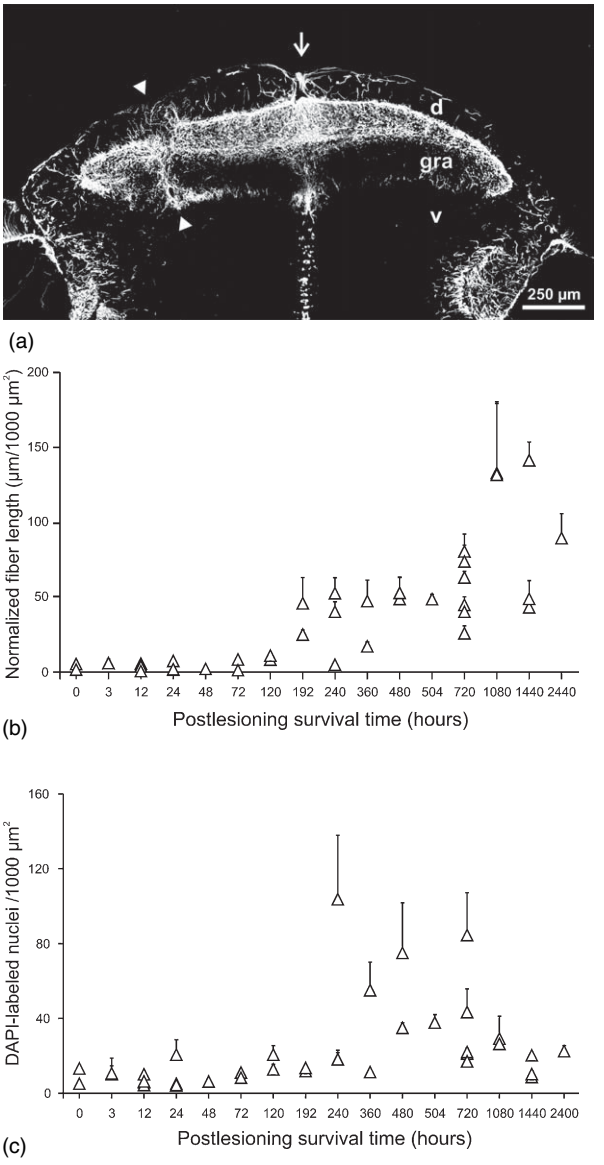

Figure 15 a, Transverse section through the corpus cerebelli of *A. leptorhynchus* 10 days after application of a mechanical lesion. The path of the lesion is indicated by arrowheads. The density of GFAP-labeled fibers is markedly higher at the site of the lesion and in the remaining ipsilateral dorsal molecular layer than in the contralateral dorsal molecular layer. The GFAP-stained radial glial fibers are also prominent at the midline (arrow), where they extend over considerable distances laterally in each hemisphere. d, dorsal molecular layer; gra, granule cell layer; v, ventral molecular layer. b, Areal density of lengths of GFAP-labeled fibers at the site of the lesion after various postlesioning survival times. c, Areal density of cells visualized by staining with the nuclear dye DAPI at the site of the lesion after various postlesioning survival times. The values plotted in (b) and (c) are means of individual fish. The survival time of '0' indicates the results of unlesioned fish. The vertical bars represent standard errors. Reproduced from Clint, S. C. and Zupanc, G. K. H. 2001. Neuronal regeneration in the cerebellum of adult teleost fish, *Apteronotus leptorhynchus*: Guidance of migrating young cells by radial glia *Dev. Brain Res.* 130, 15–23, with permission from Elsevier.

this study the granule cell neurons have been identified by deposition of the tracer substance into the molecular layer of the corpus cerebelli and through subsequent retrograde transport of the tracer to somatic regions, this result also shows that these cells have established axonal projections with the associated molecular layer.

2.24.8 Identification of Regeneration-Associated Proteins

One of the central goals of research on neuronal regeneration in fish is to gain an understanding of the molecular mechanisms mediating this phenomenon. A large-scale identification of protein candidates involved in the underlying processes in the teleostean brain has been carried out by combining the cerebellar lesion paradigm with differential proteom analysis (Zupanc *et al.*, 2006). In an initial study, a postlesion survival time of 3 days was chosen because a previous investigation has demonstrated the importance of this time point, especially for the recruitment of newly generated cells replacing the ones lost to injury (Zupanc and Ott, 1999). At 3 days following lesion, cell proliferation is dramatically upregulated, both in areas near the lesion and in proliferation zones that continuously generate new cells in intact fish.

For the proteome analysis, abundance of proteins in tissue from the site of the lesion was compared with the abundance of the same proteins in an equivalent region of the intact cerebellum. Two-dimensional gel electrophoresis of protein extracts from these two types of tissue samples revealed nearly 800 protein spots (Figure 16). Out of these total number of spots, spot intensity was significantly increased at least twofold in 30 spots and decreased to at least half the intensity of intact tissue in 23 spots (Figure 17). Since this investigation was restricted to the examination of cytosolic proteins, as well as proteins with isoelectric points between 4 and 7, the result indicates that the total number of proteins potentially involved in regeneration of the injured cerebellum at the postlesion survival time examined may well exceed 100.

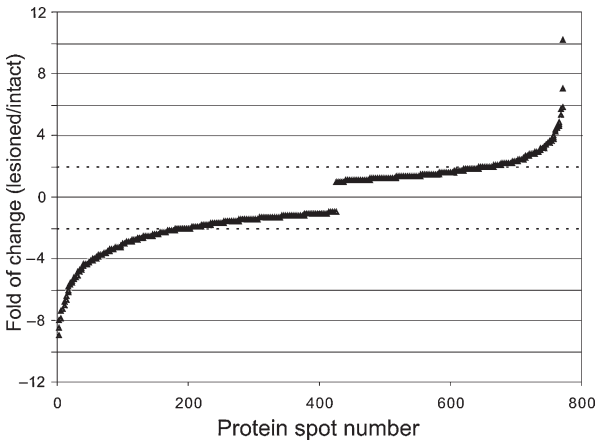

Figure 17 Scatter plot of the fold changes of 772 polypeptide spots in the corpus cerebelli of *A. leptorhynchus* 3 days after a lesion compared to unlesioned controls. Decrease in protein abundance is indicated by negative changes, increase by positive changes. Proteins are sorted in ascending order of the spot intensity change. The analysis is based on a comparison of the averaged spot intensities of four 2D gels from lesioned brains and three 2D gels from intact brains. The dotted lines indicate the +2-fold and −2-fold threshold. Reproduced from Zupanc, M. M., Wellbrock, U. M., and Zupanc, G. K. H. 2006. Proteome analysis identifies novel protein candidates involved in regeneration of the cerebellum of teleost fish. *Proteomics* 6, 677–696, with permission from Wiley-VCH.

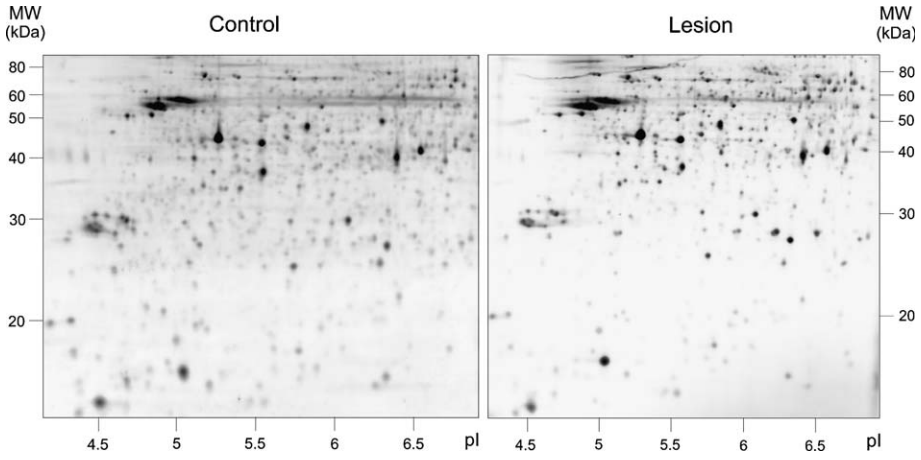

Figure 16 Images of two-dimensional gels of proteins from the intact corpus cerebelli (control) and lesioned corpus cerebelli (lesion) at a postlesion survival time of 3 days in *A. leptorhynchus*. Reproduced from Zupanc, M. M., Wellbrock, U. M., and Zupanc, G. K. H. 2006. Proteome analysis identifies novel protein candidates involved in regeneration of the cerebellum of teleost fish. *Proteomics* 6, 677–696, with permission from Wiley-VCH.

The proteins associated with 24 of the 53 spots that showed significant intensity differences after application of a lesion to the cerebellum in *A. leptorhynchus* could be identified by peptide mass fingerprinting and mass spectrometry/mass spectrometry fragmentation. Table 1 lists these identified protein spots, together with the changes found in the lesioned corpus cerebelli, relative to an equivalent area in the intact corpus. Also included in this list are the proposed functions of these proteins in the process of tissue regeneration. Detailed analysis has shown that these proteins can be divided into three major groups:

1. The first group includes cytoskeletal proteins essential for the formation of new cells, such as β-actin and β-tubulin. The transient upregulation of β-actin expression appears to relate to the regeneration of injured axons in the area of the lesion and the development of axons of the newly generated cells, as suggested by immunohistochemical experiments (Zupanc *et al.*, 2006; see Section 2.24.9.1). The transient increase in abundance of β-tubulin is closely associated with the appearance of thin capillaries at the site of the lesion several days after application of the stab wound, presumably reflecting the generation of new endothelial cells (Zupanc *et al.*, 2006; see Section 2.24.9.2). Also included in this first group are proteins that mediate the correct assembly of these structural

Table 1 Differentially expressed proteins 3 days after application of a lesion to the corpus cerebelli of *A. leptorhynchus*. The proteins were identified by proteome analysis

Protein name	*Fold change*	*M_r/pI*	*Proposed function*
β-Actin	2.1	42/5.3	Cytoskeletal protein of axons
β-Tubulin	2.9	50/4.8	Cytoskeletal protein of axons and endothelial cells
β1-Tubulin	3.6	50/4.8	Cytoskeletal protein related to neurogenesis
Keratin-10	0.5	59/5.1	Intermediate filament protein; negative regulation of cell proliferation
Chaperonin containing tailless-complex polypeptide 1, subunit ε	2.4	60/5.4	Chaperoning of cytoskeletal proteins
Tropomodulin-3 and -4	2.3	40/4.7	Capping of slow-growing ends of actin filaments
Bullous pemphigoid antigen 1	4.2	38/6.4	Linking of actin with intermediate filament proteins
Myosin heavy chain	2.5	22/5.5	Cellular motility of axons
B2-Lamin	2.9	68/5.4	Nuclear assembly during mitosis
78 kDa glucose-regulated protein	3.7	73/5.0	Neuroprotection by reducing apoptotic cell death
Glutamine synthetase	2.2	42/6.4	Neuroprotection by converting glutamate into glutamine
Cytosolic aspartate aminotransferase	0.5	47/6.7	Neuroprotection by reduction of glutamate and aspartate
α-Enolase	2	48/6.2	Energy metabolism
β-Enolase	2.1	47/7.1	Energy metabolism
F-ATP synthase β-subunit	2.1	55/5.1	Energy metabolism
Vacuolar adenosine triphosphatase	2.5	69/5.4	Possibly regulation of cellular growth
Calcineurin	0.4	60/5.6	Regulation of apoptotic cell death
70 kDa heat-shock cognate protein	2.8	71/5.3	Protein folding; possibly regulation of development
Phosphoglycerate kinase	2.1	43/5.9	Glycolysis; modulation of DNA polymerization
Creatine kinase	2.3	43/5.5	Energy metabolism; regulation of cell proliferation
Bone marrow zinc finger 2	0.4	85/9.7	Transcriptional regulation
Regeneration-associated protein 1 (similarities to steroid sensitive gene-1 protein from *Danio rerio*)	4.2	100/9.7	?
Regeneration-associated protein 2 (similarities to protein CG9699-PG from *Drosophila melanogaster*)	0.4	50/6.2	?

M_r, molecular weight (kDa); pI, isoelectric point.

Reproduced from Zupanc, M. M., Wellbrock, U. M., and Zupanc, G. K. H. 2006. Proteome analysis identifies novel protein candidates involved in regeneration of the cerebellum of teleost fish. *Proteomics* 6, 677–696, with permission from Wiley-VCH.

proteins. Among the latter are chaperonin-containing tailless-complex polypeptide 1, subunit ε, which might mediate chaperoning of cytoskeletal proteins; tropomodulins-3 and -4, which are known to perform a capping of slow-growing ends of actin filaments; and bullous pemphigoid antigen 1, which has been proposed to link actin with intermediate filament proteins.

2. The second group is comprised of proteins potentially involved in cell proliferation (such as keratin-10), cellular motility (such as myosin heavy chain), neuroprotection (such as 78 000-Da glucose-regulated protein, glutamine synthetase, and cytosolic aspartate aminotransferase), and energy metabolism (such as α- and β-enolase, as well as F-ATP synthase β-subunit). Remarkably, the abundance of glutamine synthetase is increased after cerebellar lesions in fish, whereas this enzyme is downregulated under traumatic conditions in the mammalian central nervous system. Such differences in the expression pattern of proteins between fish and mammals are particularly interesting because they are likely to provide a molecular explanation for the enormous difference in the regenerative potential between the two vertebrate groups.
3. The third group consists of a single protein, bone marrow zinc finger 2. This protein was first identified from a screen of zinc finger proteins expressed in the hematopoietic system (Han *et al.*, 1999). Although little is known about its expression pattern and function, its molecular structure and some experimental evidence suggest that it acts as a transcriptional regulator. Such evidence includes studies on Wilms' tumor, a pediatric kidney cancer. As has been shown, bone marrow zinc finger 2 associates with Wilms' tumor suppressor, another zinc finger protein, and inhibits transcriptional activity of the latter (Lee *et al.*, 2002). Nothing is known about its role in the nervous system. The proteome analysis of regeneration-associated protein in the cerebellum of *A. leptorhynchus* has provided the first indication of an expression of the gene encoding bone marrow zinc finger 2 in the brain and of a possible role in the process of brain repair. Since transcription factors function as master regulators of downstream proteins, bone marrow zinc finger 2 is an important candidate to examine the upstream mechanisms controlling regeneration of brain tissue. Interestingly, increase in abundance of several zinc finger proteins has also been found in a recent proteome analysis in the rat after spinal cord transection (Ding *et al.*, 2006).

2.24.9 Immunohistochemical Characterization of Regeneration-Associated Proteins

Based on the above-described proteome analysis and on immunohistochemical screening of candidate proteins in cryosections taken from the lesion site in the corpus cerebelli after various postlesion survival times, a number of regeneration-associated factors have been identified in *A. leptorhynchus*. The spatiotemporal expression pattern of four of them has been examined in detail: β-actin, β-tubulin, calbindin-D_{28k}, and somatostatin. The results of these investigations will be compared with the time course of the major regenerative processes, as described above and summarized in Figure 18.

2.24.9.1 β-Actin

Actin, one of the major constituents of the cytoskeleton, is highly conserved between species (for review, see Schoenenberger *et al.*, 1999). Together with other cytoskeletal proteins, it provides an intracellular scaffolding of the cell. In addition, actin is involved in cell motility and the transport of intracellular cargos to their destinations, particularly along the axon. One of its three major isoforms, β-actin, is a 42-kDa protein enriched in actively growing structures, or structures with a high capacity for morphological modifications, such as growth cones, filopodia, migrating cell bodies, axonal tracts, and dendritic spines (Kaech *et al.*, 1997; Bassell *et al.*, 1998; Micheva *et al.*, 1998). Similarly, regeneration studies have demonstrated an upregulation of actin within a few days after axotomy, reflecting the emergence of axonal sprouts and the concomitant needs to lengthen the axon shaft and to support membrane expansion at the growth cone (Tetzlaff *et al.*, 1991; Lund and McQuarrie, 1996; Lund *et al.*, 2002). Congruent with this finding, induction of axonal sprouting by treatment of the spinal cord of adult rats with antibodies against the neurite outgrowth inhibitor Nogo-A led to an increased transcription of α-actin (Bareyre *et al.*, 2002).

In *A. leptorhynchus*, Western blot analysis of tissue lysates from the area around the lesion in the corpus cerebelli has revealed a single protein band at approximately 42 kDa recognized by antibodies directed against a conserved β-cytoplasmic actin N-terminal peptide (Zupanc *et al.*, 2006). This molecular weight is virtually identical to that of the protein recognized by the same antibody in lysates from mouse cerebellum, thus suggesting that in the corpus cerebelli of *A. leptorhynchus* a fish orthologue of β-actin is expressed.

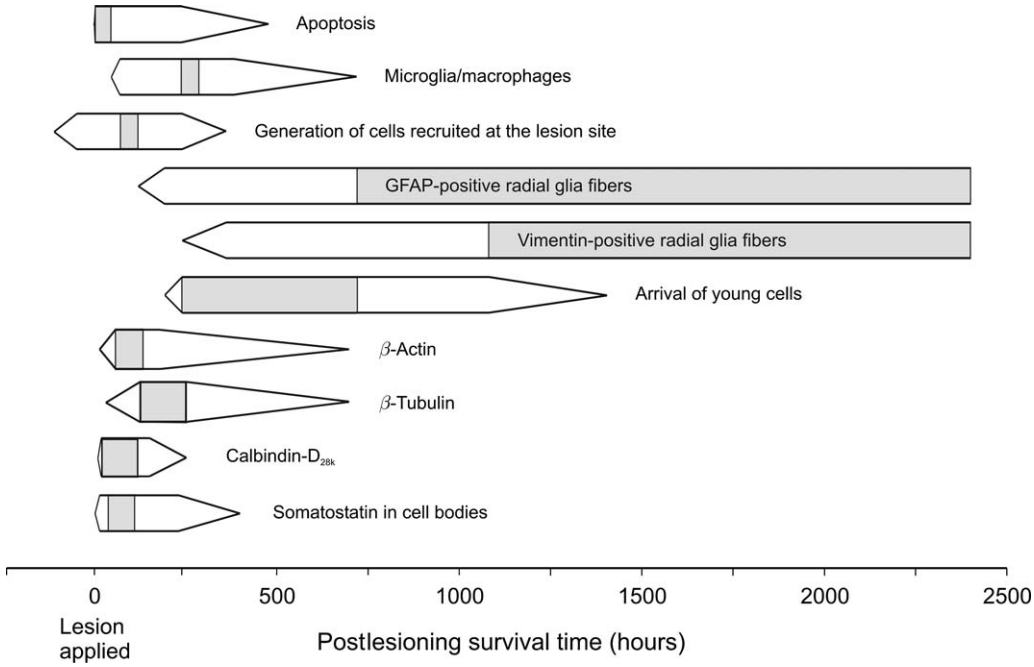

Figure 18 Schematic diagram of the time course of events in response to mechanical lesions applied to the corpus cerebelli of *A. leptorhynchus*. From the top these are: (1) Occurrence of apoptotic cell death at and near site of lesion (Zupanc *et al.*, 1998); (2) increase in density of microglia/macrophages throughout the corpus cerebelli (Zupanc *et al.*, 2003); (3) generation of new cells recruited to site of lesion 28 days after the injury (Zupanc and Ott, 1999; note that even cells born up to 2 days before the injury participate in the process of neuronal regeneration); (4) increase in density of GFAP-positive radial glia fibers at and near site of lesion (Clint and Zupanc, 2001); (5) increase in density of vimentin-positive radial glia fibers at and near site of lesion; (6) arrival of young cells at site of lesion (Clint and Zupanc, 2001); (7) upregulation of β-actin immunoreactivity at and near site of lesion (Zupanc *et al.*, 2006); (8) upregulation of β-tubulin immunoreactivity and appearance of new capillaries at and near site of lesion (Zupanc *et al.*, 2006); (9) upregulation of calbindin-D_{28k} immunoreactivity at and near site of lesion (Zupanc and Zupanc, 2006b); (10) upregulation of somatostatin immunoreactivity in somata of three cell types at and near site of lesion (Zupanc, 1999b). The first slope of each bar represents the time period during which the event shows an increase from background levels. The shaded area represents the peak period of the event. The second slope represents the time period during which the event decreases to background levels.

Immunohistochemical studies have shown that the fish orthologue of β-actin is exclusively expressed in fibers within the corpus cerebelli of *A. leptorhynchus* (Zupanc *et al.*, 2006). However, while such fibers are virtually absent in the intact corpus cerebelli (with the exception of an area at the midline of the granular layer; Figure 19a), β-actin-immunoreactive fibers appear in the area of the lesion as early as a few hours after the lesion. Between 1 and 5 days after lesion, these fibers develop into a dense, intensively labeled plexus (Figures 19b and 19c). After approximately 10–15 days, both the number of fibers and the intensity of the immunolabel gradually return to background levels (Figure 19d).

As in mammalian systems, it is likely that the transient upregulation of β-actin in the corpus cerebelli of *A. leptorhynchus* is associated with the regeneration of injured axons and the development of axons of newly generated cells. Such a notion is compatible with both the labeling pattern and the time course of the increase in protein abundance (Figure 18).

2.24.9.2 β-Tubulin

Tubulin, the principal component of microtubules, is a heterodimer of two closely related proteins, α- and β-tubulin, each with a molecular weight of approximately 50 kDa. Both the α- and β-subunit consist of a family of homologous isoforms, which appear to be related to different microtubule functions. Western blots using brain lysates of *A. leptorhynchus* have, in addition to the protein band at 50 kDa, revealed a second protein band at approximately 30 kDa (Zupanc *et al.*, 2006). The significance of this phenomenon is unclear because such an additional band has not been described in mammalian systems. Another remarkable feature of the anti-β-tubulin antibodies employed in this study was that they immunostained exclusively blood vessels and capillaries, both of which form a rich vascular bed in the granular and molecular layers (Figure 20a).

After application of lesions to the corpus cerebelli, both the composition of the immunolabeled

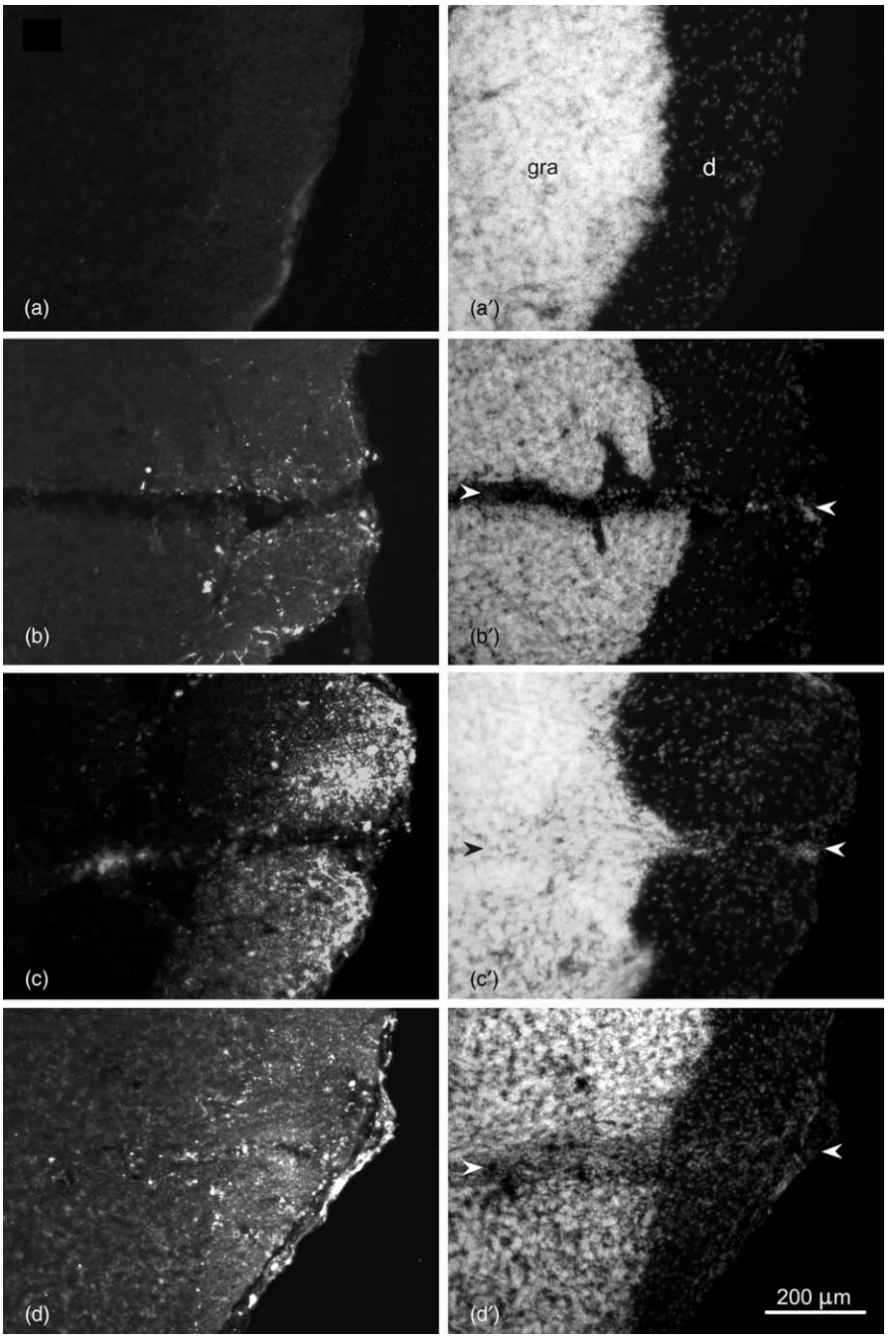

Figure 19 Pattern of immunostaining against β-actin in the intact (a) and injured (b–d) corpus cerebelli of *A. leptorhynchus*. In the DAPI counterstain of the transverse sections (a′–d′), the right hemisphere is shown with its granular layer (gra) and dorsal molecular layer (d) (dorsal: right; ventral: left; medial: up; lateral: down). The lesion path is indicated by arrowheads. Whereas in the intact corpus cerebelli immunolabeling is virtually absent (a), immunoreactive fibers have developed at 1 day after lesion in the area around the lesion within the dorsal molecular layer (b). At 3 days after lesion, the size of this fiber plexus and the intensity of immunostaining of fibers has increased (c). At 15 days after the lesioning of the cerebellum, both the number and the intensity of immunolabeling has declined compared to the pattern observed at the 3-day survival time (d). Reproduced from Zupanc, M. M., Wellbrock, U. M., and Zupanc, G. K. H. 2006. Proteome analysis identifies novel protein candidates involved in regeneration of the cerebellum of teleost fish. *Proteomics* 6, 677–696, with permission from Wiley-VCH.

structures and the intensity of the label exhibit pronounced changes (Zupanc *et al.*, 2006). In the first few days, the intensity of immunolabeling increases at the lesion site, compared to more distant regions or the equivalent areas contrallaterally or in the intact corpus cerebelli (Figure 20b). Five days after the lesion, the increase in the intensity of immunolabeling is paralleled by a pronounced rise in the

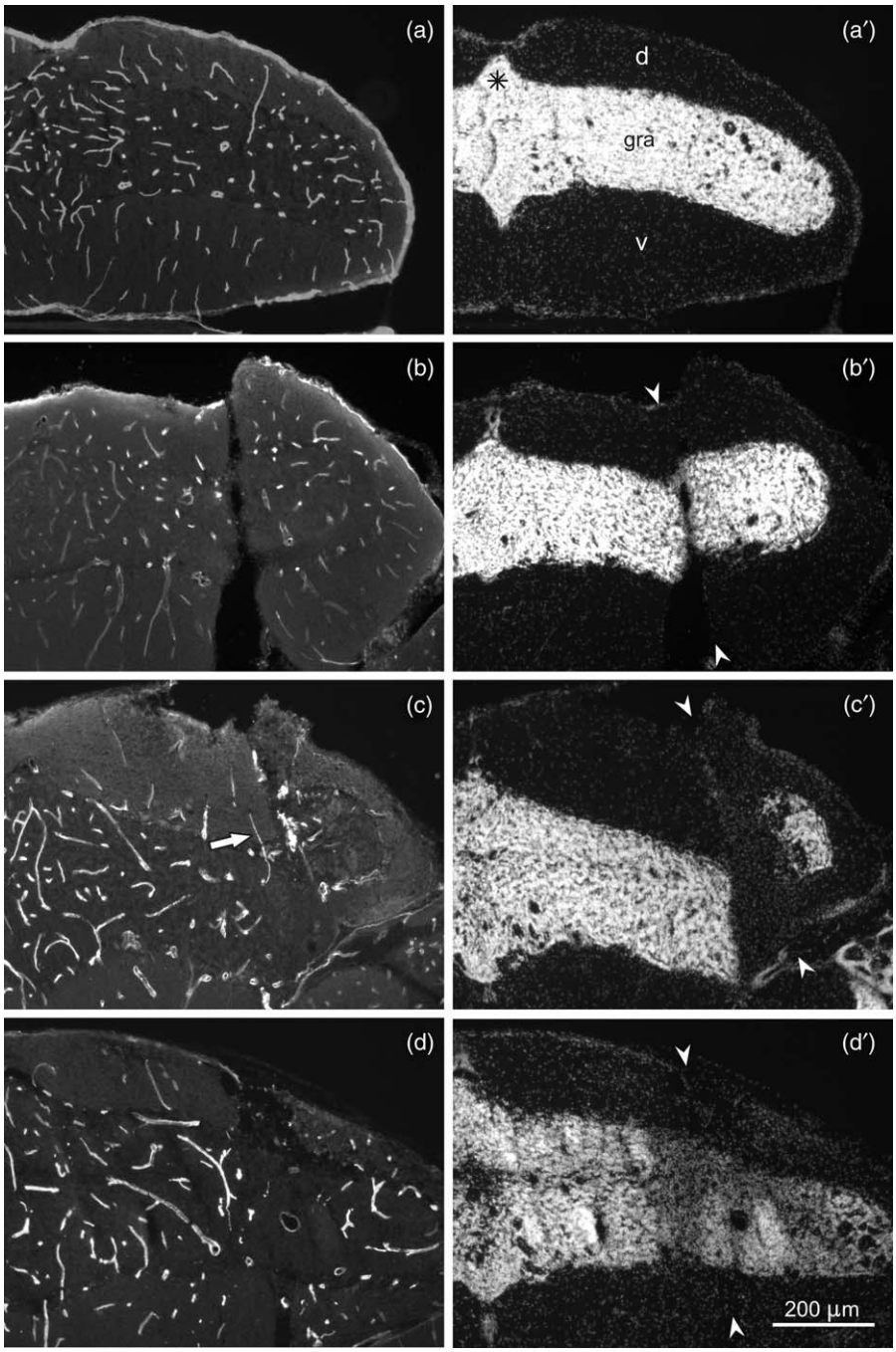

Figure 20 Blood vessels and capillaries immunostained against β-tubulin in the intact (a) and injured (b–d) corpus cerebelli of *A. leptorhynchus*. In the DAPI counterstain of the transverse sections (a′–d′), the right hemisphere is shown with its granular layer (gra), the dorsal molecular layer (d), and the ventral molecular layer (v) (dorsal: up; ventral: down; medial: left; lateral: right). The dorsal tip, a protrusion of the granule cell layer into the dorsal molecular layer at the midline, is indicated by * (a′) and the path of the lesion by arrowheads (b′–d′). Whereas in the intact CCb mostly large blood vessels are immunostained, and the intensity of immunolabeling is similar throughout the granular layer and the dorsal molecular layer (a), this pattern changes after application of a lesion (b–d). At 1 day after lesion, an increase in the intensity of immunolabeling is restricted to the area around the lesion (b). At 5 days after lesion, the intensity of immunolabeling remains elevated in the lesion path, but is also increased in other areas of the right hemisphere (c). In addition, the number of thin capillaries is increased in the area of the lesion. At 30 days after lesion, the lesion path and its immediate vicinity has become largely devoid of immunolabeled larger blood vessels, whereas, the number of immunolabeled thin capillaries in this area is higher than in the remaining regions of the CCb (d). Two of these thin capillaries are indicated by arrows. Note the restoration of a major part of the granular and molecular layers in the area of the lesion at the latter survival time, as evident through the DAPI counterstain (d′). Reproduced from Zupanc, M. M., Wellbrock, U. M., and Zupanc, G. K. H. 2006. Proteome analysis identifies novel protein candidates involved in regeneration of the cerebellum of teleost fish. *Proteomics* 6, 677–696, with permission from Wiley-VCH.

number of immunopositive vascular structures; the latter is mainly caused by the appearance of many thin capillaries (Figure 20c). At postlesion survival times of 10, 15, and 30 days, a gradual decline in the number of larger blood vessels is found in the area of the lesion, whereas the number of thin capillaries, particularly in the immediate vicinity of the lesion path, remains larger than in other areas of the corpus cerebelli (Figure 20d).

This pattern of immunolabeling suggests two major processes following a lesion: First, the blood vessels in the area of the lesion undergo cell death within a few weeks after the injury. Second, the depletion of large blood vessels is paralleled by a repopulation of the lesion area by thin capillaries, probably due to angiogenesis.

2.24.9.3 Calbindin-D_{28k}

Calbindin-D_{28k}, originally discovered as a vitamin D-dependent calcium binding protein with a molecular weight of approximately 28 kDa in the chick intestinal mucosa (Wasserman and Taylor, 1966), has been found in many types of tissue, including brain, in a variety of vertebrate species (for review, see Christakos *et al.*, 1989). Within the cell, this protein plays an important role as a calcium buffer in the maintenance of calcium homeostasis (for review, see Baimbridge *et al.*, 1992).

Western blot analysis of brain lysates of *A. leptorhynchus* has demonstrated the presence of a protein band with a molecular weight of approximately 28 kDa recognized by anti-rat-calbindin-D_{28k} antibodies. This suggests that this protein in the brain of *A. leptorhynchus* is similar to the mammalian form of calbindin-D_{28k} (Zupanc and Zupanc, 2006b). Immunohistochemical staining has indicated the absence of calbindin-D_{28k} immunoreactivity in most parts of the intact corpus cerebelli of *A. leptorhynchus*, including the region corresponding to the wider area of the lesion site in lesioned individuals.

Unlike the intact corpus cerebelli, calbindin-D_{28k} immunoreactive cell bodies and fibers are evident in the lesion path and the immediate vicinity of the lesion in the period between 16 h and 7 days after the lesion (Zupanc and Zupanc, 2006b; Figure 21). Both the number of immunolabeled cells and the intensity of the label are most pronounced 1–3 days after lesion. Analysis of the morphology of the immunostained cells by confocal microscopy suggests that most, and perhaps all of them, are granular neurons.

Since the transient upregulation of calbindin-D_{28k} is paralleled by a decline in the number of cells undergoing apoptotic cell death (Figure 18), it has been hypothesized that this protein exerts a neuroprotective function by buffering free intracellular Ca^{2+}, the concentration of which is commonly elevated after brain insults. Such a role of calbindin-D_{28k} has also been proposed in a variety of other brain systems and is supported by several lines of evidence, including age-related decreases in calbindin-D_{28k}-expressing neurons, particularly in brain areas selectively vulnerable in neurodegenerative diseases of the elderly (Iacopino and Christakos, 1990; Kishimoto *et al.*, 1998; Bu *et al.*, 2003; Geula *et al.*, 2003); reduced levels of calbindin-D_{28k} expression in the brains of humans suffering from neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's, as well as in animal models mimicking such diseases (Iacopino and Christakos, 1990; Iacopino *et al.*, 1992; Ng *et al.*, 1996; Thorns *et al.*, 2001); the relative resistance of calbindin-D_{28k}-expressing neurons to neurotoxicity induced by glutamate, calcium ionophore, or acidosis (Mattson *et al.*, 1991); and an increase in the survival of neurons after various types of insults by overexpression of the gene for calbindin-D_{28k} (Ho *et al.*, 1996; Phillips *et al.*, 1999; Monje *et al.*, 2001; D'Orlando *et al.*, 2002). Such a neuroprotective effect is paralleled, and probably mediated, by the Ca^{2+}-buffering capacity of calbindin-D_{28k}, as hippocampal neurons expressing this protein, which are relatively resistant to neurotoxicity, exhibit a greater capability to reduce free intracellular Ca^{2+} levels than calbindin-D_{28k}-negative neurons in cell culture (Mattson *et al.*, 1991).

2.24.9.4 Somatostatin

Somatostatin (= somatotropin-release inhibiting factor, SRIF) is one of the most abundant neuropeptides in the mammalian brain, displaying concentrations between 1000 and more than 4000 pmol g^{-1} protein (Crawley, 1985). In mammals, the biologically active forms of somatostatin, a tetradecapeptide (SRIF-14), and an N-terminally extended form of SRIF-14 consisting of 28 amino acids (SRIF-28), are synthesized as part of a large precursor molecule (prepro-somatostatin) comprising 116 amino acids (for review, see Patel, 1992). This molecule, encoded by a single gene, is rapidly cleaved into the prohormone form (pro-somatostatin; 92 amino acids), which is further processed into various smaller molecules including SRIF-14 and SRIF-28. SRIF-14 is totally conserved from fish to human (Vale *et al.*, 1976; King and Millar, 1979). Additional forms of somatostatin have been identified in several fish species. These fish-specific

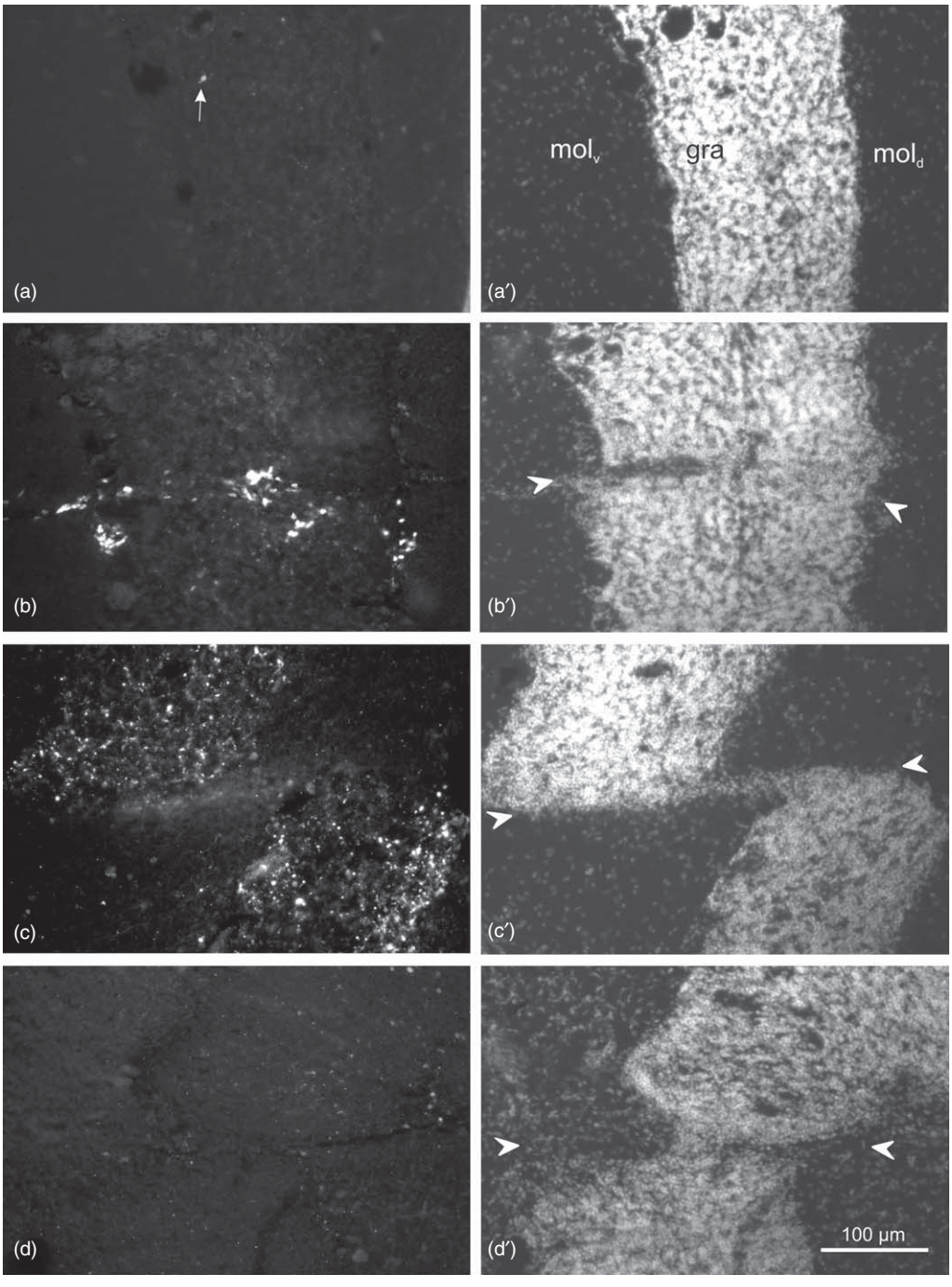

Figure 21 Calbindin-D_{28k} immunoreactivity in the intact corpus cerebelli (a) and the lesioned corpus cerebelli after survival times of 1 day (b), 2 days (c), and 7 days (d). The cytoarchitecture (gra, granular layer; mol_d, dorsal molecular layer; mol_v, ventral molecular layer), including the lesion path (arrowheads), are shown in the corresponding DAPI counterstains (a′–d′). In the intact corpus cerebelli, immunolabeling is sparse (a). The arrow points to a single immunopositive cell body. Intensively labeled cell bodies are associated with the path of the lesion at a postlesion survival time of 1 day (b). Labeled somata intermingle with an intensely labeled fiber plexus at 2 days postlesion (c). At 7 days postlesion, the number and intensity of immunolabeled structures are dramatically reduced and have almost reached control levels (d). Reproduced from Zupanc, G. K. H. and Zupanc, M. M. 2006b. Upregulation of calbindin-D_{28k} expression during regeneration in the adult fish cerebellum. *Brain Res.* 1095, 29–34, with permission from Elsevier.

forms are derived from a second gene, which apparently has been lost in the course of evolution leading to higher vertebrates (for review, see Patel, 1992).

In *A. leptorhynchus* and the closely related genus *Eigenmannia*, somatostatin-like immunoreactivity (Sas and Maler, 1991; Zupanc *et al.*, 1991a; Stroh and Zupanc, 1993, 1995, 1996) and somatostatin

messenger RNA (Zupanc *et al.*, 1991b) are widely distributed within the brain. The endogenous ligand interacts with specific receptors, as indicated by the demonstration of somatostatin-receptor binding sites (Zupanc *et al.*, 1994) and the molecular cloning and pharmacological characterization of a somatostatin receptor resembling the mammalian sst_3 receptor subtype (Zupanc *et al.*, 1999; Siehler *et al.*, 1999, 2005).

In the intact corpus cerebelli, very few cells displaying somatostatin-like immunoreactivity are found. However, their numbers increase dramatically at the lesion site 1 day after application of the stab wound and reach peak levels at 2 days, followed by a rapid decline to background levels between 5 and 10 days after lesion (Zupanc, 1999b; Figures 22 and 23).

Since the time period during which somatostatin is upregulated coincides with the period during which most of the cells later incorporated at the site of the lesion are generated (Figure 18), it is possible that this neuropeptide is involved in the regulation of cell proliferation. Indeed, a growth-inhibiting action of somatostatin is well established for numerous tumors (for review, see Pollak and Schally, 1998) and for the regeneration of several peripheral organs (Kokudo *et al.*, 1992; Thompson *et al.*, 1993; Zieleniewski and Zieleniewski, 1993; Bufalari *et al.*, 1996). Somatostatin appears to exert this function though a cell-cycle block due to G_1 arrest (Mascardo and Sherline, 1982) or through induction of apoptosis (Szende *et al.*, 1989; Srikant, 1995).

Alternatively, somatostatin may act as a trophic factor-like substance by influencing the early stages of differentiation of the young granule cell neurons. Evidence for such a function stems from developmental studies in mammals. In the cerebellum, somatostatin-binding sites are transiently expressed during a restricted period shortly after birth when the cerebellar granule cells are formed. Medium supplemented with somatostatin induces neurofilament synthesis in cultured granule cells (Taniwaki and Schwartz, 1995; Schwartz *et al.*, 1996), thus supporting the notion that this neuropeptide could act as a differentiation factor.

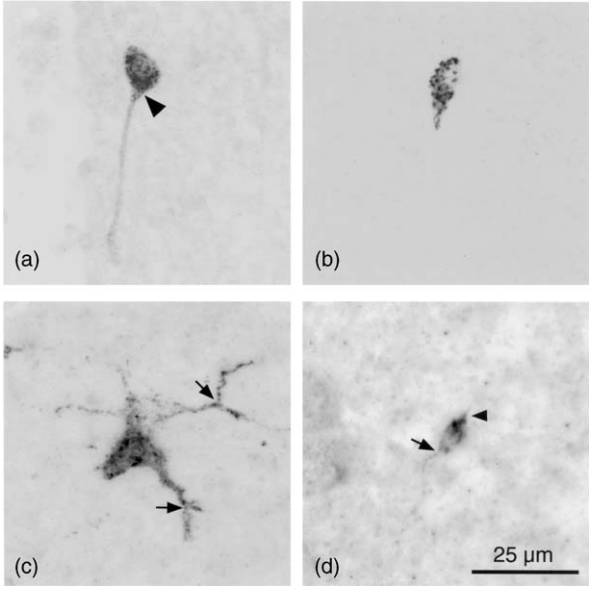

Figure 22 Confocal images of cell types in the corpus cerebelli expressing somatostatin after lesions. a, Cell type-1 is comprised of ovoid-shaped cells from which one process emerges (arrowhead). b, The cytoplasm of cells of type-1 is distinguished by grains filled with intensely labeled immunoreactive material. c, The typical feature of type-2 cells is the large, multipolar morphology. Their processes are relatively thick and bifurcate shortly after having emerged from the soma (arrows). d, Cells comprising type-3 are small. One process originating from the cell shown in the confocal image is clearly labeled (arrow). At the opposite pole, the initial segment of another process is visible (arrowhead). Based on their morphological features, type-1 cells are probably granular neurons, type-2 cells astrocytes, and type-3 cells microglia. Reproduced from Zupanc, G. K. H. 1999b. Upregulation of somatostatin after lesions in the cerebellum of the teleost fish *Apteronotus leptorhynchus. Neurosci. Lett.* 268, 135–138, with permission from Elsevier.

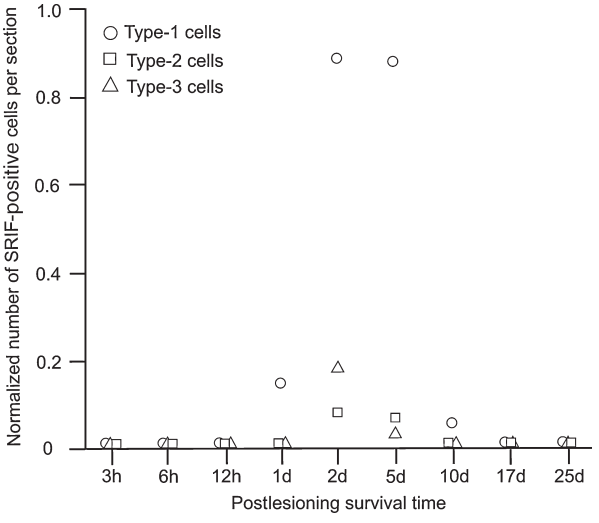

Figure 23 Temporal pattern of antisomatostatin immunoreactivity expressed by three different types of cells after application of lesions to the corpus cerebelli. In each of the three cell types, the level of SRIF is very low within the first 12 h following the lesion. Then, over the next few days, somatostatin expression, as assessed by the normalized number of somatostatin-positive cells per section taken through the rostrocaudal extent of the lesion, dramatically increases. This number has returned to background levels by day 17 after the lesion. Reproduced from Zupanc, G. K. H. 1999b. Upregulation of somatostatin after lesions in the cerebellum of the teleost fish *Apteronotus leptorhynchus. Neurosci. Lett.* 268, 135–138, with permission from Elsevier.

2.24.10 Toward an Evolutionary Understanding of Adult Neurogenesis

Although adult neurogenesis has been intensively studied over the last decade, particularly since its existence was demonstrated in the human brain (Eriksson *et al.*, 1998), an insufficient amount of comparative data is available to reach a comprehensive understanding of the evolution of this phenomenon. This applies not only to vertebrates as a whole, but also to any of the other major taxonomic groups. In teleosts, for example, a detailed mapping of the proliferation zones in the whole adult brain has been conducted in just three out of an estimated 25 000 species: *A. leptorhynchus* (Zupanc and Horschke, 1995), the three-spined stickleback (Ekström *et al.*, 2001), and the zebra fish (Zupanc *et al.*, 2005). In other important clades, such as hagfishes, lampreys, or cartilaginous fishes, thus far no detailed investigation of adult neurogenesis has been published at all.

Despite these limitations, it appears likely that adult neurogenesis is a trait shared by all teleost fish (see Section 2.24.4). Moreover, in any of the teleostean species examined thus far, neurogenesis occurs in many regions of the adult brain, and the number of new neurons is immense relative to the total number of brain cells.

In contrast, in mammals adult neurogenesis is limited to two brain regions, and the overall rate of neurogenesis relative to the number of cells in the whole brain is very low. These limits of neurogenesis could be causally related to the fact that the number of sensory receptor cells and muscle fibers remains constant in mammals during adult life. The only exception is the generation of new primary sensory cells in the olfactory epithelium, which may prompt neurogenesis in the olfactory bulb and the hippocampus (see Section 2.24.6).

One possible explanation for this difference between teleost fish and mammals is that neurogenesis in the adult brain is a primitive trait that already existed some 400 Mya in the common evolutionary ancestors shared by modern mammals, birds, reptiles, amphibians, and ray-finned fishes. One major function of this trait has been to ensure a constant ratio of motor (sensory) elements versus central elements within motor (sensory) pathways when the number of peripheral elements grows. However, as soon as the pattern of muscle growth shifted from hyperplasia more toward hypertrophy in the course of the evolution of mammals, the neurogenic potential of brain structures forming part of the motor pathway was reduced in parallel. A similar reduction in the number of neurogenic brain regions and in the rate of neurogenesis occurred when the formation of new sensory cells was more and more abandoned. According to this hypothesis, neurogenesis would have continued only in the olfactory bulb and the hippocampus as a consequence of the continued mitotic activity in the olfactory epithelium.

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3.01 What Fossils Tell Us about the Evolution of the Neocortex

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Glossary

allometric Applied to morphology, the measures of two different organs or of an organ and the whole body.

archaic orders Orders of mammals that are entirely extinct.

encephalization Enlargement of the whole brain relative to its expected size.

encephalization quotient (EQ) Gross brain size relative to expected brain size; expected brain size is determined by the regression of brain size on body size in an appropriate set of species on which data are available, the empirical quotient is the residual from the regression on log-log coordinates.

endocast Cast molded by the cranial cavity in vertebrates. Also called endocranial cast.

foramen magnum The hole at the entry of the spinal cord into the cranial cavity. It may be thought of as 'medulla' in an endocast, but is actually larger, containing blood vessels and sinuses in addition to medulla and anterior spinal cord.

fossil brains Fossil endocasts interpreted as brains.

holarctic Northern Hemisphere.

neocortex A thin sheet of many millions of nerve cells in the telencephalon of the brain of all living mammals, often considered as involved in conscious thought. All sensory information (vision, hearing, smell, taste, etc.) is known to be represented in various localized regions in the neocortex, frequently in many 'projection areas'. Higher functions such as intention, motivation, and attention are correlated with neocortical activation. It is a uniquely mammalian structure, and in histological sections, in living species, it has six layers of nerve cells as distinct from other brain structures with fewer layers or without layering.

neocorticalization Enlargement of neocortex relative to expected enlargement from brain-body analysis. It can be measured as the ratio of neocortical surface area to total brain or endocast surface area.

neotropical Southern Hemisphere (primarily South and Central America).

paleoneurology The science that deals with the fossil evidence of the nervous system and behavior. Primary evidence is from endocasts, but also evidence of behavior inferred from skeletal adaptations for running or climbing, dental patterns, animal tracks, and nesting sites.

progressive orders Orders of mammals in which living species have survived.

relative neocortical surface area Ratio of neocortical surface area to total endocast surface area.

3.01.1 Introduction

The story of the brain's evolution is told by casts of the cranial cavities of extinct species. These endocasts document much of the evolution of the mammalian brain during the past 65 million years, the Cenozoic era. A single late Jurassic fossil (Simpson, 1927; Jerison, 1973) had extended the known evidence to about 150 Mya, and other

explorations (Hu *et al.*, 2005; Kielan-Jaworowska *et al.*, 2005; Novacek, 1996) fill gaps in our knowledge of the Cretaceous period (65–145 Mya). Mammals first appeared during the Triassic period of the Mesozoic, and it may one day be possible to trace the history of the mammalian brain almost to its beginnings, perhaps 225 Mya (see The Evolution of Neuron Classes in the Neocortex of Mammals, Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications).

Encephalization is the increase in relative size of the brain as a whole over geological time. Its history was reviewed in depth in Jerison (1973) (cf. Falk, 1992; Falk and Gibson, 2001). Other recent evolutionary analysis emphasizes methodological innovations in cladistic analysis, with major revisions of mammalian phylogeny (McKenna and Bell, 1997; cf. Simpson, 1945). This article is consistent with those revisions.

Our central topic is neocorticalization, the increase of the relative amount of neocortex in mammals. ('Relative' in this case refers to the ratio of surface area to total surface area of the endocast.) Identifiable neocortex is a feature of the external morphology only of mammalian brains, but neural structures with similar functional significance have also evolved in birds and reptiles (Butler and Hodos, 2005; Karten, 1997; Reiner *et al.*, 2005). Avian and reptilian brain structures homologous with mammalian neocortex must first have appeared in the common amniote ancestor of these classes of vertebrates, but fossils are unlikely to be helpful in identifying these earlier ancestral connections. The question for this article is whether there was a change in neocorticalization within the mammals as they evolved during the past 225 million years (see The Origin of Neocortex: Lessons from Comparative Embryology). Like the avian Wulst, which is absent in endocasts of the earliest birds, evidence of neocortex may have been absent in endocasts of the earliest mammals. We don't yet know, but I review what we do know in this article.

We know that the neocortex, like the brain as a whole, became relatively larger in some but not all mammalian lineages (Jerison, 1990). Neocortex is present in all living mammals. Fossils tell their history, and it is reassuring that their evidence is consistent with inferences from the comparative neuroanatomy of living species (Butler and Hodos, 2005; Johnson, 1990; Shimizu, 2001). Opossums and hedgehogs (*Didelphis virginianus* and *Erinaceus europaeus*) can still be viewed as 'primitive', and cats and dogs and monkeys may be thought of as 'advanced', although one recognizes that this is an arbitrary dichotomy. (Spellings and orthography in this article follow the rules of taxonomic nomenclature. Genus and species are italicized, genus capitalized and species in lowercase.) From the fossils, we learn approximately when some identifiable changes in the brain occurred and how they differed in different lineages. They are the real proof of what is otherwise conjecture, that in most mammal species the brain evolved to relatively larger size and that this encephalization was usually accompanied by increased neocorticalization. Surprisingly, there appears to have been no comparable change in the olfactory bulbs other than their reduction in primates evident since the Miocene (about 20 Mya) and their complete disappearance in at least some cetaceans even longer ago.

All of the neural adaptations recognizable in the fossils are ancient, many occurring tens of millions of years ago. The most recent changes have been within the hominins, the human lineage, in which the most recent measurable increases in encephalization appeared about 250 000 years ago in the Neandertal and sapient species (*Homo neanderthalensis* and *Homo sapiens*). (References in the text to Neandertal follow the current German spelling and capitalization of nouns. The Neandertals were named for the Neander Valley in which the first specimen was found in 1856. German is famous as a language that combines words and elongates them rather than keeping them as short phrases; thus, the Neander Valley (capitalized in English as a place name) was the 'Neanderthal' before the German spelling reform of 1908, when it became 'Neandertal'. The rules of taxonomic nomenclature preserve the first published name of *Homo neanderthalensis*. The initial capital letter for the German was dropped in the species name in deference to the taxonomic usage of lower case for species. The genus '*Homo*' is as named by Linnaeus in 1758. Specimens shown here are as catalogued in AMNH (American Museum of Natural History, New York), BMNH (Natural History Museum of London, British Museum), FMNH (Field Museum of Natural History, Chicago), WISC (University of Wisconsin), and UT (University of Texas, Department of Paleontology).) The evolutionary evidence is at the generic and species level. I review a few within-species differences (Figures 3 and 4), but these are small compared to between-species effects.

3.01.2 Fossil Brains

Molded by the cranial cavity, endocasts such as those reviewed here have been called fossil 'brains' (Edinger, 1929; see Kohring and Kreft, 2003). The

brain, as soft tissue, does not fossilize, of course, but endocasts in birds and mammals resemble brains with dura intact, and they often show the superficial pattern of sulci and gyri in remarkable detail. Further analysis relies on relationships of external structures to the functional and microscopic anatomy of brains in living animals. In this chapter, brains and endocasts are treated as equivalent to one another. For most purposes, one can ignore the small differences between them in size and shape and use the same terminology for parts of an endocast as for comparable parts of the brain.

Neocortex can be distinguished from other structures visible on the surface of an endocast in mammals by using the rhinal fissure as the landmark. The evidence is exemplified by the brain of the living armadillo shown in Figure 1a. Rhinal fissure can be traced backward from the dorsal margin of the olfactory tract, and the fissure visible on the brain is also visible on endocasts. Figure 1b is a coronal section to show how the rhinal fissure serves as the boundary between neocortex and paleocortex, with paleocortex identified by the darkly stained layer of neurons in lamina II. Neocortex is taken as dorsal to the rhinal fissure on brains and endocasts.

Endocasts can be made from the cranial cavity of any skull. Those made from fossils are of special interest, because they are a physical record of the actual evolution of the brain. Figure 2, for example, presents snapshots of three-dimensional (3-D) scans of endocasts of two Eocene fossil mammals that lived about 40 Mya, a prosimian primate *Adapis parisiensis* and an even-toed ungulate (artiodactyl), *Anoplotherium commune*. Their endocasts may be compared with the brains of the living bush baby (*Galago senegalensis*) and the living llama (*Lama glama*), a camelid distantly related to *Anoplotherium*. An endocast of another Eocene fossil artiodacyl, *Bathygenys reevesi*, is shown in Figure 5 and that of an archaic Paleocene herbivore (*Phenacodus primaevus*, order Condylarthra, 58 Mya) is shown in Figure 9.

The olfactory tract and rhinal fissure are easily distinguishable on the lateral surface of the endocasts of *Anoplotherium* and *Bathygenys*; they are less clear but also identifiable in *Phenacodus*. We identify and measure neocortical surface area as dorsal to the fissure. The rhinal fissure in *Adapis* follows a course very much like that in the living bush baby, partly hidden by the temporal lobe. To be able to see the rhinal fissure in this endocast, it has to be rotated a bit to display more of the ventral surface. One objective of 3-D analysis reported here was to rotate virtual endocasts in primates to expose neocortex and include primate measurements in the analysis of mammalian data. Neocorticalization is the increase of neocortex relative to the rest of the brain, and it can be measured as a ratio of surface areas on an endocast.

This article is primarily on a quantitative analysis, but there is one interesting qualitative feature evident in Figure 2 that may be important. Comparing the Eocene fossils and their living relatives there is an obvious difference in the flexure of the brain, in the extent to which it is curved about the primary anterior–posterior axis, which is more marked in the living ungulates than the primates but evident in both. For example, in the fossils, olfactory bulbs, forebrain, hindbrain, and medulla are more or less in a line like railroad cars. The brains of their living relatives are bunched up and more globular. The difference probably results from the patterns of relative growth of the skull and skeleton compared to

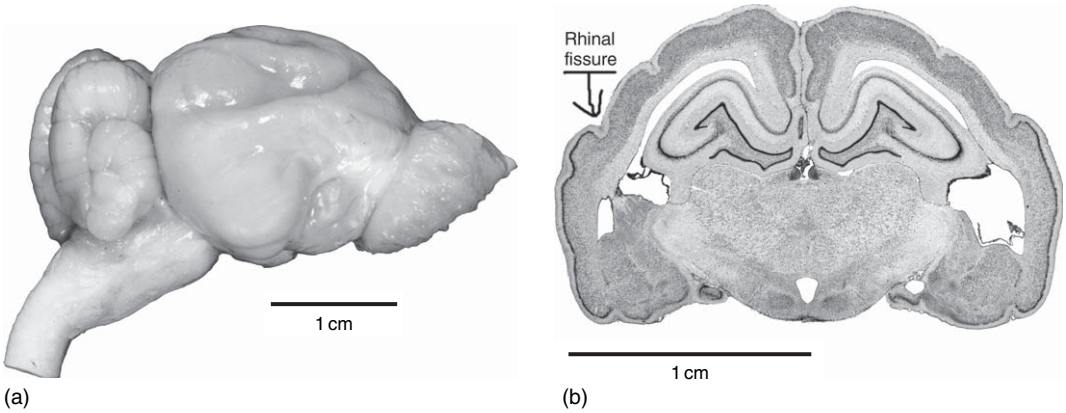

Figure 1 a, Brain of armadillo, *Dasypus novemcinctus*; rhinal fissure faintly visible dorsal to olfactory tract, then prominent further posteriorly. b, Coronal section of armadillo brain showing lamina II (dark stained layer) at border of rhinal fissure. Specimen WISC 60-465.

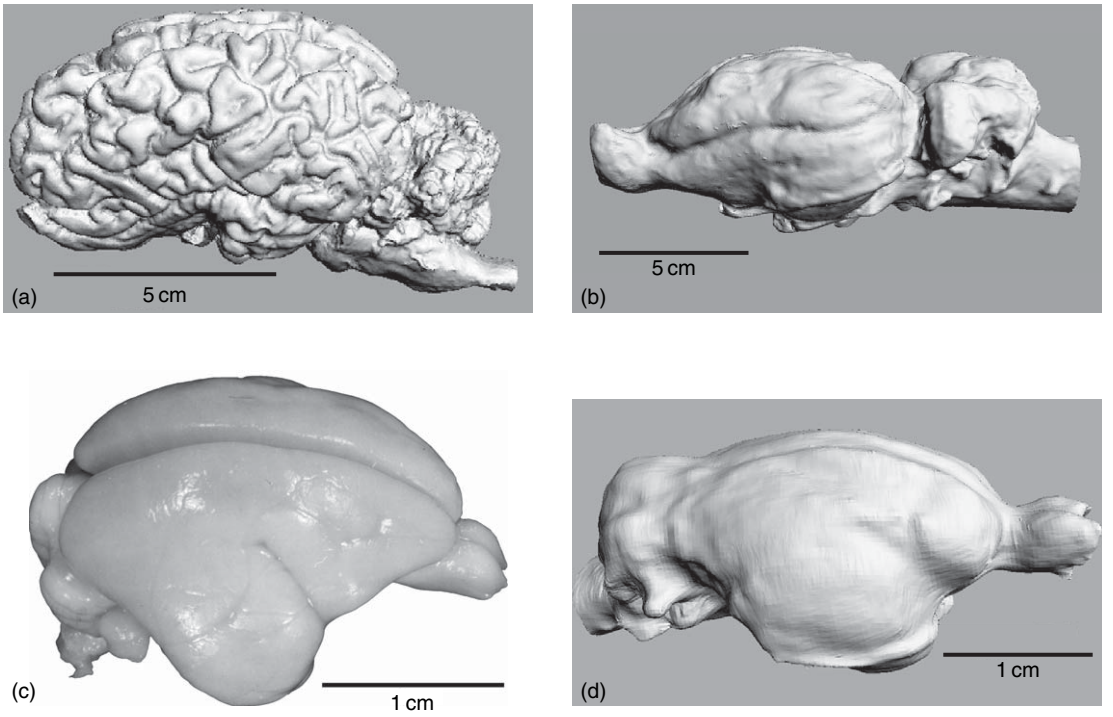

Figure 2 a, Brain and b, endocast in two artiodactyls, the living llama (*Lama glama*, WISC 65-139) and a 40-million-year-old camelid, A*noplotherium commune*, BMNH 3753. c, Brain of the living bush baby (*Galago senegalensis*, WISC 61-686) and d, endocast of a 40-million-year-old prosimian, *Adapis parisiensis*, FMNH 59259/BMNH 1340.

the growth of the brain, and is at least partly an epigenetic effect of squeezing a brain into the confines of the cranial cavity.

When an overall trend in skeletal growth resulted in enlargement of the cranial cavity, the brain can grow to fill and, to an extent, shape the cranial cavity. When trends toward encephalization became more prominent, the pressure was to maximize the amount of brain that could be packed into a given space. Later brains became more globular, primarily as an accommodation to maximize their volume relative to the space available for their growth. The change in shape as an evolutionary event would have been one of the changes that occurred at the Grande Coupure, the extinction of many species at the end of the Eocene and the beginning of the Oligocene about 33 Mya (Hooker *et al.*, 2004).

3.01.3 The Specimens and Their Measurement

The 78 ml plaster endocast of *Anoplotherium* in Figure 2 was prepared from the carefully cleaned cranial cavity of the fossil's skull (Palmer, 1913). This animal probably weighed about 80 kg, a weight comparable to that of the living llama in which the brain's volume is about 230 ml. *Bathygenys* (Figure 5) was a small artiodactyl that lived at the end of the Eocene, 35 Mya. It was about the size of the living chevrotain weighing about 5 kg. Its 12 ml natural endocast shown in Figure 5 is actually a piece of rock, but it unmistakably pictures the brain. One chevrotain (*Tragulus javanicus*) has been reported as weighing 4 kg with a 19 g brain (Nieuwenhuys *et al.*, 1998). The area of neocortex in both *Anoplotherium* and *Bathygenys* was 28% of the entire surface area of the endocast. In Oligocene species that lived about 30 Mya, such as the fossil horse Mesohippus, typical ratios are about 40% or more.

The volume of the endocast of *Phenacodus* (Figure 9) is 31 ml, and 16% of its surface area is neocortex. Its rhinal fissure is less marked than in the other endocasts illustrated here, but adequate for a measurement of the area of its neocortex. It was illustrated by Cope (1883). The endocast scanned for this analysis is a copy of the one made by Cope. Reconstructions of *Phenacodus* in life (Savage and Long, 1986) show it as living in a small herd of sheep-like five-toed animals. The weight of the fossil, estimated as 56 kg, is also appropriate for living sheep (*Ovis aries*) in which a brain weight of 130 g has been recorded.

Adapis, a prosimian primate that was a contemporary of *Anoplotherium*, weighed about 1.5 kg, and its endocast's volume was 8 ml. From skeletal

features, it has been reconstructed as a tree-dwelling lemuroid. The brain of the living galago, the bush baby, is shown for the comparison in Figure 2 because it is about the same size and shape as that of *Adapis*, but galago is a much smaller animal, weighing only about 250 g. A living lemur (*Lemur fulvus*) has been recorded with a body weight of 1.4 kg and had a 23 g brain.

The differences between the fossil and living brain sizes at comparable body sizes are examples of encephalization. Brains in most Eocene species averaged a quarter to half the size of living species in comparable niches and their relative size might be reported as 'encephalization quotients, EQs' – $0.25 < EQ < 0.50$ for Eocene fossils. Brain and body weight data on living species for comparisons are now available in many places. Some of the published data collections on living mammal species that I have seen have been in Count (1947), in Quiring (1950), and in Nieuwenhuys *et al.* (1998). I have published estimates on many fossil mammals (Jerison, 1973, 1990), and Holloway *et al.* (2004) has more data on primates in the human lineage.

EQs are not ratios of brain size to body size. They are ratios of measured brain or endocast size relative to expected size, and expected size is determined from the allometric relationship between brain and body size. That relationship is nonlinear and is usually described by the power function:

$$E = kP^{\alpha}, \qquad [1]$$

where E is brain size and P is body size in the same metric units (e.g., g or ml). There is some debate on correct values for the parameters k and α (see Jerison (2001)), but empirically the values $k = 0.06$ and $\alpha = 0.75$ are good approximate values as determined on large samples of living mammal species. When the equation is transformed logarithmically, it is

$$\log E = \alpha \log P + \log K. \qquad [1a]$$

Graphed on logarithmic coordinates, α is the slope and $\log k$ is the y-intercept of the best-fitting straight line. An encephalization quotient is the residual from that regression. For theoretical reasons (Jerison, 2001), I prefer $\alpha = 2/3$, in which case one must use $k = 0.12$ for computations. For a given set of parameters, it is an elementary exercise to compute an encephalization quotient.

To return to the specimens, the *Bathygenys* endocast in Figure 5 was made naturally. When this animal died, perhaps at a lakeside, its soft tissue decayed but its skull must have remained relatively undamaged. Sand and other debris could then pack the cranial cavity and could be covered and protected by layers of sediment. When the waters subsided, the skull and its contents eventually fossilized. Many millions of years later, the fossil was uncovered, presumably by erosion or earth movements. The fossilized skull must then have eroded, leaving only its hardened rock contents, the natural endocast. A lucky fossil hunter could find the specimen. Professor Jack Wilson of the University of Texas found the *Bathygenys* fossil, which he recognized as a natural endocast (Wilson, 1971), collected it for his paleontology department, and made it available for this report.

The plaster endocast of *A. commune* shown in Figure 2 is part of the history of anatomy and paleontology. A largely intact fossil skeleton of the whole animal was found in gypsum quarries in Montmartre, now part of Paris, and was named in 1804 by Baron Georges Cuvier. He noted that the fossil's canine teeth seemed short and ineffective as weapons – 'anoplos' is from the Greek for 'unarmed' and 'therium' for beast – hence, *Anoplotherium*. Serving under the Emperor Napoleon, Cuvier was director of the Muséum National d'Histoire Naturelle in Paris two centuries ago, and he undertook to demonstrate that fossils are evidence of the history of life.

At the time, fossils were sometimes considered to be mineral accretions that merely resembled living things. Cuvier accepted what we now recognize as the 'uniformitarian hypothesis', namely, that the present laws of nature have always been valid (Simpson, 1970). That he named the fossil according to his judgment of its teeth is a uniformitarian view that is natural for us. We share the judgment that they are not merely rocks that happened to look like teeth but were once teeth and had fossilized. The story is that in a public exhibition Cuvier 'dissected' his *Anoplotherium*, in which some of the fossilized vertebral column was exposed. The dissection was with hammer and chisel, and Cuvier pointed out that if what looked like the vertebral column had been the vertebral column of an animal that once lived, further exposure would reveal pelvic bones. It did. This was his way of proving that he had been working on the remains of an animal that was comparable anatomically to living animals.

Among the other endocasts and brains illustrated in this chapter, *Phenacodus* was collected and named by Edward Drinker Cope as mentioned earlier, and it was one of the bones of contention in the fossil feud of the late nineteenth century about discoveries in the American West (Wallace, 1999). The adapid endocast is from a skull presently at the Natural History Museum of London and was from the phosphorites of Quercy in southwest France, a Late Eocene site in which the fossils are about 40

million years old. Its endocast was first prepared a century ago under the direction of Elliot Smith (1903) and has had a prominent place in discussions of the evolution of the primate brain (LeGros Clark, 1962). This article's scan is from a later preparation for Professor Robert D. Martin, then at the Anthropology Department of University College, London (Martin, 1990). The brain of galago with which it is compared in Figure 2 is from the University of Wisconsin brain collection, a collection that I consulted for comparisons with almost all of the fossils analyzed for this chapter. At this writing, the Wisconsin collection can be viewed on the internet; see the 'Relevant Websites' section. Like the galago brain, the llama brain and the armadillo brain of Figure 1 are also in the Wisconsin collection. Some of that collection has been moved to Washington, D.C., and is now part of the National Museum of Health and Medicine of the Armed Forces Institute of Pathology.

Hundreds of fossil 'brains' have been collected throughout the world, either as natural endocasts or as latex or plaster casts made from fossil crania (Edinger, 1975), and they are available for study in the back rooms of many museums. The quality of an endocast as a model of the brain differs in different taxa. In fish, amphibians, and reptiles, the model is usually poor because when they mature their brains do not fill the cranial cavity. Semicircular canals and other auditory and vestibular structures are occasionally well preserved in many vertebrates (Rowe, 1996; Dominguez *et al.*, 2004). In mammals and birds, endocasts often provide accurate and detailed pictures of the external surface of the brain as in Figures 2, 5, and 9. Comparisons between brains and endocasts in living mammals indicate that only minor errors occur in treating endocasts as undissected brains.

Measurement of surface area in endocasts was essentially impossible until recently, when technologies were developed that enable us to scan and digitize irregular solids for computer analysis. The endocasts used for the 3-D analysis in this chapter were digitized with a laser scanner system and its associated software. After scanning, the surface areas were measured with that software to provide the data for Figures 7 and 8. At this writing, more details about the system are available on the internet; see 'Relevant Websites' section.

3.01.4 Brains and Endocasts

Why should we be concerned with simple-minded measurements of gross brain size? One obvious reason is that these are reliable measures that can be taken on fossils, and they enable us to quantify the evolution of the brain. Less appreciated is their utility for an understanding of the brain's work in living species, because gross brain size, either surface area or brain weight or volume, may estimate the total information-processing capacity of brains in living mammals. That relationship is inferred from Figure 3, which graphs surface area of living brains as a function of brain size. The number of neurons underneath a specific amount of surface appears to be constant in many species (Rockel *et al.*, 1980, but see also Haug, 1987 and Hofman, 1985, 1988). Since the total processing capacity of a neural system is related to the number of neurons in the system, total surface area must estimate the total number of information processing units in a brain. Analogously, the surface area of neocortex as a part of the brain measures the contribution of neocortex to the total amount of information processed by the brain. The surface–volume relationship as shown in Figure 3 is almost perfect with a product–moment correlation coefficient $r = 0.99$. Uniformitarianism suggests that this almost deterministic relationship was as true for fossil endocasts as it is for living brains, although there are questions raised at the end of this article among the 'caveats'.

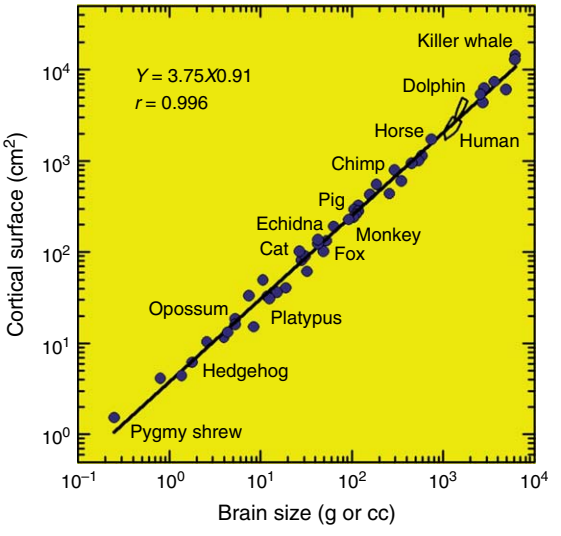

Figure 3 Total cortical surface area (including 'buried' cortex) as a function of brain size in 50 species of living mammals. Correlation: $r = 0.996$; regression: $Y = 3.75X^{0.91}$. A few of the species are labeled to suggest the diversity of the sample. Human and dolphin data are presented as minimum convex polygons enclosing 23 points for humans and 13 points for dolphins to suggest within-species diversity. Data from Brodmann (1913), Elias and Schwartz (1971), Ridway (1981), and Ridway and Brownson (1984). From Jerison, H. J. 1991. Brain size and the evolution of mind. 59th James Arthur Lecture on the Evolution of the Human Brain. American Museum of Natural History.

Figure 3 also provides information about within-species variability compared to that between species. In two of the species, humans (*H. sapiens*) and dolphins (*Tursiops truncatus*), it was possible to show the full range of individual data by enclosing those data in convex polygons that incorporate the complete samples. It is evident that the polygons are only slightly larger than the individual points graphed for the other species, each of which is a single datum for the species.

How good is an endocast as a representation of a brain? The obvious answer is in the endocasts and brains illustrated in this article. The relationship has been quantified for gross size in the human species and is shown in Figure 4. Although partly obscured by the well-known variability in human brain size, there is a strong relationship between brain and endocast (cranial capacity) as indicated by the high correlation coefficients. Endocasts and brains are equivalent to one another for information on size, with a small difference (about 7%) due to the fluids and meninges that surround the brain. The regression lines are parallel to one another, showing that the difference between endocast and brain follows the same rule for women and for men. This article is not concerned with the sex differences in human brain size, but that difference is also shown to complete the graphic summary of the data as published by Davis and Wright (1977).

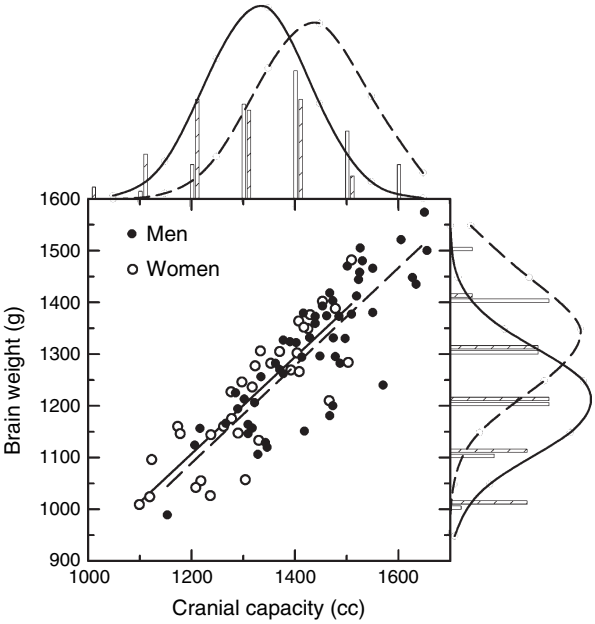

Figure 4 Relationship between brain size and cranial capacity (endocast volume) in 54 human male and 33 female cadavers. Solid lines and normal curves for males; dashed lines and normal curves for females. Mean brain size: male = 1308 g; female = 1221 g. Mean cranial capacity (endocast volume): male = 1431 ml; female = 1322 ml. Correlation coefficients, $r = 0.84$ for men; $r = 0.85$ for women. Regression equations: male, $Y = 0.94X - 44$; female, $Y = 0.94X - 16$. Marginal distributions shown by fitted normal curves. Data from Davis, P. J. M. and Wright, E. A. 1977. A new method for measuring cranial cavity volume and its application to the assessment of cerebral atrophy at autopsy. *Neuropathol. Appl. Neurobiol.* 3, 341–358.

3.01.5 Two-Dimensional Analysis of Neocorticalization

The first quantitative analysis of neocorticalization presented measurements of the areas of neocortex and olfactory bulbs in 2-D lateral projections in a sample of 35 fossil and 24 living species of carnivores and ungulates (Jerison, 1990). It was based on profiles of the endocasts in which rhinal fissures were visible, and the measure was the area of neocortex dorsal to the fissure. The 2-D analysis was also performed on the areas of the olfactory bulbs. Data for the analysis are illustrated in Figure 5a on the endocast of *Bathygenys reevesi*, discussed earlier; Figure 5b sketches the the areas that were measured.

The 2-D results are graphed in Figure 6 and show how the neocortical and olfactory bulb quotients in this sample changed with geological age during the Cenozoic era. The quotients are ratios of measured brain areas relative to their expected areas, with the latter determined by the regression of brain areas on the height of the foramen magnum (medulla). The measure on the foramen magnum followed a suggestion by Radinsky (1967) to use the foramen measure to control for body size differences in different species. The quantitative analysis was limited to neocortex and olfactory bulbs. Paleocortex and hindbrain were not analyzed because the curvature of the brain hides much of the paleocortex and because hindbrain regions such as the cerebellum are partly hidden under overlying neocortex. (Regression analysis such as this is often referred to as allometric analysis, the analysis of the measures of two organ sizes relative to one another.)

The results of the 2-D analysis as discussed in the original report (Jerison, 1990) were, first, that neocorticalization occurred, which is shown by the significantly positive slope of the regression of the neocortical quotient on geological age. Second, the olfactory bulbs did not change in relative size in these species during the Cenozoic. Taken together, these two results validated the method in that it could discriminate between the change and absence of change. A third result was that the 'archaic' fossil species, that is, species from orders of mammals that are now entirely extinct, were

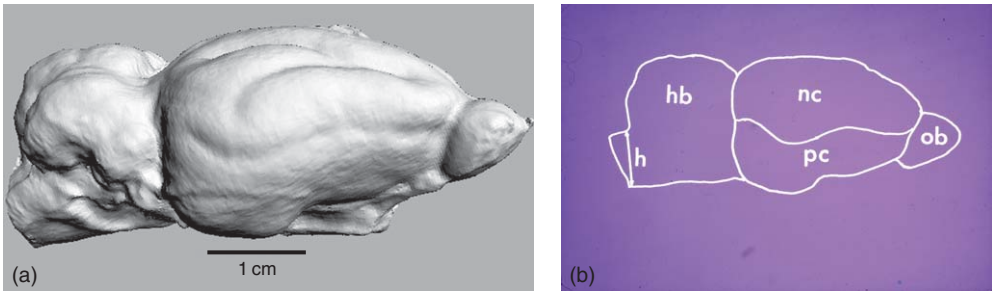

Figure 5 a, Natural endocast of *Bathygenys reevesi* (UT 40209-431). b, Profile of endocast, showing areas measured for Figure 6. nc, neocortex; ob, olfactory bulbs; h, height of foramen magnum. Not measured: pc, visible paleocortex; hb, visible hindbrain, including cerebellum.

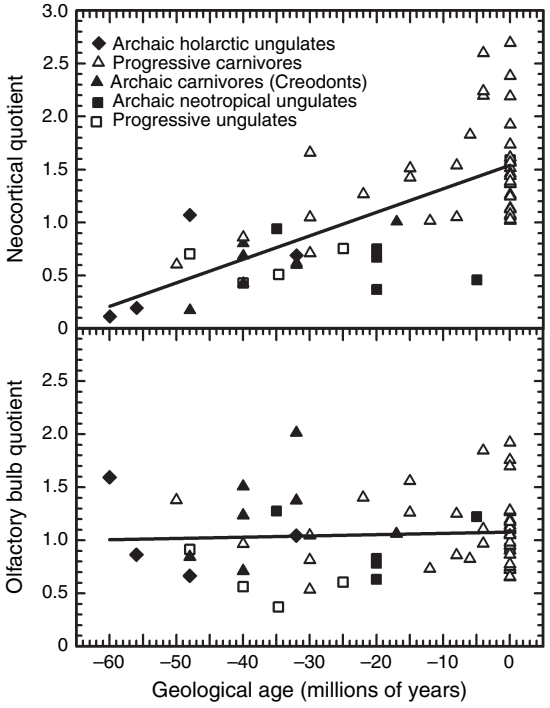

Figure 6 The 2-D analysis showing increased relative neocortical surface area (top) and stasis in olfactory bulbs (bottom) during the past 60 million years; see Figure 5. Quotients are residuals from regression of neocortex area and olfactory bulb area on foramen magnum height. They are interpreted as ratios of measured areas relative to expected areas on the basis of body size in this sample. 'Progressive' neocortical change noted here (positive slope of regression line; $r = 0.75$) demonstrates neocorticalization over time. Each point is a species. Archaic carnivores are from the order Creodonta. Other groups are discussed in the text. Redrawn from Jerison, H. J. 1990. Fossil evidence on the evolution of the neocortex. In: Cerebral Cortex (*eds.* E. G. Jones and A. Peters), vol. 8A, pp. 285–309. Plenum.

significantly below average in neocorticalization, falling below the regression line determined for the entire sample. This suggests positive selection for neocorticalization, that it improved fitness in an evolutionary sense.

The 2-D data on brain size are flawed because they are limited to profiles of the brain and do not measure the actual areas of the curved surfaces of the endocasts. They are also limited to species in which rhinal fissure is visible on the lateral surface, and this excluded primates from the analysis. When the 2-D analysis was published, it was not possible to perform an equivalent 3-D analysis with the technology available at that time. Such a technology has since been developed, and the analysis of 3-D images of endocasts is published here for the first time.

3.01.6 Three-Dimensional Analysis: Neocortex

The analysis of the newly acquired 3-D data on a larger sample of fossil and living mammals, which includes primates (Figure 7), confirms that there was progressive neocorticalization in mammals during the Cenozoic. The positive slope of the regression (Figure 7) is similar to that found in the 2-D analysis. The sample of 106 mammals included 84 fossil species and 22 living species. There were seven fossil primates: two hominins and five prosimians, including the *A. parisiensis* shown in Figure 2. The hominins were two Plio-Pleistocene australopithecines, *Australopithecus africanus* (the Taung specimen discovered by Raymond Dart in 1923) and *Australopithecus robustus* (SK 1585) from South Africa (see Tobias, 1971; Holloway *et al.*, 2004). There are partial endocasts for Oligocene and Miocene anthropoids (*Aegyptopithecus*, *Libypithecus*, and *Proconsul*; see Radinsky, 1979), which were sufficient to indicate that frontal and temporal lobes were in a primate-like configuration (Jerison, 2006), but they were too incomplete otherwise for this quantification. The 22 living mammal species included eight anthropoids (simians) and two humans. Primates have evidently always been above average in neocorticalization,

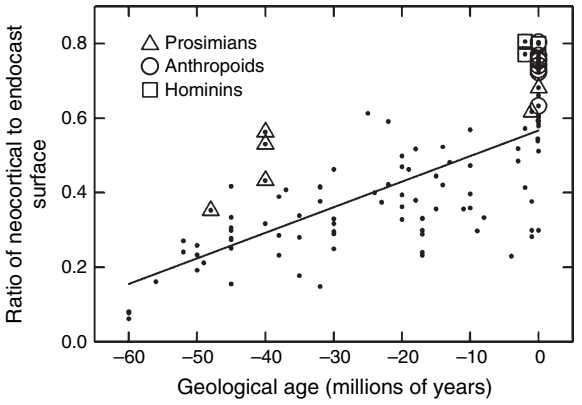

Figure 7 Relative size of neocortex measured in the 3-D analysis of the endocasts of 106 species of mammals during the past 60 million years. Points for prosimians are marked by triangles, hominins (including australopithecines) by squares, and anthropoids by circles. (All 106 species are shown as points, and identifying symbols surround the points.) Regression of neocortical ratio on geological age: $Y = 0.007X + 0.57$; $r = 0.72$. In living mammals, 57% of the endocast surface area is devoted to neocortex; the increase was about 7% per 10 million years. In living monkeys and living and fossil hominins, the ratio averages about 75%.

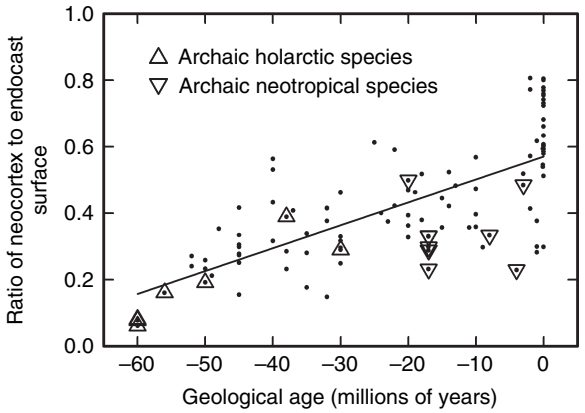

Figure 8 The same mammal species as in Figure 7, marked to distinguish 'archaic' from 'progressive' species, that is, species that are members of presently extinct orders or suborders of mammals from those that are members of surviving groups. Upright triangles for holarctic species, inverted triangles for neotropical species. Regression line is for the entire sample, and is the same as in Figure 7. Archaic species on average fall below the regression line.

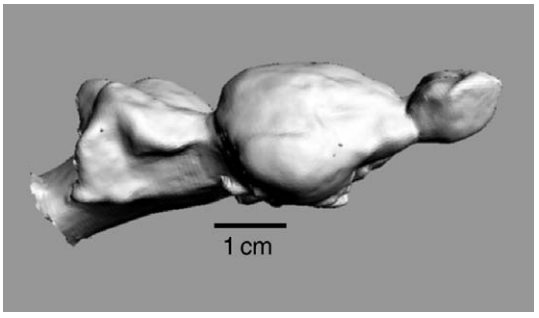

Figure 9 Endocast of *Phenacodus primaevus* (FMNH 59042/AMNH 4369), a late Paleocene archaic holarctic species (58 Mya).

that is, their data lay above the regression line determined for the entire sample as shown in Figure 7. Living and fossil hominins are typical primates on these measures. The highest ratio of neocortex to endocast surface area was a langur's (*Cercocebus albigena*) at 80.4% followed by one living human at 80.0%. A second living human endocast measured 77.7% and was topped by two other monkeys.

As in the 2-D analysis, it was possible to compare species from archaic orders with those from 'progressive' orders, and the data showing the difference are presented in Figure 8. The species in which endocasts were illustrated earlier in Figures 2 and 5 were progressive in that there are even-toed ungulates (order Artiodactyla) and primates (order Primates) living today.

Several orders of Miocene and Pliocene South American mammals, originally discovered as fossils by Charles Darwin in the 1830s on the voyage of the *Beagle*, are 'archaic', having no surviving species. Their data are included in Figure 8 and marked with inverted triangles. Erect triangles mark holarctic species from Europe and North America, also from extinct orders. Thirteen of the 15 archaic species fell below the regression line. The probability that this was a random departure from 'average' is less than 0.05 (chi-square test). The 3-D analysis thus supports the conclusion that neocorticalization contributes to fitness, that is, there was positive selection for neocorticalization.

Endocasts of archaic species are not superficially unusual. That of *P. primaevus* discussed earlier is shown in Figure 9. It might be distinguished from the other fossil endocasts because of slight differences in appearance, but it is also different quantitatively. At the animal's estimated body weight (56 kg), its expected endocast volume is 176 ml according to my preferred parameters of eqn [1]. The measured volume of the endocast at 31 ml results in an encephalization quotient of 0.18. Its ratio of neocortical area to total endocast area is 0.16, one of the lowest in the sample, and it is an example of the grade of encephalization and neocorticalization in most Paleocene mammals.

3.01.7 Three-Dimensional Analysis: Olfactory Bulbs

The 3-D quantitative analysis of neocorticalization reported here supplements but does not entirely replace the 2-D analysis. It omits the olfactory bulbs, which could not be measured reliably on too many of the fossil endocasts. There were obvious artifacts in many of them in the representation of olfactory bulbs. In preparing plaster endocasts from a skull, the region of the olfactory bulbs is cleaned out, and it is easy to make mistakes. The cribiform plate and the region of the turbinals has sometimes been excavated, resulting in artificially enlarged bulbs. In others, the olfactory bulbs may be incompletely excavated in preparing latex endocasts. Many natural endocasts, unlike the *Bathygenys* endocast illustrated in Figure 5, are also obviously distorted in the region of the olfactory bulbs. There were enough uncertainties in the sample of endocasts that were scanned for this article to make it inappropriate to present 3-D data on the olfactory bulbs without further study. Olfactory bulbs in the 2-D analysis were all sketched by neurobiologists familiar with normal living brains, who used that information in their reconstructions (see Jerison, 1990). The sketches were all published prior to the later quantitative analysis, and the areas in the 2-D analysis were measured independently of the sketching. The result that showed no change in the relative size of the olfactory bulbs was unexpected and unanticipated. Clearly unbiased, the conclusion of the 2-D analysis can be accepted at least provisionally, namely that the relative size of the olfactory bulbs remained more or less unchanged during the Cenozoic.

The evidence of the reduction of olfactory bulbs in primates and cetaceans is from comparative anatomy. The fossils suggest that their reduction in primates occurred after the Oligocene, when *Aegyptopithecus* lived; Radinsky's (1979) sketches indicate olfactory bulbs in *Aegyptopithecus* that were comparable to those in fossil prosimians (cf. *Adapis* in Figure 2) and relatively larger than in later anthropoid species. In Miocene and Pliocene anthropoids, the olfactory bulbs appear as reduced as in living species, and australopithecine olfactory bulbs are reduced comparably to those of living chimpanzees and humans. Fossil data on cetaceans were reviewed in Jerison (1973) and indicate either reduced or completely absent olfactory bulbs.

3.01.8 Caveats and Conclusions

Neocorticalization occurred in many lineages, and there appeared to be some increase in all mammal species after the Paleocene epoch. The overall increase is evident in the positive slope of the regression lines of the neocortical ratio on geological age. The increase was most dramatic in primates, where it is evident in the earliest record of their brains in the Eocene epoch, but even in 'primitive' living marsupials such as the koala (*Phascolarctos cinereus*), neocorticalization to the extent of 30% of the endocast surface is in advance of the Paleocene grade of the archaic *Phenacodus*.

Another conclusion is about the diversity of neocorticalization. The range between 30% in the living marsupial koala and 80% in living humans and langurs suggests the variety of niches for which neocorticalization could be selected. When I published the 2-D data 15 years ago, I thought that the correlation of 0.7 between geological age and neocorticalization and the scattered points in its graph (Figure 6) might be due to the inadequacies of 2-D measurements. The better method for determining and measuring surface area, and the larger sample for the measures in Figures 7 and 8, indicate that the variability is real and reflects the true diversity of adapations for neocorticalization in mammals.

The third conclusion which also verified the 2-D analysis indicated that neocorticalization contributed to fitness. The evidence is in the fate of archaic species, which were on the average less neocorticalized than progressive species. This kind of conclusion may seem obvious and hardly worth special mention, but it is difficult to find reasonable evidence for the fitness of quantifiable traits that evolved to different extents in different taxa. The unusual history of the olfactory bulbs in mammals is as instructive as that of the neocortex. Based on this trait living anthropoid primates (including *H. sapiens*) are a 'degenerate' order, and one can interpret the reduction in their olfactory bulbs as evidence of the relative unimportance of olfactory information in their lives. Stasis in the evolution of the olfactory bulbs is presumably the norm, and if primates had been included in the 2-D analysis their degeneracy might have been clearer. But humans write the histories, and in our accounts of the history of the brain, large olfactory bulbs have erroneously been taken as a sign of primitiveness rather than normality. Olfactory bulbs, large or small, are adaptive to specialized niches.

The final caveat is to be wary of conclusions based on endocasts rather than brains and to be wary of conclusions based on externals rather than on the fundamental structure and function of the brain. On the other hand, conclusions based only on the fundamentals have to follow a cladistic analysis of data on living species, comparing apparently

homologous traits and taking into account their differentiation in different living species. Johnson (1990) has reviewed cladistic analyses of neural evolution, and there have been several other important publications on phylogeny, which were cited in the opening paragraphs of the article. The conclusions, based on endocasts and limited to externals, provide a time dimension for the brain's evolution and broadly date the events.

I have emphasized the place of endocasts as providing direct evidence on the evolution of the brain and that the relevance of the evidence comes partly from relationships between superficial data such as brain size and more fundamental measures of the brain's structure and function. Figure 3, showing the relationship between the gross size of the brain and the extent of its cortical surface area, is a good example of the approach, illustrating a likely relationship between the gross measure of the brain itself and neural information processing capacity. In that graph, the dependent variable was total cortical surface, including surfaces buried in the fissures. For a closer look, consider Figure 10, which graphs the measured surface area of endocasts rather than brains as a function of their volume. Although the relationship is equally strong for brain measures and endocast measures in which cortex buried in the fissures is not measured, the difference between the slopes on logarithmic coordinates (the allometric exponent) is instructive.

In the endocasts (Figure 10), the slope is about two-thirds, which is the expected relationship among similar solids of different size. For example, in graphs of the surface–volume relationship in spheres of different size, the slope is exactly 2/3, as it is in cubes or any other solid object of any shape if shape is conserved as size changes. In an equation like eqn [1], $\alpha = 2/3$ for a given solid, and the differences among solids are in the parameter k. Regardless of their sizes, for all spheres, $k = 4.84$; for all cubes, $k = 6$. Figure 10 tells us that our endocasts were more alike in shape than we might have guessed, at least with respect to this aspect of their geometry. Figure 3, on the other hand, tells us that had we been able to work with the brains of these fossils rather than their endocasts we should have expected convolutedness to increase as volume increased, that is, convolutedness would be greater in larger brains. The change in convolutedness is reflected by the exponent $0.91 > 2/3$. That information is lost in working with endocasts. The high correlation coefficients save the day for a uniformitarian view. They indicate that the surface areas of portions of the neocortex buried in the sulci and fissures are also related in an orderly way to brain or endocast size. It is, therefore, likely that like actual brain surface area, the surface area of endocasts also estimates the information processing capacity.

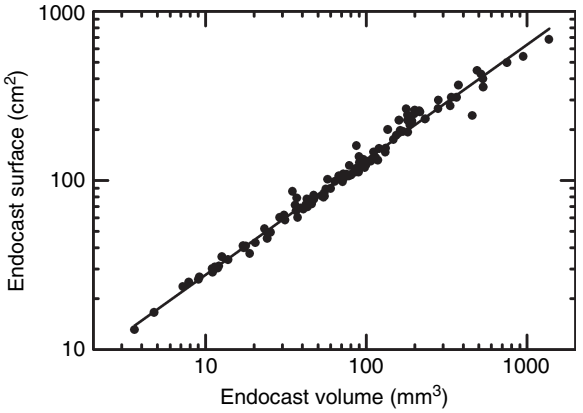

Figure 10 Surface–volume relations in endocasts as measured in the sample of 106 species used in the 3-D analysis. Product–moment correlation, $r = 0.992$; regression, $Y = 5.8X^{0.68}$.

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3.02 The Origin of Neocortex: Lessons from Comparative Embryology

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Glossary

cell migration — The cortex comprises two types of neurons: glutamatergic pyramidal projection neurons and GABAergic interneurons. The two cortical cell populations are generated at different sites within the forebrain. The pyramidal neurons are generated in the local cortical germinal zone and migrate radially (perpendicular to the pial surface) to reach their final destination in the cortex. In contrast, most of the interneurons are born in the distant ganglionic eminence (neuroepithelium of the embryonic pallidum) and migrate tangentially (parallel to the pial surface) to reach the cortex.

germinal zone — In the embryonic brain, epithelium lining the ventricles (the ventricular zone) is a neurogenic source. In mammals, there is an additional adjacent layer of mitotic activity termed the subventricular zone. These compartments differ in size, organization, and in their modes of division. The proliferative cells in the ventricular zone undergo interkinetic migration, and have radially aligned nuclei. The subventricular zone contains cells with randomly oriented nuclei.

infragranular and supragranular — The mammalian neocortex (or isocortex) consists of six layers. Layer 4 is also called the granular cell layer. Layers 5 and 6 are referred to as infragranular and layers 2 and 3 are also called supragranular layers. Different cortical areas show considerable variations in the proportions of these components.

layers of the cortex — These are cells spanning the entire thickness of the wall of the embryonic forebrain extending perpendicular to the pial surface. These cells were previously assumed to act merely as scaffolds for newly born neurons to migrate along. They are now considered to be the source of most neurogenesis in the developing cortex.

radial glia — At the ventricular zone, radial glia divide to yield a new glial cell and a daughter cell which migrates away from the ventricular surface. This is termed asymmetrical division. The subventricular zone also participates in neurogenesis. Here the intermediate progenitors mostly undergo division to produce two identical daughter neurons, which migrate to the cortex. This is termed symmetrical division.

symmetrical and asymmetrical divisions

3.02.1 Constant Features of the Mammalian Isocortex

The mammalian six-layered cortex (neocortex or isocortex) is a great achievement of cortical

development and evolution (see The Evolution of Neuron Classes in the Neocortex of Mammals, Organization of a Miniature Neocortex – What Shrew Brains Suggest about Mammalian Evolution, Sparse Coding in the Neocortex, What Fossils Tell Us about the Evolution of the Neocortex). Cortical neurons are arranged into distinct layers according to their input and output in a very specific and conserved manner (Lorente de No, 1949; Toyama *et al.*, 1974; Peters and Jones, 1985). In spite of the enormous variations in cortical surface area, and in the sulci and gyri (Krubitzer, 1995; Krubitzer and Kahn, 2003), the basic cortical circuitry is similar. The laminar allocation of cells connecting to the thalamus, spinal cord, or intracortical areas is remarkably conserved among all mammals studied. Rockel *et al.* (1974) counted the number of neuronal cell bodies in a narrow radial strip (30 μm wide) through the depth of the neocortex in several different functional areas (motor, somatic sensory, area 17, frontal, parietal, and temporal) in different mammalian species (mouse, rat, cat, monkey, and human) and found that the numbers were surprisingly constant. The same absolute number (congruent to 110) even characterized all areas of all species, with only one exception (see below).

This conserved cortical cell number dogma was established before the availability of various neuronal markers. It would be important to revisit this idea with modern neuroanatomical tools. The principal neuronal types of the cerebral cortex are the excitatory pyramidal cells, which project to distant targets, and the locally projecting inhibitory nonpyramidal interneurons (Peters and Jones, 1985). Pyramidal neurons are generated in the cortical neuroepithelium and migrate radially to reach the cortex following an inside-first outside-last gradient (Rakic, 1995, 2005). Interneurons produced from the neuroepithelium of the embryonic pallidum also contribute to the formation of the cerebral cortex (Parnavelas, 2000; Corbin *et al.*, 2001). These cells migrate tangentially through the striatocortical junction to reach the cortex (Marín and Rubenstein, 2003). In rodent, only a few nonpyramidal cells are generated in the cortical ventricular zone (VZ). It is known that around 70–85% of cortical neurons are excitatory glutamatergic pyramidal neurons, while the rest are GABAergic interneurons. These basic proportions also seem to be constant in all mammals (DeFelipe, 2002).

In spite of the constant number and neuronal cell types in the cortex, the mammalian cortices exhibit remarkable variations in their thickness, relative proportions of their layers, and patterning of cells and fibers (Ramón y Cajal, 1911; Figure 1). The differences within the same brain across various cortical areas are equally fascinating (Brodmann, 1909). The transition between primary and secondary visual cortical areas in primate is perhaps the most striking change in cortical organization (Rakic, 1988), as the binocular part of area 17 of primates (macaque and human) has approximately 2.5 times more neurons (Rockel *et al.*, 1974, 1980). The structure of layer 4 is so different in primate primary visual cortex that it can be seen as the line of Gennari with the naked eye (Figure 1e). However, this transition should be considered as an exception rather than the rule, since this is the only currently known aerial boundary where the numbers of cortical cells in a unit column changed. The cytoarchitectonic differences between cortical areas reflect the subtle changes in different components of the cortical circuits needed to perform the computational function of that particular area. For example, motor cortical areas have a more prominent output layer, layer 5, while the granular layer is nearly absent. On the other hand, in primary sensory areas there is more emphasis on granular and supragranular layers (layers 4 and 2–3) at the expense of the infragranular layers (Brodmann, 1909).

3.02.2 Dorsal Cortex Contains Fewer Neurons in Nonmammalian Vertebrates

The major divisions of the brain are comparable and are remarkably well conserved across sauropsids (birds and reptiles) and mammals (Puelles *et al.*, 2000). The exception to this is the organization of telencephalic derivatives (Striedter, 1997, 2005). There are several hypotheses about the evolutionary rearrangement of different sectors of the forebrain to produce the neocortex in mammals (Northcutt and Kaas, 1995; Molnár and Butler, 2002a, 2002b; Butler and Molnár, 2002). In spite of the emerging anatomical and gene expression data (Fernandez *et al.*, 1998), identifying structures at the striatocortical junction of the forebrain of various vertebrates and elucidating homologies remains difficult (Bruce and Neary, 1995; Butler and Hodos, 2005; Molnár *et al.*, 2006). In contrast to the six-layered mammalian cortex, reptiles possess a three-layered cortex which is similar to layers 5 and 6 of mammals

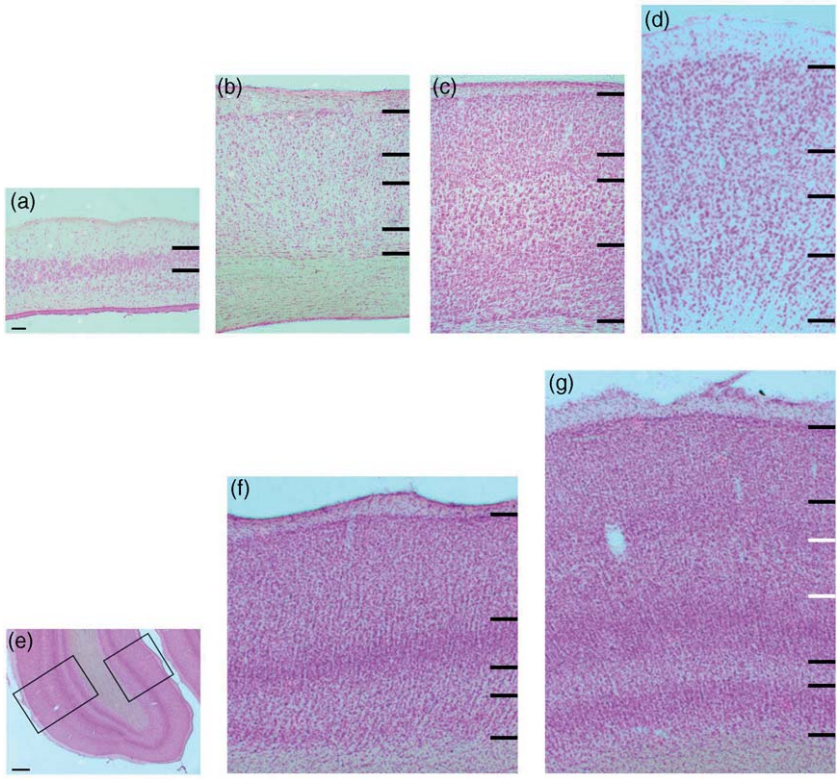

Figure 1 Nissl-stained coronal sections of four different amniote brains that demonstrate the similarities and differences in the cortical lamination in: a, Crocodile (Australian); b, hedgehog; c, mouse; d, cat; e–g, rhesus monkey. e, Low-power image was taken from the border of 17–18. Boxes in (e) depict the location of the images taken from areas 17(g) and 18(f). Bars mark the layering of the different cortices. White bars in (g) mark the partitioning of layer 4 into sublayers (a)–(c). Scale bar: 100 μm (a–d, f, g); 500 μm (e).

(Goffinet, 1983; Goffinet *et al.*, 1986; Reiner, 1991; Figure 1a). The isocortical homologue of birds is a pseudolayered structure, which is considerably different in organization to that of mammalian neocortex (Medina and Reiner, 2000). The most notable difference is that the mammalian cortex contains dramatically more neurons. Therefore, a question arises: where did the extra cortical cells come from in the mammalian brain?

3.02.3 What are the Major Changes in Cortical Neurogenesis in Mammals?

Much debate exists about the progression from the postulated primitive ancestor to the modern-day mammalian and reptilian cortices (Northcutt and Kaas, 1995). There are two theories on the (total) increase of mammalian cortical neurons. Both theories suggest that there are accessory sites of neurogenesis for the mammalian cortex. First, the equivalent cell migration hypothesis suggests that a considerable population of mammalian neurons are generated outside the neocortex and migrate into the cortex during development. This theory predicts relocation of corresponding cell groups in ancestral species at the reptilian mammalian transformation (Karten, 1997). Second, the dorsal cortical germinal zone elaboration hypothesis suggests that the generation of extra cortical neurons for the expanding sheet of cortical neuroepithelium and elaboration of the granular and supragranular cortical layers in mammals required an accessory site of proliferation within the cortical subventricular zone (SVZ) and the appearance of an intermediate progenitor population (Martínez-Cerdeno *et al.*, 2005; Molnár *et al.*, 2006).

Although one cannot directly study ancestral brains, study of comparative cortical development of extant species can still reveal the developmental mechanisms that change most considerably in mammals and other vertebrates, elucidating the steps of evolutionary progression. Unfortunately, current neurodevelopmental studies are limited to very few vertebrate species, making generalizations and comparisons difficult. Our own comparative

developmental analysis is based on six species: (1) turtle, (2) chick, (3) mouse, (4) rat, (5) macaque, and (6) human.

3.02.4 Are Cortical Neurons Produced Outside the Mammalian Cortex Supportive for the Equivalent Cell Migration Hypothesis?

It has been argued that equivalent circuits are present in the visual and auditory pathways in avian and mammalian telencephali (Karten, 1968, 1997). Although the basic components of these circuits (thalamic recipient cells, interneurons, and efferent projection neurons) are present in both avian and mammalian cortical circuits, their arrangement is very different. While these components are arranged into cortical layers in mammals (layers 4, 2–3, and 5–6 respectively), they are mostly situated subcortically in birds (Karten, 1991). In lizard, turtle, and bird, this subcortical structure is a large mass of cells protruding into the lateral ventricle above the striatum, called the dorsal ventricular ridge (DVR). The DVR hosts most of the neurons required for information processing in the equivalent circuits. As shown by a study in iguana (*Iguana iguana*), highly organized multiple representations involved in visual processing were observed in the DVR (Manger *et al.*, 2002). In comparison to the DVR, the three-layered dorsal cortex in the iguana appears rudimentary compared to the neocortex of mammals (Figure 1).

The recent discovery that the subpallium, a region outside the cortical neuroepithelium, contributes tangentially migrating neurons to the mammalian cerebral cortex generated a good deal of excitement (de Carlos *et al.*, 1996; Anderson *et al.*, 1997; Tamamaki *et al.*, 1997). In rodents, most of these neurons are derived from the medial ganglionic eminence (Lavdas *et al.*, 1999; Sussel *et al.*, 1999; Wichterle *et al.*, 1999; Xu *et al.*, 2004). This region of the forebrain did not correspond to the sector which was suspected to be homologous to the DVR. Moreover, these neurons are exclusively GABAergic interneurons and not excitatory pyramidal cells, which one would expect for the equivalent projection circuits.

3.02.5 Generation and Mode of Migration of the GABAergic Interneurons

The proportion of GABAergic neurons in avian pallial regions and their rather uniform distribution closely resemble the patterns seen in other vertebrates, including mammals (Veenman and Reiner, 1994; Jarvis *et al.*, 2005). In both cases, generation and differentiation of GABA neurons in the ventral forebrain regions specifically require *Dlx* family transcription factor expression (Stuhmer *et al.*, 2002). Likewise shared are *Emx* genes expressed in the cortical domain, which primarily generates excitatory glutamatergic neurons (Anderson *et al.*, 2002; Gorski *et al.*, 2002). The molecular mechanisms and the genetic pathways are conserved in phyla as distant as amphibians, reptiles, birds, and mammals. Orthologues of *Dlx* genes have been cloned in these vertebrates (Fernandez *et al.*, 1998; Puelles *et al.*, 2000), and these genes define homologous ventral forebrain domains. The DVR, which contains the neuronal elements of the equivalent circuit, according to Karten's (1997) hypothesis, lies between the *Dlx* and *Emx* expression domains (Fernandez *et al.*, 1998; Puelles *et al.*, 2000; Jarvis *et al.*, 2005) (Figure 2).

Though the dorsal cortex of sauropsids lacks several cell types found in the mammalian isocortex, both structures are comprised of two basic components required to build a functional cortex: the excitatory glutamatergic projection neurons and the inhibitory GABAergic interneurons (Goffinet *et al.*, 1986; Blanton *et al.*, 1987; Reiner, 1991, 1993).

During embryonic development, GABAergic neurons originate in the ventral subpallium and progressively colonize the dorsal pallium following distinct routes (Nadarajah and Parnavelas, 2002; López-Bendito *et al.*, 2004; Métin *et al.*, 2006). These streams are generally oriented tangentially to the brain surface throughout their path, but at the pallium/subpallium boundary, or within the VZ before reaching the cortical plate, where some cells reorient radially (Nadarajah *et al.*, 2002).

Comparative analysis of tangentially migrating neurons in birds revealed that, just as in mammals, most GABAergic interneurons originate in the ventral telencephalon (Cobos *et al.*, 2001). In slice cultures prepared from embryonic chick brains, GABAergic cells follow similar tangential routes in both subpallium and pallium, and show similar branched leading processes (Tuorto *et al.*, 2003). The similarities in the migration route and morphology to mammalian tangentially migrating interneurons suggest common mechanisms of development (Bellion *et al.*, 2005). Accordingly, the developmental sequence of GABAergic interneurons in the turtle cortex is reminiscent of tangential migration from ventral territories (Blanton and Kriegstein, 1991). In bird and turtle, the relative contribution of pallidum (or medial ganglionic eminence of the telencephalon) and paleostriatum (or lateral ganglionic eminence of the telencephalon) to the GABAergic population is debated (Cobos *et al.*, 2001; Tuorto

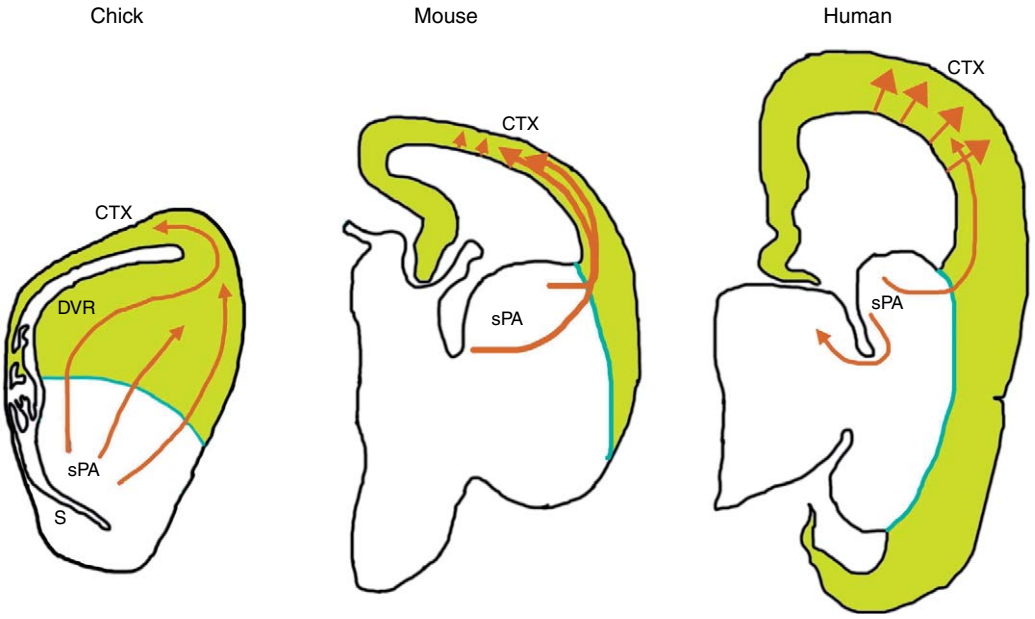

Figure 2 Common mechanism of subpallial origin and tangential migration of GABAergic neurons in bird, rodent, and human. Schematic outlines represent the cross sections through chick, mouse, and human forebrains. Orange arrows depict the migratory patterns of GABAergic neurons from subpallium (sPA). S, septum; CTX, dorsal cortex. See text for details. The left panel was inspired by Cobos *et al.* (2001) and the right panels by Tan (2002). Adapted from Molnár, Z., Métin, C., Stoykova, A., *et al.* 2006. Comparative aspects of cerebral cortical development. *Eur. J. Neurosci.* 23, 921–934, with permission from Blackwell Publishing.

et al., 2003). In rodent and primate, there also seem to be great differences in the proportion of GABAergic neurons generated locally in the pallium and the striatum/pallium (lateral and medial ganglionic eminences). In human, gene expression evidence suggests that a substantial fraction (65%) of cortical interneurons are generated by the pallium (Letinic and Rakic, 2001), whereas in rodents this estimate is only 5% (Letinic *et al.*, 2002; Tan, 2002).

In spite of all these current uncertainties, the neuronal production observed outside the cortex in mammals is not supportive of the equivalent cell migration hypothesis. Several expectations were not fulfilled by the tangentially migrating neurons. (1) The migrating neurons are purely GABAergic and do not contain any excitatory pyramidal neurons. (2) The origin of the migrating cells in mammals does not coincide with the domain which is considered homologous to the DVR. (3) Tangential migration is not unique to mammalian brains. In avian and probably reptilian brains, GABAergic interneurons also arise from a *Dlx* domain and migrate tangentially to the dorsal cortex. Therefore, it is more likely that changes in the local dorsal cortical neurogenetic program, together with some major rearrangements at the striatocortical junction (Molnár and Butler, 2002a), provided the foundation for remodeling the mammalian cerebral cortex (Molnár *et al.*, 2006).

3.02.6 Basic Pattern of Cortical Neurogenesis

The predecessor of the mammalian cortical plate is the preplate (or primordial plexiform zone), which contains a heterogeneous population of the earliest-born neurons of the cortex, and is considered to be the reptilian component of the mammalian neocortex (Marin-Padilla, 1978). The first neurons of the rodent and human cortex probably originate from subpallium and not from cortex (Bystron *et al.*, 2005). This is in line with the notion that Cajal–Retzius cells migrate in from various sources (Bielle *et al.*, 2005). Neurons subsequently generated from the cortical plate split the preplate into an outer plexiform layer and an inner subplate (Marin-Padilla, 1978; Smart and McSherry, 1982; Smart and Smart, 1982; Luskin and Shatz, 1985). Newly produced neurons migrate out of the germinal zone from the VZ towards the pial surface according to a strict timetable. In mammals, the cortical plate is destined later to become the six-layered structure of the mature cortex. The cortex is formed in an inside-first outside-last neurogenic gradient (Angevine and Sidman, 1961) where younger cohorts migrate beyond previously generated neurons to settle at the upper border of the cortical plate. Consequently, the oldest neurons of the cortex occupy the deep layers, whereas the upper layers are made of late-born neurons.

In all veterbrates, embryonic neurogenesis provides the majority of neurons that compose the adult brain. In the embryonic brain, epithelium lining the ventricles (the VZ) has long been known to be a neurogenic source (Sauer, 1936; Sauer and Walker, 1959; Sidman *et al.*, 1959). In mammals, an additional, adjacent layer of mitotic activity was also observed (termed the SVZ). It was believed that gliogenesis commences during late corticogenesis and continues perinatally in the SVZ (Privat, 1975), and hence neurogenesis and gliogenesis were thought to occur in the VZ and SVZ respectively (Sturrock and Smart, 1980; Bayer and Altman, 1991). These compartments differ in size, organization, and in their modes of division. The proliferative cells in the VZ undergo interkinetic migration, and have radially aligned nuclei. The SVZ contains cells with randomly oriented nuclei (Smart, 1973).

Radial glia were previously assumed to act merely as scaffolds for newly born neurons to migrate along, and until recently, their importance was unappreciated. They are now considered to be the source of most neurogenesis in the developing cortex (Malatesta *et al.*, 2000; Noctor *et al.*, 2002). At the VZ, radial glia divide asymmetrically to yield a new glial cell and a daughter cell which migrates away from the ventricular surface. Elegant time-lapse photography of individual neurons migrating in brain slices demonstrated that the SVZ also participates in neurogenesis and the VZ is not the sole neurogenic compartment (Noctor *et al.*, 2004). Noctor and his colleagues also showed that the daughter cells generated from asymmetrical VZ divisions head directly to their locations in the future cortex, while others choose to arrest in the SVZ, where they are termed intermediate progenitors. Here the intermediate progenitors mostly undergo symmetric division to produce two identical daughter neurons, which migrate to the cortex (Kriegstein and Noctor, 2004; Noctor *et al.*, 2004) (Figure 3). This work was further confirmed *in vivo* using direct labeling of neurogenic progenitors lying outside the VZ (Wu *et al.*, 2005), which demonstrated that cells marked in the SVZ subsequently gave rise to upper-layer pyramidal neurons.

Analysis of gene expression in the germinal zone revealed that the VZ and SVZ progenitor cells are controlled by different genes. Embryonic expression of *Svet1* (Tarabykin *et al.*, 2001), *Cux1*, and *Cux2* (Nieto *et al.*, 2004) are only seen in the SVZ but expression of these genes is absent in the VZ. Postnatally, these genes are confined to the supragranular and granular layers of the cortex. On the other hand, the transcription factor Otx-1 and Er81 labels cells in the VZ exclusively during embryonic stages, and later is confined to layer 5 and 6 or layer 5, respectively (Frantz *et al.*, 1994; Tarabykin *et al.*, 2001; Yoneshima *et al.*, 2006). Thus, the SVZ gives rise to neurons that go on to populate the upper cortical layers (2–4), whereas the VZ produces the earlier-born neurons which give rise to the deeper layers (5 and 6) of the cortex. Differential fate is a product of transcription factor action on divergent dividing and migratory properties.

In addition to the proliferation in the VZ and SVZ, it was recently found that there are further scattered divisions within the intermediate zone, cortical plate, and marginal zone in rodent and human. These abventricular divisions compose less than 10% of all divisions at any stage of any cortical area during development (Carney *et al.*, 2004; Carney, 2005). Nevertheless, it shows that scattered progenitor cells are also capable of division outside the two main proliferation compartments (Figure 4).

In summary, we favor the dorsal cortical germinal zone elaboration hypothesis on two accounts. By examining the cortical development in macaque, where the variety of supragranular layer cells is much more diverse, the germinal zone differentiates

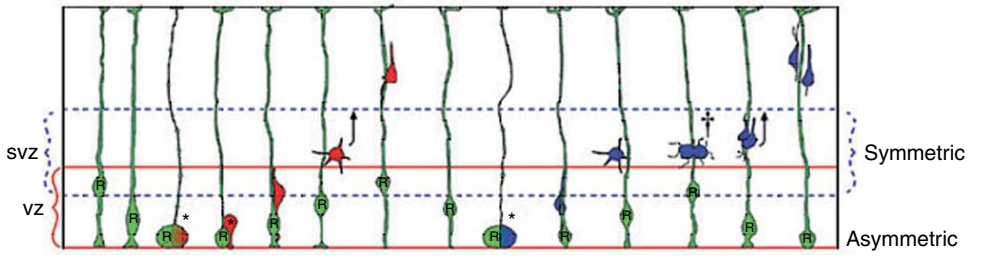

Figure 3 Two distinct programs of division and migration are observed in the germinal zone. Radial glia (R) self-renew and directly give rise to neurons (red) through asymmetric division in the VZ (*). Other neurons are generated from intermediate progenitor cells (blue) in the subventricular zone (SVZ) with terminal symmetric divisions (dagger). Reprinted by permission from Macmillan Publishers Ltd: *Nat. Neurosci.* (Noctor, S. C., Martinez-Cerdeno, V., Ivic, L., and Kriegstein, A. R. 2004. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat. Neurosci.* 7, 136–144), copyright (2004).

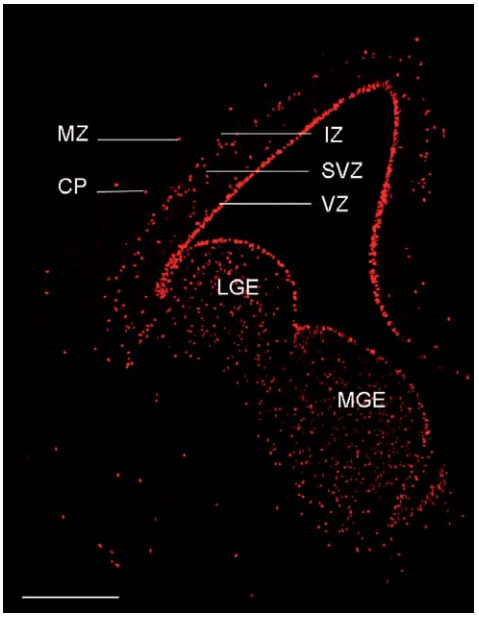

Figure 4 Coronal section through the right hemisphere of an E14 rat brain stained with phosphohistone H3 antibody to reveal the sites of cell divisions. Most of the divisions occur in the VZ lining the cortical neuroepithelium. There is a second major row of divisions in the SVZ, but there are further scattered divisions in the marginal zone (MZ), cortical plate (CP), and intermediate zone (IZ). There are large number of divisions in the medial and lateral ganglionic eminences (LGE, MGE). Scale bar: 100 μm. Unpublished figure from Carney *et al.* (2004) and Carney (2005).

further, with unique features not seen in rodents. On the other hand, the dorsal cortical neuroepithelium of nonmammalian vertebrates (turtle and chick) does not contain subventricular zone.

3.02.7 Neurogenesis in Primate Cortical Neuroepithelium

The developing primate cortex contains a unique compartment of the SVZ termed the outer SVZ (OSVZ) (Smart *et al.*, 2002). This site of proliferation has been shown to produce the majority of the supragranular layers (Lukaszewicz *et al.*, 2005). In rat and mouse, the supragranular layers are significantly smaller compared to macaque; thus, correspondingly, the SVZ is also much smaller (Smart *et al.*, 2002).

The work of Kennedy, Dehay, Smart, and colleagues produced some very interesting comparisons between mouse and macaque in cortical development (Smart *et al.*, 2002; Lukaszewicz *et al.*, 2005; Figure 5a). In mouse, the VZ is the major source of proliferation up until E15, after which it begins to regress in size. The SVZ appears at E13 but lags behind in proliferative terms, and begins to regress after E15 (Smart, 1973; Smart and McSherry, 1982). The relatively low germination activity in the SVZ is mirrored in the smaller proportion of the upper layers in the mouse neocortex. The SVZ in rodents accounts for no more than 35% of cortical proliferative population at E15 (Takahashi *et al.*, 1995). Accordingly, the supragranular layers occupy no more than a third of the thickness of the mature neocortex (Smart *et al.*, 2002). In contrast, the monkey has a very different process of proliferation. At a gross histological level, the size of the proliferative compartment (i.e., VZ + SVZ) rapidly increases in size from E65 onwards (Figure 5b). At this point, on the basis of histological appearance, the SVZ appears to have two distinct components: an inner SVZ (ISVZ) and a larger, OSVZ. Between E65 and E72, the OSVZ rapidly increases in size and becomes the major proliferative area of the SVZ, with the ISVZ contributing little. The dense, radially oriented precursors of the OSVZ constitute a unique feature, and birth-dating experiments showed that it generates the supragranular layers of the neocortex (Lukaszewicz *et al.*, 2005). The predominance of OSVZ in macaque could be due to the increased importance of the corticocortical connections and therefore the supragranular layers, where most corticocortical connections are formed. After E72, the OSVZ begins to decrease in size, accompanied by a corresponding increase in cerebral wall thickness, suggesting that the postmitotic cells are migrating to their future home in the cortex. By E78 the proliferative compartment has been fully split by the complete appearance of the inner fiber layer, with the ISVZ now attaching to the VZ but not the OSVZ (Figure 5) (Smart *et al.*, 2002).

It is possible that localized transcription factor expression of the SVZ and the VZ is responsible for the creation of certain neuronal subtypes. It is also possible that further compartmentalization of SVZ in primates is a correlate of higher neuronal diversity of supragranular layers (DeFelipe *et al.*, 2002). The contribution of SVZ in neuronal production seems to grow in evolution as the complexity of the cortex increases. The emergence of an additional proliferation zone and its diversification during cortical evolution might have been triggered by the necessity to produce more neuronal subtypes in different morphological compartments.

3.02.8 Cortical Neurogenesis in Nonmammalian Cortex

Our group became interested in examining the dorsal cortex of nonmammalian (turtle and chick) brains because these vertebrates do not possess a

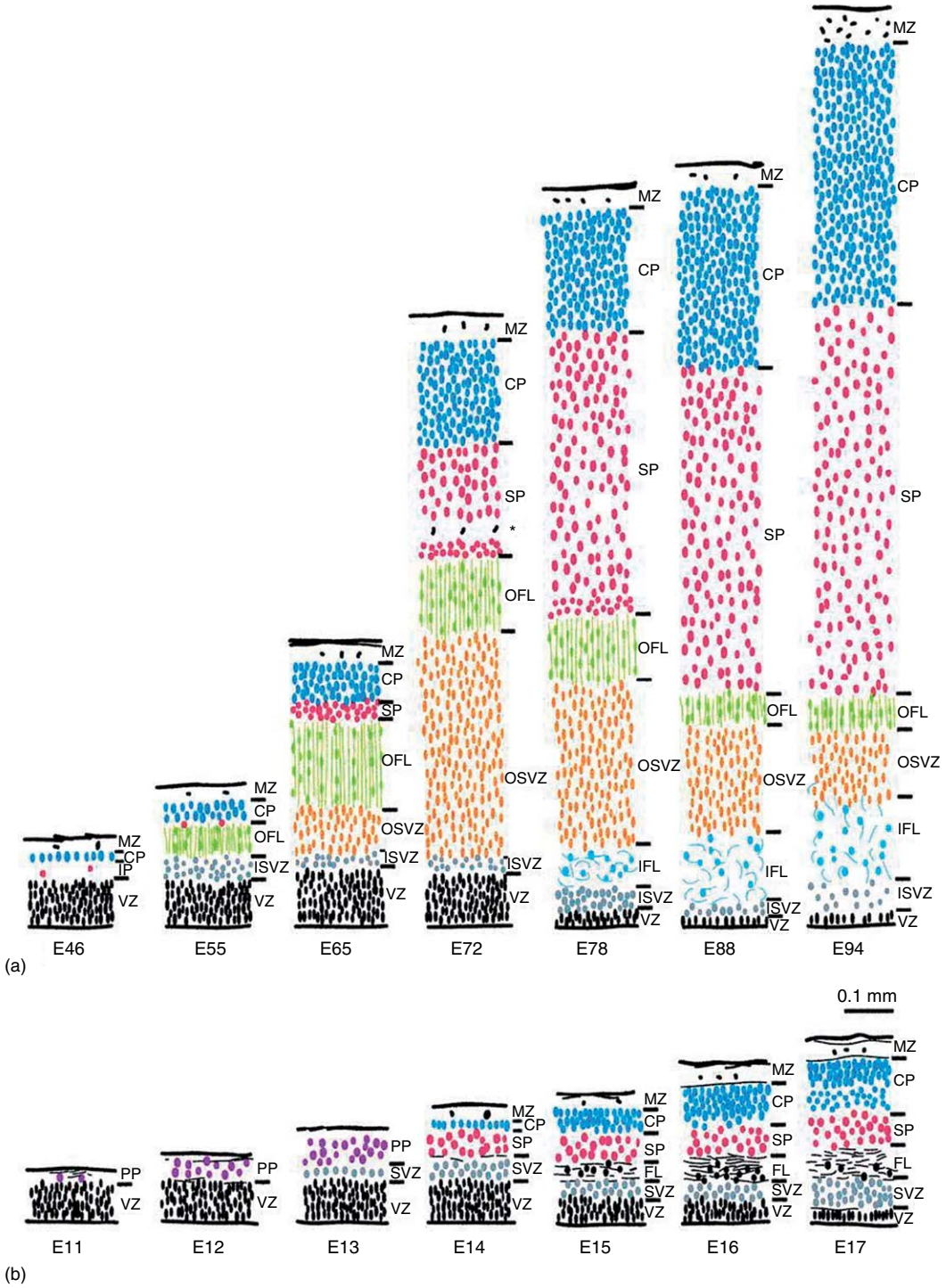

Figure 5 Comparison of histological sequences in the developing mouse and monkey telencephalic wall. These drawings are of transects through putative area 17 in monkey (a) and mouse (b) at comparable developmental stages. The depth of each layer is drawn to a common scale. The internal detail of each layer is not to scale but depicts the orientation, shape, and relative packing density of nuclei in each layer. The vertically aligned pairs have been chosen with reference to birth-dating experiments to illustrate corticogenesis at equivalent developmental stages. A curiously conspicuous clear layer marked by an asterisk (*), located in the deep subplate (SP), is transiently present at E72. At later stages it appears to merge into the SP. CP, cortical plate; IFL, inner fiber layer; ISVZ, inner subventricular zone; MZ, marginal zone; OFL, outer fiber layer; OSVZ, outer subventricular zone; SP, subplate proper; VZ, ventricular zone. Reproduced from Smart, I.H., Dehay, C., Giroud, P., Berland, M., and Kennedy, H. 2002. Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. *Cereb. Cortex* 12, 37–53, Oxford University Press.

six-layered cortex, and the complexity and variety of neuronal types within them is more limited than in mammals. If indeed the generation of the upper layers of cortex found in mammals required an accessory site of proliferation, such as the SVZ, in addition to the VZ (Martínez-Cerdeno *et al.*, 2005; Molnár *et al.*, 2006), then this zone should be rudimentary or nonexistent in the reptilian and avian dorsal cortical neuroepithelium during embryonic development.

We examined the site of mitotic divisions in embryonic turtle and chick brains, paying special attention to the investigation of the presence or absence of abventricularly generated cells and the SVZ. Using phosphohistone H3 (a G2 and M-phase marker), we found that the distribution of mitotic cells in embryonic turtle and chick is fundamentally different from each other, and from rodent and primate (Lukaszewicz *et al.*, 2005). In turtle and chick cortex, there is a single major zone of proliferation with additional scattered abventricular divisions within the dorsal cortex (Figure 6). However, in certain regions of the chick brain (mesopallium, nidopallium, and striatum), two distinct proliferative zones are visible, whereas the turtle has only one (ventricular) organized zone of mitotic activity throughout the brain (Figures 6c and 6d). In turtle a significant proportion of division occurs abventricularly (highest in the striatum and septum), but these are scattered across the entire depth of the forebrain structures and never align into a distinct zone. VZ mitosis peaks in earlier stages (S18.5 and S20) before shifting to an increasingly abventricular site of proliferation in later stages (S23 and S25). In turtle, the major zone of activity is the VZ. Using Nissl-stained preparations, Martínez-Cerdeno *et al.* (2005, 2006) also suggested that the majority of the divisions are in the VZ, but noted the presence of a rudimentary SVZ. According to our own observations on the distribution of phosphohistone H3 immunoreactivity, there appears to be no organized zone outside the VZ anywhere in the embryonic turtle brain. Thus, one must conclude that the SVZ is absent in turtle (Figure 6a).

In the chick the situation is more complicated. An SVZ-like structure has been identified (Striedter and Keefer, 2000), but this sector appears restricted to the dorsolateral portion of the basal telencephalon. This finding remains controversial, especially as the SVZ is only found in this region. In the homologue of the dorsal cortex of the chick brain, the hyperpallium has a similar distribution of phosphohistone H3-labeled cells to turtle (i.e., VZ only; Figure 6b). Outside the cortex there appears to be a clear and distinct secondary zone of proliferation in the mesopallium and nidopallium (and, to a certain extent, in the striatum) above the VZ. This is clearly

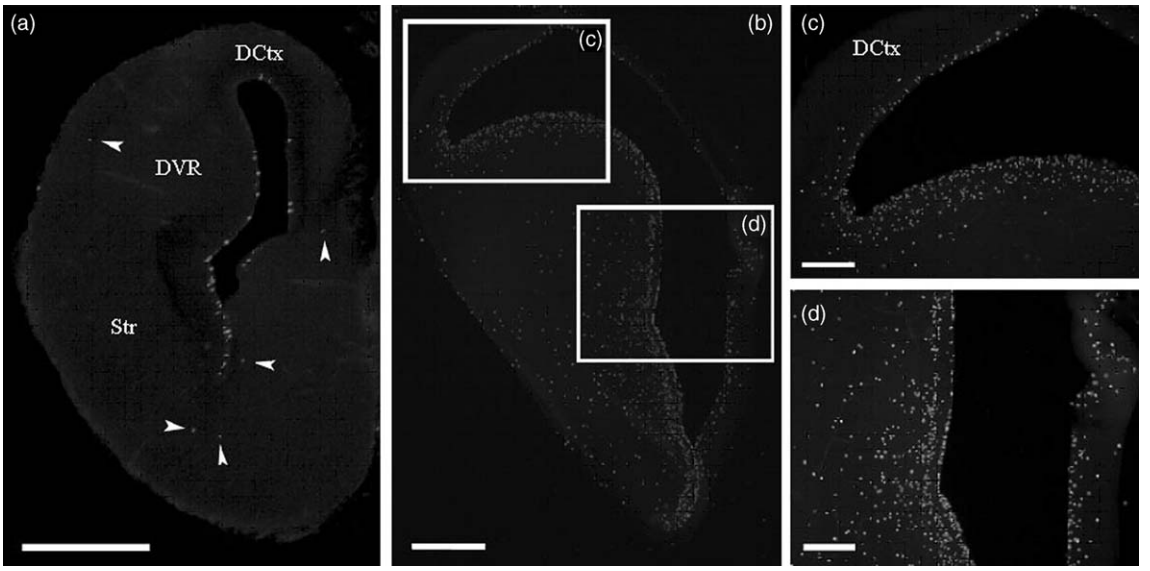

Figure 6 The lack of SVZ in turtle and chick dorsal cortex has been demonstrated with antiphosphohistone H3 immunohistochemistry. H3 immunofluoresence demonstrates mitotic activity in S18.5 turtle (a) and E8 chick (b) in the VZ and occasionally scattered abventricular proliferations (arrowheads in (a)). In turtle there is no organized SVZ in any part of the neuroepithelium. In chick, higher magnification (c, d) demonstrates an additional layer of proliferation superficial to the VZ in mesopallium and nidopallium in addition to more numerous scattered abventricular proliferation profiles. DCtx, dorsal cereberal neocortex; Str, Striatum. Scale bars: 500 μm (a, b); 200 μm (c,d).

demarcated by the presence of a band which separates the two zones, lacking in phosphohistone H3-labeled cells. Interestingly, this arrangement is not detected at any stages in the hyperpallium. This suggests that the SVZ is not a purely mammalian phenomenon in the forebrain, but it is unique for mammalian dorsal cortex together with the six-layered isocortex, to which SVZ provides neurons for more superficial layers (Noctor *et al.*, 2004; Wu *et al.*, 2005). As SVZ was absent in the hyperpallium, it seems likely that the presence of a precortical SVZ is an exclusive hallmark of mammals (Molnár *et al.*, 2006). This is one of the forces driving their cerebral complexity over other taxonomic classes. In support of this, recent data also suggest the SVZ is absent from amphibians (Wullimann *et al.*, 2005).

3.02.9 Evolving Cortical Progenitor Populations in VZ and SVZ

The absence of SVZ in turtle and chick hyperpallium indicates that the VZ directly provides the cells that subsequently form the dorsal cortex of the postnatal brain. Indeed, by examining the neurotransmitter organization and connections of turtle cortex, Reiner (1993) suggested that only the infragranular layers produced by the ventricular progenitors are present. It would be interesting to study further whether the fate of the reptilian or avian ventricular progenitors could be modified by providing appropriate gene expression patterns in the SVZ. Perhaps the turtle and chick dorsal cortical progenitor cells do have some innate ability to arrest in an SVZ-like region for additional symmetrical division. Moreover, several transcription factors show strong regional expression and could be used to map out VZ (*Pax6*) and SVZ (*NeuroD* and *Tbr2* co-expression) (Hevner *et al.*, 2006). The role of *Svet1* could be explored further by electroporating DNA into the VZ of turtle and chick isocortical homologues. The cell cycle parameters in different sectors of the turtle and chick germinal zone should be further studied. Measurements made using proliferating cell nuclear antigen (PCNA) immunohistochemistry and ^{3}H-thymidine pulse labeling revealed that cell cycle differs in duration in the germinal zones of monkey (Lukaszewicz *et al.*, 2005). The cell cycle is longer in the monkey OSVZ than in VZ (or comparable mouse), which may allow for the precise generation of a greater diversity of neurons that compose the supragranular layers (Figure 7) (Smart *et al.*, 2002; Molnár *et al.*, 2006).

Further study of the different cortical progenitor populations and their evolutionary origin could have general clinical implications. Various developmental diseases have a background of disrupted cortical formation (Francis *et al.*, 2006) and examining these cases could help expound the principles of cortical neurogenesis. The mechanisms involved in the formation of sulci and gyri of the brain are not fully understood (Van Essen, 1997). The lissencephalic cortex of mouse has been demonstrated to be a result of a less active SVZ. Drawing from this observation, lissencephaly in human may also result from inadequate SVZ proliferation. However, generalizations should currently be avoided until cortical neurogenesis is examined in gyrencephalic rodents and lissencephalic primates. According to the dogma on the cell numbers within a unit column of mammalian cerebral cortex, all mammalian cortices in all areas possess the same number of cells, with the exception of the primate primary visual cortex (Rockel *et al.*, 1974, 1980). If so, then the compartmentalization of the germinal zone should correlate more with the size of the cortical sheet and with the proportions and cell diversity of the supragranular cell layers. The cortical SVZ appears to be crucial for generating the increased cortical size and complexity (particularly the diversity of the upper layers) seen through the progression of mammalian evolution. While the SVZ is not unique to mammals, the development of a cortical SVZ appears to have been a crucial step in cortical evolution.

3.02.10 Summary and Conclusions

Comparative studies contribute to the debate on the possible evolutionary progression from the developmental mechanisms present in the postulated primitive ancestor to the modern-day mammalian and other vertebrate cortices (Northcutt and Kaas, 1995). We examined two theories that address the increased number of mammalian cortical neurons. Both suggest that there are accessory sites of neurogenesis for the mammalian cortex. The analysis of embryonic development does not support the equivalent cell migration hypothesis, although there is indeed a considerable population of mammalian neurons generated outside the cortex that migrate into the cortex during development; these neurons are exclusively GABAergic. More importantly, this process is not unique to mammals. We believe an increasing volume of work supports the dorsal cortical germinal zone elaboration hypothesis. In light of recent comparative investigations on embryonic cortical neurogenesis in frog, turtle, chick, rat, mouse, and macaque, it is more likely

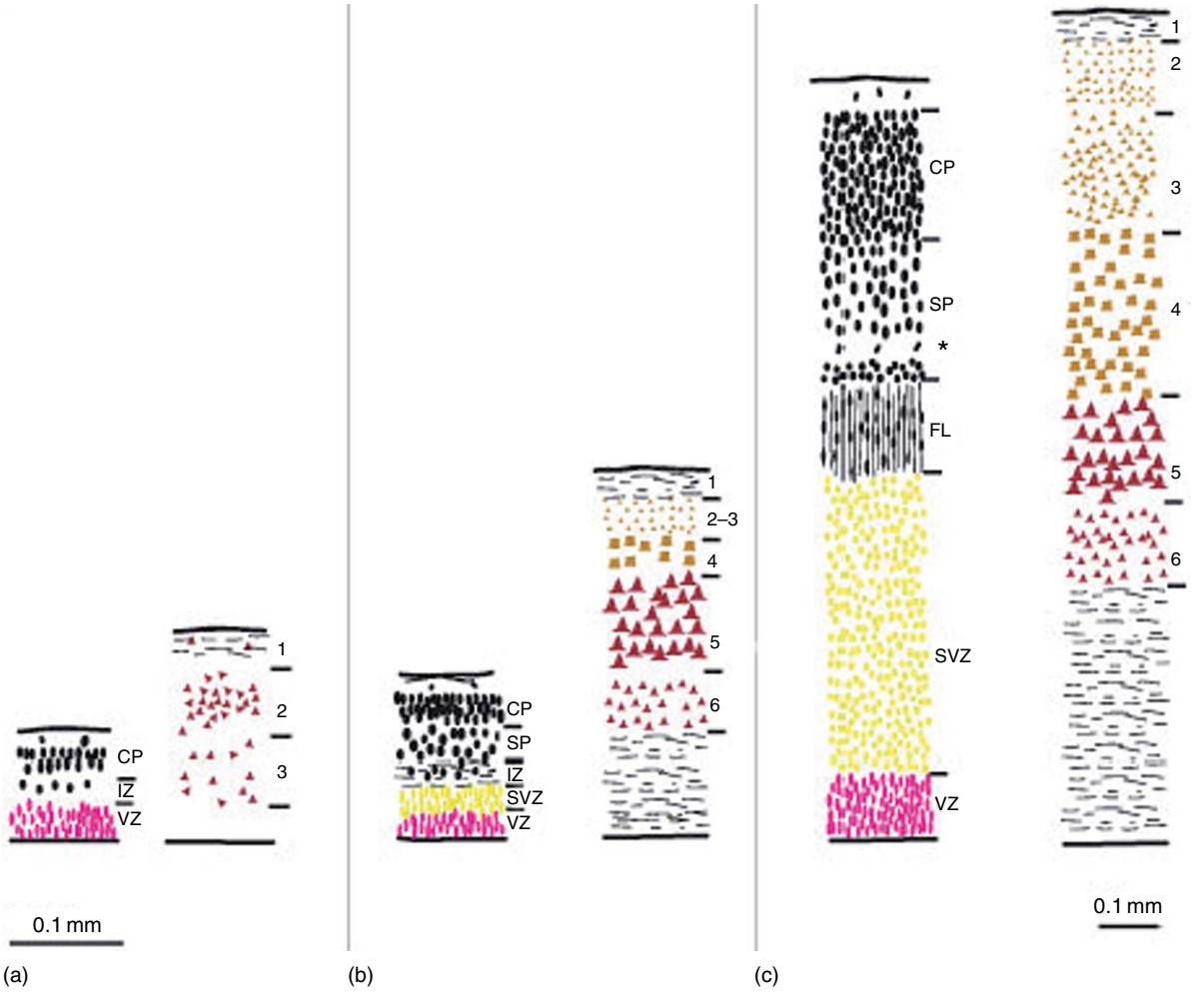

Figure 7 There is a strong correlation between the increase in supragranular layer complexity and the increase in subventricular zone between Turtle and chick (a); mouse (b); and monkey (c). The left panels for mouse and monkey are from Figure 5 from an E15 mouse and an E72 monkey. The right panels represent the layering in the adult. VZ and infragranular layers (6 and 5) are labeled red; SVZ and supragranular layers (2 and 3) are colored yellow. Note that the increase in the complexity of supragranular layers is accompanied by an increase in the SVZ during development. For clarity, SVZ includes ISVZ and OSVZ in the monkey panel. Adapted from Molnár, Z., Métin, C., Stoykova, A. *et al.* 2006. Comparative aspects of cerebral cortical development. *Eur. J. Neurosci.* 23, 921–934, with permission from Blackwell Publishing.

that the generation of extra cortical neurons for the larger cortical sheet and increasingly elaborate granular and supragranular cortical layers in mammals required the adoption of an accessory site of proliferation within the cortical SVZ, as well as the appearance of an intermediate progenitor population (Smart *et al.*, 2002; Kriegstein and Noctor, 2004; Noctor *et al.*, 2004; Martínez-Cerdeno *et al.*, 2005; Molnár *et al.*, 2006).

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3.03 Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications

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Glossary

area	A major subdivision of neocortex. Each area performs a specific set of functions. Areas were called the "organs of the brain" by Brodmann (1909).
column or module	Subdivisions of areas that mediate a function or functions that are repeated many times within other modules of the same type within the area. Areas may have two or more types of intermixed modules.
cortical magnification	The greater representation in cortical areas of important parts of sensory surfaces in proportion to receptor density.
motor cortex	Subdivisions of cortex that are specialized to elicit and control body movements. Movements can be evoked by electrically stimulating motor cortex.
representation	Areas are said to represent a sensory surface, such as the retina, skin, or cochlea, when stimulation in different parts of the sensory surface activates neurons in different parts of the area in a matching or isomorphic pattern. Muscles and movements are also represented in areas of motor cortex. Some areas may have more abstract, higher-order representations.

3.03.1 Introduction

The evolution of the large human brain intrigued early investigators, such as Smith (1906) and Clark (1959), but their efforts to describe this evolution were greatly constrained by the limited information on brain organization and the few techniques to evaluate brain function available at that time. What they did have were extensive collections of brains, preserved in jars of fixative, and thin sections of brains stained for the cell bodies of neurons or for the myelin that wraps the axons of these neurons. Thus, they could carefully observe ways in which the brains of extant mammals varied greatly in size, the locations of fissures that indent the cortex and even in the architectonic appearance of cortex and other parts of the brain. Such early investigators recognized that the neocortex was a part of the brain that varied the most in size and that some portions of neocortex could be recognized as similar

enough that they were likely to be homologous across species. They concluded that early mammals had small brains with little neocortex, and mammals leading to humans had brains that were progressively larger and more complexly organized, with proportionally more neocortex. While these deductions were based on the appearances of the brains of a large array of species, they primarily depended upon detailed considerations of the brains of a few key mammals that were thought to represent stages or levels in the course of the evolution of human brains (for review see Kaas, 2002; Preuss, 2000).

Today, we are in a position to greatly expand on the efforts of such early investigators. Most importantly, we know so much more about the organization of the brains of a wide range of present-day mammals. The traditional Nissl, myelin, and Golgi stains have been supplemented by an ever-increasing array of histochemical and immunochemical protocols for revealing the architecture of brains and their structurally distinguishable parts. Electrophysiological approaches, especially microelectrode recording and stimulation procedures, have greatly expanded our understanding of brain organization, and this knowledge has been fortified and advanced by newer methods such as the optical imaging of patterns of evoked neural activity. We also know something about the sizes and shapes of the brains of long extinct mammals from the ever-increasing fossil record based on the endocasts of the brain cases of the preserved skulls (Jerison, 1973; Kielan-Jaworowska *et al*., 2004). Although this record tells us little about the functional organization of the brains of extinct mammals, it does yield information about brain sizes and fissure patterns.

Conceptual advances have been important as well (see Hodos and Campbell, 1969; Preuss, 1995a; Striedter, 1998). Early investigators assumed that various extant species could be used to represent stages or levels of mammalian evolution. The problem with this assumption is that any extant mammal is likely to contain a mixture of ancestral (plesiomorphic) and newer, apomorphic (specializations derived from an earlier state) traits or features. Of course, some mammals appear to have mainly primitive brain features, while others have many obviously advanced features. Thus, methods were needed to distinguish primitive from advanced traits. Otherwise, an advanced trait in a generally primitive brain could be mistaken for a primitive trait and vice versa. The current approach to this problem is to use cladistic analysis (see Eldredge and Cracraft, 1980; Wiley, 1981). In brief, some brain traits are recognized as present or absent in members of a clade of mammals (any group of mammals that have all descended from a common ancestor). The distribution of the trait across the phylogenetic tree of related mammals, the cladogram, tells you whether it is more likely (more parsimonious to assume) that the trait was present in a common ancestor, and was retained in many or all of the descendents, or subsequently evolved in one or more lines. Given this approach, there is a logical way to distinguish primitive from advanced (derived) traits, other than from their appearance or association with other traits. A common mistake of early and even current investigators was to consider simple or undifferentiated traits as primitive. For example, a few current investigators still support the theory of Sanides (1970) that poorly differentiated regions of neocortex are older and highly differentiated regions are newer. This may generally be the case, but it can be demonstrated not to be the case in some instances. For example, primary visual cortex (V1 or area 17) in the hedgehog has poorly differentiated cell types and cell layers are not very distinct (Kaas *et al*., 1970), while primary visual cortex in tarsiers is perhaps more distinctly laminated than in any other mammal (Collins *et al*., 2005). Yet, V1 is an equally old area in both mammals, having been present in the reptilian ancestors of mammals (see below). The histological structures of V1 had simply differentiated more in the line leading to tarsiers than in the line leading to hedgehogs (see The Evolution of Visual Cortex and Visual Systems).

To be fair, early investigators such as Eliott Smith and Le Gros Clark had some appreciation of the advantages of broad comparisons across members of a clade. They recognized that the easily identified corpus callosum, the bundle of axons that interconnects the two cerebral hemispheres, was a new feature in placental (Eutherian) mammal brains because all members of the placental clade have a corpus callosum, no members of the monotreme or marsupial clades have a corpus callosum, and no reptiles or other vertebrates have a corpus callosum. But only easily identified traits could be examined across a wide range of mammals at the time of their investigations. Recognizing many brain characters is still difficult, and thus premises about brain evolution still depend on too few observations (see Kaas, 2002). Fortunately, we have procedures to correct mistakes, and powerful ways of determining brain organization. A great aid to current cladistic studies of brain evolution is the progress that has occurred in understanding the details of the phyletic radiation of mammals. This understanding is based on both the fossil record, and the results of recent

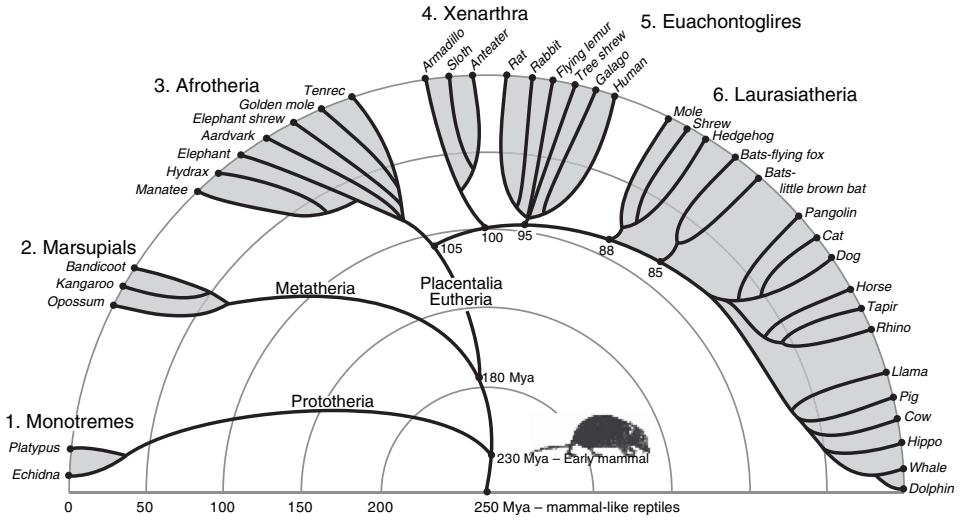

Figure 1 A broad overview of current understanding of the phyletic radiation of mammals. While uncertainties remain, current evidence supports the division of extant mammals into six major clades or superorders (1–6). The molecular evidence, together with the fossil evidence, allows estimates of the times of divergence for these major branches of the radiation and their sub-branches. Thus, early mammals emerged from mammal-like reptiles around 230 Mya. Most early mammals were shrew-like in appearance, and they changed little until the extinction of dinosaurs some 65 Mya. An early mammal is depicted at the center of the radiation, the starting point for mammals. Based on Murphy, W. J., Pevzner, P. A., O'Brien, J. O. 2004. Mammalian phylogenomics comes of age. *Trends Genet.* 20, 631–639.

molecular studies of phylogenetic relationships. As an example, we know from the discovery of fossil whales (Cetacea) with retained hind limbs (Gingerich *et al*., 2001), that whales evolved from a branch of even-toed ungulates (Artiodactyls), and molecular evidence supports the same conclusion (Shimamura *et al*., 1997). A version of a modern phylogenetic tree of the mammalian radiation, showing only the main branches, is shown in Figure 1. This depiction usefully guides the following discussion of brain evolution in mammals, which focuses on neocortex as a flexible structure that has been modified in many ways.

3.03.2 The Basic Structure of Neocortex and the Transition from Dorsal Cortex of Reptiles

In order to understand the different ways that neocortex has changed in the evolution of various mammals, it is useful to briefly review the basic organization of neocortex, and contrast it with its homologue, the dorsal cortex of reptiles (Figure 2; Northcutt and Kaas, 1995). The cortex is the outer sheet of tissue of the forebrain. In reptiles, three major regions are generally distinguished (Figure 3). A dorsomedial region forming the medial walls of the cerebral hemisphere has long been identified as a homologue (the same structure) of the mammalian hippocampal formation (e.g., Smith,

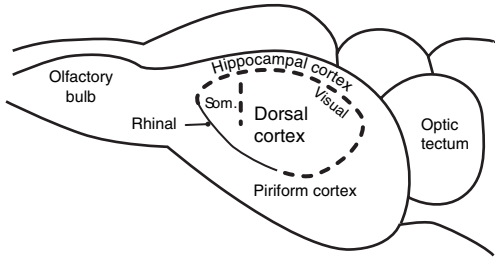

Figure 2 A dorsolateral view of the brain of a turtle showing subdivisions of the forebrain. The large olfactory bulb provides input to the lateral cortex (cf. Figure 3) that is the homologue of piriform cortex of mammals. A small dimple, referred to as the rhinal sulcus in mammals, separates dorsal cortex from lateral cortex, the homologue of piriform cortex. Dorsal cortex is the homologue of neocortex of mammals. Dorsal cortex has visual inputs from the lateral geniculate nucleus, as in mammals, and there is evidence for somatosensory (som.) inputs as well. The medial hippocampal cortex is the homologue of the mammalian hippocampus. The optic tectum is the homologue of the mammalian superior colliculus. Based on Hall, W. C., Ebner, F. F. 1970. Thalamotelencephalic projections in the turtle (*Pseudemys scripta*). *J. Comp. Neurol.* 140, 101–122.

1910; see Striedter, 1997 for review). In reptiles, this tissue does not have the coiled form of the mammalian hippocampus, but its location and appearance, with a single dense row of cell bodies sandwiched between layers of fibers, help identify it as the hippocampus. A dorsal sector, the dorsal cortex of reptiles, is the homologue of mammalian neocortex. Nevertheless, dorsal cortex is only

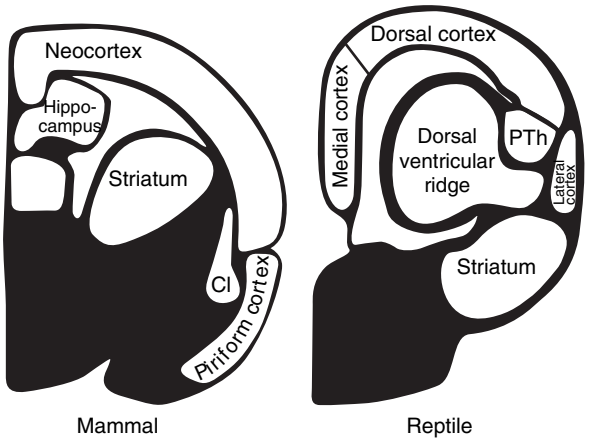

Figure 3 Schematic cross sections through the forebrain of a reptile and a small-brained mammal. Note that the dorsal cortex of reptiles, although smaller, corresponds in position to neocortex of mammals. PTh, pallial thickening; lateral (piriform) cortex; Cl, claustrum. Based on Striedter, G. F. 1997. The telencephalon of tetrapods in evolution. *Brain Behav. Evol.* 49, 179–213.

slightly modified in appearance from the medial hippocampal cortex. Dorsal cortex has a thin deep layer of fusiform neurons with horizontal dendrites, and a more superficial layer of more pyramid-like neurons with dendrites extending vertically into an overlying fiber layer (e.g., Ulinski, 1986). Together these two cell layers are rather thin, only a few cells thick, and unimpressive. Afferents from the thalamus enter from the lateral margin of dorsal cortex and course in the outer fiber layer to contact the dendrites of the cells below (Hall and Ebner, 1970). The ventrolateral part of dorsal cortex, termed the pallial thickening, is now thought to be the reptilian homologue of the mammalian claustrum (a thin sheet of cells internal to layer 6 of ventrolateral neocortex with interconnections with neocortex (see Dinopoulos *et al.*, 1992 for review). The lateral cortex of reptiles is considered the homologue of piriform (olfactory) cortex of mammals. The point of this brief description is to stress that mammalian neocortex is not really a new structure in mammals. Hence, many comparative neuroscientists prefer to call it isocortex for its uniform appearance in mammals. However, a comparison of dorsal cortex with neocortex illustrates that neocortex has new features and in that sense can be called new.

In contrast to dorsal cortex of reptiles, neocortex in all mammals is a rather thick structure, packed with neurons, that is traditionally described as having six layers of different functions (Figure 4). Across these layers, a species variable numbers of fewer than 100 to over 200 neurons are aligned in a vertical row. As neurons in this row (sometimes called a minicolumn) are densely interconnected, they constitute a functional unit that integrates the functional roles of neurons across all the layers. The functionally dominant inputs for such minicolumns consist of only a few closely related inputs from a location in the thalamus or other parts of cortex, which terminate largely on the small stellate or granular neurons of layer 4. Large pyramidal neurons of layer 5 provide outputs over long distances, mainly to subcortical structures such as the superior colliculus and spinal cord, but also to the other hemisphere and to other parts of cortex in the same hemisphere. Smaller pyramidal neurons in layer 3 provide most of the connections between different sectors of cortex while pyramidal and spindle shaped neurons in layer 6 provide 'feedback' projections to whatever thalamic nucleus or cortical area provides the driving input to layer 4 neurons, and projections to the claustrum (see Dinopoulos *et al.*, 1992). Other inputs to any sector of cortex include modulating feedback inputs to superficial (1, 2, and 3) and deep (5 and 6) layers from other areas of cortex, modulating inputs from the thalamus, and modulating inputs from various neurotransmitter specific nuclei (dopamine, serotonin, acetylcholine). Many of these modulating inputs terminate on the distal ends of dendrites of pyramidal cells. Neurons in different rows or minicolumns of neurons interact via horizontal connections stemming largely from layer 3 pyramidal cells. Layer 2 has small pyramidal cells. All layers contain stellate-shaped inhibitory neurons (~20% of the total) of several types that inhibit nearby neurons when they are activated by inputs from neurons in other structures or by nearby neurons. The great computational power of neocortex comes, in part, from this basic arrangement of neurons with specialized roles within and across layers, and the activation of a complete row of neurons by a few powerful inputs. All this while this activity is subject to modification by nearby inhibitory neurons, horizontal intrinsic connections, feedback connections from other areas of cortex and the claustrum, and various neurotransmitter modulating inputs from the brainstem and thalamus. All these connections allow the outputs of such a row of neurons to be modified by their activating patterns. Many of the modifications are instantaneous and short-lived, but highly active inputs can induce long-term changes in responsiveness as well (plasticity and perceptual learning). The result is great flexibility. In addition to this flexibility based on sensory experience and activity patterns unique to each individual, natural selection can genetically modify all the features of

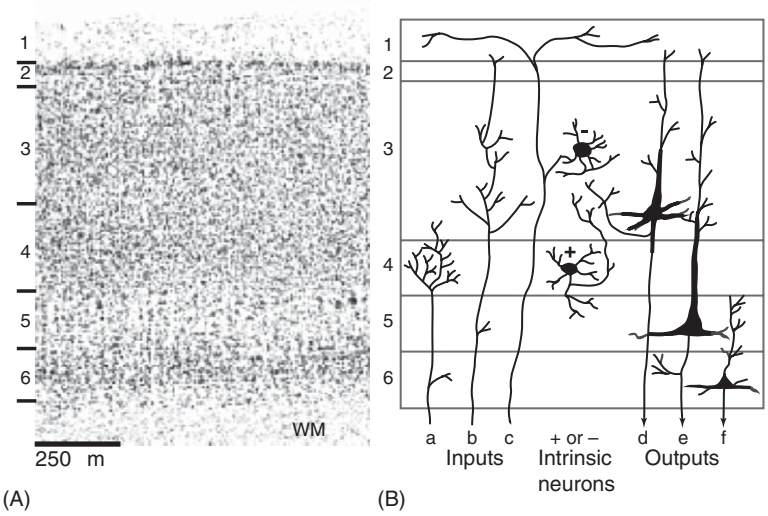

Figure 4 The laminar organization of neocortex in mammals. A, A thin slice of neocortex through primary visual cortex (V1) of a prosimian primate (galago) that has been stained to reveal the cell bodies (dark dots) of neurons, but not axons or dendrites. The cell bodies vary in shape and packing density across the thickness of cortex in a laminar pattern. Traditionally, six layers have been recognized, along with varying numbers of sublayers. Different cortical areas within a species vary in the appearance of the layers, from more to less distinct, and the same (homologous) area across species varies in appearance, but often they retain enough similarities to be identified. This cortex is just over 1 mm thick, with the cortical surface at the top of the photomicrograph and the white matter (WM) or fibers (axons) that connect cortex with other areas and structures at the bottom. B, A schematic of the cortical layers showing some of the types of neurons and connections. The major activating inputs are axons (a) from other areas of cortex or from the thalamus that terminate on stellate or granule cells in layer 4 (and inner layer 3). These 'spiny' stellate cells, thought to be modified pyramidal cells, have short dendrites within the layer, while sending axon branches to activate neurons in layers 3 and 5. Typically, the axons (a) activating neurons in layer 4 also have a branch that provides some activation of neurons in layer 6. Other inputs (b) include feedback, activating axons from other areas of cortex that terminate in layers 1–3 and layer 5. Other modulating inputs (c) from the thalamus and brainstem terminate in layer 1, and in layers 2 and 3. Most of the neurons in layers 2, 3, 5, and 6 are pyramidal neurons with short basal dendrites in the same layer, and a long apical dendrite that usually reaches layer 1. All layers have a portion of inhibitory stellate neurons (–) with short nonspiny dendrites and short axons. Axon inputs, spiny stellate cells (+) and pyramidal cells activate other such cells and the inhibitory neurons, which synapse on excitatory neurons to dampen their activity. Layer 3 pyramidal cells project to other areas of cortex and to subcortical targets. Layer 5 pyramidal cells project mainly to subcortical targets. Layer 6 cells provide feedback connections to the thalamus or other areas of cortex, as well as to the claustrum.

such rows of cortical neurons over generations, to alter and adjust functions. For example, the functions of any row of neurons can be changed by evolving different activating inputs, or different targets for the outputs.

There are two other basic features of neocortex that have contributed to it being such an important part of the brain in all mammals, and the dominant part of the brain in most mammals. The sheet of cortex that is made up of vertical rows of cells that form layers is subdivided across its surface into functionally distinct and specialized regions called areas that are often divided into smaller functionally distinct regions called columns or modules (the term, module will be used here). The concept of a cortical area goes back to early investigators, such as Brodmann (1909), who defined cortical areas as the 'organs of the brain', and identified areas by structural differences in the layers and neurons between sectors of cortex. The early evidence for functionally specialized areas came from observing impairments in abilities of humans and other mammals after damage to specific regions of neocortex. Areas can be small or large, depending on functional role and overall size of neocortex, but the general concept is that they constitute a region of cortex with sharp boundaries where neurons are related in function as a consequence of having specific types of inputs and specific targets for outputs (Kaas, 1982). The prototypical example of an area is V1, also known as area 17 or striate cortex, which receives most of the output of the dorsal lateral geniculate nucleus of the visual thalamus, and provides activating visual inputs to other areas of cortex. Each row of cells in V1 deals with inputs corresponding to a specific location in visual space, and adjoining rows deal with adjoining locations in space so that the V1 of each cerebral hemisphere forms a map or representation of the contralateral visual hemifield. Other areas receive inputs from other sensory modalities, or from other cortical areas, and have outputs to other types of structures. This allows areas to have great flexibility in function, while relying on similar minicolumns as the basic computational unit. Most

importantly, the relatively simple computations of cortical minicolumns, can result in very complex outcomes, just by the process of reiteration. The outputs of any cortical area provide inputs to other cortical areas where the computational functions are repeated. This means that the more cortical areas there are, the more steps in processing are possible, resulting in more sophisticated computations. Mammals with large brains and large sheets of neocortex generally have more cortical areas and more variable and sophisticated behavior. Cortical evolution in mammals is largely characterized by modifications that change the functions of cortical areas, and by the addition of cortical areas, thereby adding steps to the information processing sequences.

A further feature of cortex that adds to its flexibility is the cortical column or module (Kaas, 1982). Many, perhaps all, cortical areas are not uniform in function. Relative to its surface, a cortical area may be divided into a tile-like or band-like pattern of alternating blocks of neurons of related, but slightly different functions. Sensory receptors, and the computations based on inputs from these receptors, create different functional classes of neurons at various levels of processing in the nervous system. The projections of these classes to other structures can be combined for various types of integration, or segregated for further processing. Modules, which include neurons across cortical layers, are one way of maintaining functional segregation. As an unusual (perhaps unique) example of modular segregation, the ancestors of the highly specialized monotreme, the duck-billed platypus, evolved electroreceptors in the skin of its nose (bill), which is also highly sensitive to touch. What to do with this new type of input? The platypus uses the somatosensory system for analyzing the electroreceptor input by dividing the structures for processing tactile inputs into modules for tactile receptor inputs and modules for electroreceptor inputs. Thus, the part of primary somatosensory cortex of the platypus that represents the bill is subdivided into alternating modules of neurons processing information form either electroreceptors or tactile receptors (Krubitzer *et al.*, 1995). The reorganized and subdivided somatosensory system can now be used to detect the electrical activity of the muscles of prey as the platypus feeds in the water. Mammals have evolved different types of modules within cortical areas in the process of acquiring new and expanded functions. This ability of cortex to be modified by forming modules is another reason why cortex has been so important in brain evolution.

As another sign of the ability of cortex to evolve in different ways, separate classes of input to a cortical area may be segregated in sublayers rather than in modules (see Visual Cortex: Evolution of Maps and Mapping). For example, in primates, two classes of visual input, stemming from two classes of retinal ganglion cells (M and P), form segregated pathways to primary visual cortex, where they terminate either in the upper or lower half of layer 4, forming two functionally and morphologically distinct sublayers (Casagrande and Kaas, 1994). As another example, a different type of segregation occurs in the visual system of tree shrews, where the classes of retinal ganglion cells that respond to light onset (ON cells) or light offset (OFF cells) form segregated pathways that terminate in upper or lower sublayers of layer 4 of primary visual cortex (Norton *et al.*, 1985). Thus, different types of sublayers of the same layer can form in the same cortical area in different lines of mammalian evolution. Given this and other modes of modification, no wonder that neocortex has played such a critical role in mammalian evolution.

Before ending this section, we need to consider another variable feature of neocortex, the sensory representation. In some sense, cortical sensory areas constitute maps or representations of peripheral arrays of sensory receptors: the mechanoreceptors of the skin, the photoreceptors of the retina, and the row of sound sensitive hair cells of the inner ear. Mammals evolve different processing systems for these receptors by distributing them differently in the receptor sheet, and by providing some of them with proportionally more neurons in representations. Thus, behaviorally significant parts of receptor surfaces evolve greater numbers and densities of receptors, and by maintaining a set number of cortical neurons for each receptor, these parts of the receptor sheet activate larger parts of the cortical representations of the receptor sheets than do receptor surfaces with fewer receptors. Traditionally, this has been called the 'cortical magnification' of important sensory surfaces. Thus, the tongue and fingers are 'magnified' in the representation of body receptors in somatosensory cortex of humans, while the whiskers of the face are magnified in S1 of rats and mice. The auditory hair cells of echolocating bats that respond to the echo frequency have a large cortical magnification in primary auditory cortex, and many mammals have a magnified representation of the receptors of the central retina, used for detailed vision. The cortical processing machinery is reassigned in evolution as the distribution of receptors in the receptor sheet is changed to allow various behavioral specializations.

A related modification of the representations in cortex has been called 'afferent magnification' (Catania and Kaas, 1997) when some afferents, those with enhanced behavioral significance, gain more cortical space and cortical neurons than do others. Thus, when the receptors of the fovea of the retina, via their ganglion cells, project to the thalamus, they activate a larger cortical territory in V1 than predicted from the number of afferents (Azzopardi and Cowey, 1993). As another example, afferents from the behaviorally important 11th ray of the nose of the star-nosed mole activate more of primary somatosensory cortex than predicted from the number of afferents (Catania and Kaas, 1997). Thus, sensory systems can evolve to devote more cortical space and neurons for some inputs than for others in the same system. Both cortical magnification of receptor-dense sensory surfaces and afferent magnification of behaviorally important receptors and afferents have led to many well-recognized modifications of cortical representations (see Johnson, 1990).

In summary, we have outlined some of the major ways in which neocortex can be modified in the course of evolution to accommodate the behavioral specializations of various mammals. Examples indicate that these modifications do occur, but present understandings of brain organization in most mammals are too limited to allow an extensive survey of the brains of different species and a listing of derived features that have emerged as adaptations. Instead, this section of the review serves to remind us that neocortex is a uniquely organized but highly variable part of the mammalian brain, and suggests that this is the case because neocortex emerged in early mammals as an extremely flexible part of the brain where functions could be modified and extended in so many useful ways. Now we go on to a discussion of how neocortex in early mammals was probably organized, and then to some of the modifications that have occurred in some of the lines of descent. In doing this, we will briefly consider the implications of increasing the size of neocortex and cortical areas, something that has occurred repeatedly in cortical evolution. Even more briefly, we will note the implications of decreasing the size of the cortex from that of ancestors.

3.03.3 The Fossil Record: How Much Neocortex Did Early Mammals Have?

Mammals evolved from cynodonts, mammal-like reptiles, at least 230 Mya (Figure 1). The transition involved a loss of body mass, as early mammals were quite small, mostly shrew to mouse size, but occasionally cat size to coyote size (Hu *et al*., 2005; Kielan-Jaworowska *et al*., 2004). This small size dominated for over 150 million years, during which time the three major surviving lines of mammalian evolution emerged. The egg-laying Prototherian monotremes diverged very early from Therian mammals some 230 Mya, and the Metatherian marsupials diverged more recently from the Eutherian (placental) mammals some 180 Mya. Dental structures suggest that most early mammals were carnivorous and their small size indicates that they likely ate mostly insects. They may have foraged during the night, as most small mammals do today. Along with their small size, early mammals had small brains and little neocortex. Mammals maintained this body type until 65 Mya, when the massive dinosaur extinction took place, after which they rapidly diverged to occupy a diverse range of ecological niches via modifications in body size (see Falkowski *et al*., 2005) and form, including modifications in the brain. Today, there are over 4600 species of mammals. Here we briefly consider some of the fossil remains of some of the early mammals in order to develop some concept of what their brains were like. While there have been a number of publications describing these fossils, they have been collectively described in an extensive review (Kielan-Jaworowska *et al*., 2004).

Brain size varies with body size, as large mammals generally have larger brains than small mammals. Thus, comparisons of brain size across taxa to see if the brains have gotten bigger or smaller depend on comparisons of brain size of a given mammal to the predicted brain size for that mammal's body mass based on averages of mammals of given body masses (Jerison, 1973). For their body sizes, mammal-like cynodont reptiles had brains that were smaller than current mammals, and early stem mammals also had brains smaller than that predicted from body size (brain sizes were based on the size of the brain cavity in the skull). The reconstructed brain from a 85-million-year-old placental mammal is shown in Figure 5. The brain of this early mammal was obviously small. A hint of a rhinal fissure marks the transition from piriform (olfactory) cortex to neocortex. The high position of rhinal fissure indicates that neocortex was quite small. In addition, neocortex did not extend caudally to cover the midbrain, as it does in most extant mammals. The proportionally large olfactory bulb and piriform cortex indicate that much of the forebrain was olfactory in function, while the small neocortex apparently had a limited role in behavior.

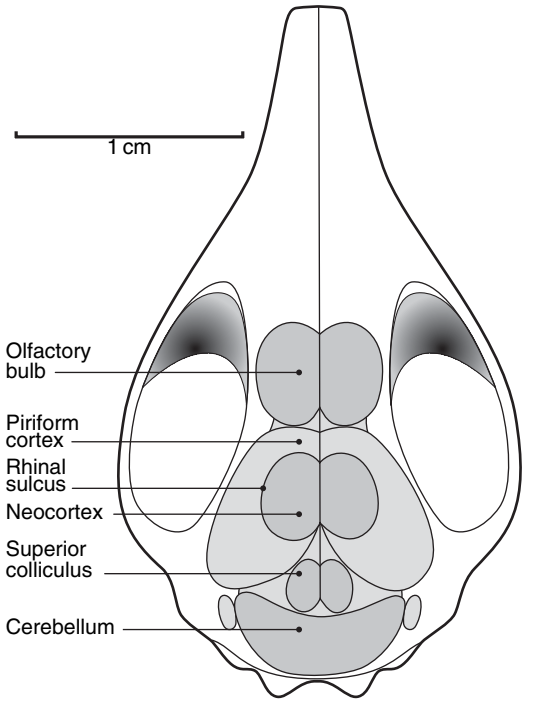

Figure 5 A reconstruction of the skull and brain (from the endocast) of an early (Late Cretaceous) Eutherian mammal (85 Mya). Primitive features of the brain include proportionally little neocortex compared to the olfactory bulbs and piriform cortex. The hemispheres are widely separated posteriorly, and they fail to cover the superior colliculus of the midbrain. Modified from Kielan-Jaworowska, Z., Cifelli, R. L., Luo, Z.-X. 2004. Mammals from the Age of Dinosaurs. Columbia University Press.

In summary, the fossil record provides some information about brain size in early mammals. Their brains were generally smaller for their size than the averages for current-day mammals, and they were not much different from the sizes of the mammal-like reptiles from which they emerged. Much of their forebrains were devoted to olfactory (piriform) cortex and the olfactory bulb. The suggestion of a rhinal fissure high on the lateral surface of some brain endocasts provides evidence that neocortex was proportionally very small.

3.03.4 How was the Neocortex of Early Mammals Subdivided into Functionally Distinct Areas?

As mentioned earlier, modern mammals are not necessarily completely modern. That is, some parts or features of their brains are likely to have been retained from ancient ancestors, while others have been greatly modified or created in more recent ancestors. It is said that evolution proceeds by tinkering, rather than by redesign (Jacob, 1977). Allman (1999) likened brain evolution to the process he noted when he visited a giant power plant of a major city. As greater and greater demands were placed over the years on the power plant, modifications and modernizations were needed to keep up with the demands. Yet, the power plant could never be shut down for a complete redesign. Thus, new control systems were added to old systems and a mixture of ancient to new features were integrated into one functional system. If we can identify the retained parts of brains in the brains of extant mammals, then we can reconstruct the major features of the brains of ancestors. The problem is how to do this. It was tempting for early investigators to infer that simple and undifferentiated brain features are the old ones retained from an ancient ancestor, while structurally complex and internally subdivided features are relatively new. This inference may generally work, as the assumption behind it seems logical, but it can lead to big mistakes, as the same (homologous) cortical area may range from highly differentiated to poorly differentiated in different extant mammals. Only the changes in the differentiation of the area, and not the area itself, differ in age. To avoid such mistakes, rules for reconstructing ancestral character traits have emerged (e.g., Brooks and McLennan, 1991) that assign widely shared traits among members of a clade (any group of mammals that have descended from a common ancestor) to a common ancestor. As members of any such group would have diverged from another at different times in the past and from different shared ancestors, each previous time of divergence is called a node in a cladogram, and characters that diverged from a node are compared, as well as characters inferred for a node from those of other previous nodes. By proceeding backwards in time, characters can be inferred for the last common ancestor of the clade. The optimization of the reconstruction, based on maximum parsimony criterion, can be a bit more complex, using downpass, uppass, and final optimizations (Cunningham *et al.*, 1998). In any case, the questionable assumption of simple-means-primitive is avoided.

Here we use the cladistic approach in a less formal way, largely because many brain features are difficult to identify without extensive investigation. The costs and time in investigation mean that few members of any clade have been well investigated. The difficulties in using a cladistic approach in studies of brain evolution, and the use of a truncated approach have been outlined elsewhere (Kaas, 2002). Here, we simply use the comparisons available to infer some of the major organizational features of neocortex of the first mammals.

A basic assumption of our truncated cladistic approach is that information about the organization of brains of extant mammals is not all equally useful. We know from the fossil record that early mammals had small brains with little neocortex. A number of current mammals, such as opossums, hedgehogs, tenrecs, and to a lesser extent, even rats and mice, have small brains with little neocortex, while several others, such as ourselves, have large brains with proportionally huge amounts of neocortex. Obviously, the large brains must have changed a lot, while the small brains may have changed relatively little. Thus, it should be easier to find the common features in small brains, if only because the needle is in a small haystack. Of course, more extensive cladistic comparisons are important, and they remain the ultimate test of any proposition. For instance, the small brains of echolocating bats have highly specialized (derived) areas of auditory cortex, and studying auditory cortex in these bats alone would lead to a highly misleading idea of how auditory cortex was organized in early mammals. Nevertheless, a productive approach seems to be to study small-brained mammals in as many of the major branches of the mammalian radiation as is practical, make inferences about the brains of early mammals from this data set, and then see how consistent these inferences are with what is more widely known about mammalian brains, including human brains.

We start our analysis by considering the organization of neocortex in the brains of members of four of the six major branches or superorders of the mammalian radiation (Figure 1). The earliest branch (230 Mya) with surviving members, the monotremes, is barely surviving as they are represented today by only two families, one genera of platypus and two of echidna. As these survivors are highly specialized in body form and brain organization as adaptations to unusual niches, we will return to them later. The marsupials or Metatherians, a line some 180 million years old, have been more successful, representing today about 7% of extant species in 16–18 families. They vary in brain size and shape, but most of the American opossums and Australian possums have small brains, with little neocortex, and the brains of some of these, especially the North American opossums, have been well studied. The highly varied superorder, Afrotheria, over 100 million years old, includes the very impressive and very large-brained elephants, but also the small-brained tenrecs, now almost completely restricted to the island of Madagascar. Tenrecs look like the reconstructions of stem mammals as if they stepped out of the distant past (Figure 6). Tenrecs were once

Figure 6 A photograph of the tenrec, *Echinops telfairi*, which is found in southwestern Madagascar. This small member of the Superorder, Afrotheria, retained many primitive characteristics in body form and appearance, so that it was previously classified with the insectivores of the Laurasiatherian superorder. Reconstructions of the appearance of early mammals look a lot like current day tenrecs. Reproduced from, figure 1, Krubitzer, L. A., Kunzle, H., Kaas, J. H. 1997. Organization of sensory cortex in a Madagascan insectivore, the tenrec (*Echinops telfairi*). *J. Comp. Neurol.* 379, 399–414, with permission from Wiley-Liss, Inc.

placed in the Insectivore order with hedgehogs and shrews, but molecular evidence indicates that they are only distantly related, and their many shared features are retentions of ancient mammalian features. Thus, their brains are small with very little neocortex. Another superorder that is nearly as old, Xenarthra, includes sloths, anteaters, and armadillos. These mammals do not have much neocortex, but little is known about how their neocortex is organized. However, somatosensory (S1) motor (M1), visual (V1 plus more temporal visual cortex) and auditory areas have been identified (e.g., Royce *et al.*, 1975). The highly varied members of the Laurasiatherian superorder include the large-brained whales and dolphins, as well as small-brained moles, shrews, and hedgehogs. As quite a bit is known about their brains, we will use them to represent the superorder. The other remaining superorder, Euarchontoglires, includes humans and other primates, and thus, they are of special interest. It also includes tree shrews and flying lemurs as our closest nonprimate relatives, and lagomorphs and rodents. As rats have rather small brains that have been intensively studied, they represent the superorder as we deduce common features across the superorders.

3.03.4.1 Primary Sensory Areas

The brain of the tenrec (Figures 7a and 7b) closely resembles that of early stem mammals (Figure 5) in

external appearance. In a dorsolateral view of the intact brain, a small cap of neocortex, marked by the shallow indentation of the rhinal 'sulcus', rests over the much larger olfactory brain, the piriform cortex, olfactory tubercle, and olfactory bulb. As with early mammals, olfactory processing dominated the forebrain. This is even more evident in the flattened brain. In tenrecs and other mammals,

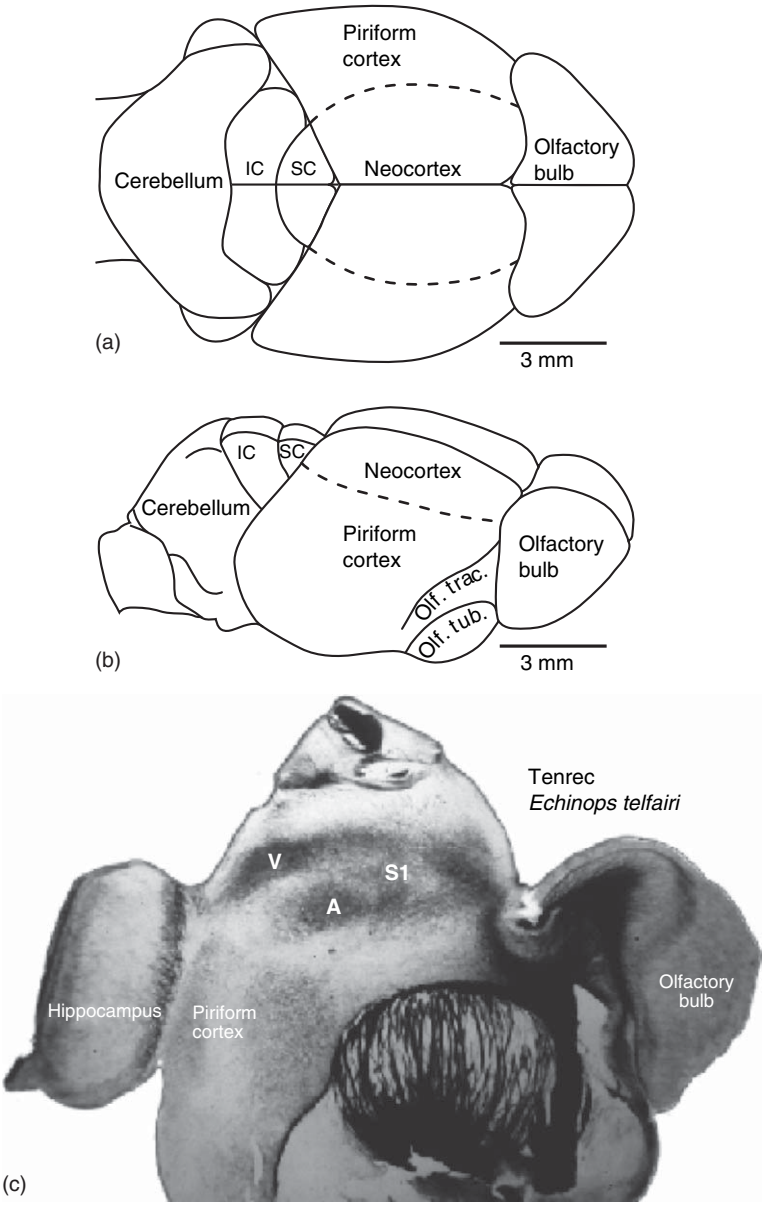

Figure 7 The brain of a Madagascan tenrec (*Echinops telfairi).* a, A dorsal view of the brain showing the small amount of neocortex relative to the large olfactory bulbs and piriform (olfactory) cortex. The small neocortex fails to cover the midbrain, as in the brains of early mammals (compare with Figure 5). b, A lateral view of the brain, showing the greater extent of piriform compared to neocortex. The line dividing neocortex from piriform cortex represents the rhinal sulcus, which is no more than a dimple in tenrecs. The olfactory tract (olf. trac.) and tubercle (olf. tub.) are apparent. c, A brain section stained for myelin that has been cut parallel to the surface after the cortex, hippocampus, and olfactory bulb have been separated from the rest of the brain and flattened. This preparation allows all of neocortex to be viewed as a single sheet, containing piriform cortex and the hippocampus. As in other mammals, the primary visual (V), auditory (A), and somatosensory (S1) areas stain darkly for myelin and are easily identified. Other areas of neocortex of tenrecs are shown in Figure 8b. At the top of the figure, neocortex of the medial wall of the cerebral hemisphere has been unfolded, and the fornix, a bundle of axons, is very dark, while the corpus callosum, a bundle of axons connecting the hemispheres, is less so. Next to the corpus callosum, the cingulate (limbic) cortex is lightly stained. The myelin dense region rostral to S1 may be primary motor cortex, M1. IC, inferior colliculus; SC, superior colliculus. Adapted from, figures 2 and 8, Krubitzer, L. A., Kunzle, H., Kaas, J. H. 1997. Organization of sensory cortex in a Madagascan insectivore, the tenrec (*Echinops telfairi*). *J. Comp. Neurol.* 379, 399–414, with permission from Wiley-Liss, Inc.

it is possible to separate the cortical sheet from the underlying basal ganglia and thalamus, flatten the whole sheet, cut it into thin sections parallel to the cortical surface, and stain these sections to reveal architectonically distinct subdivisions. For the small tenrec brain, it is relatively easy to also include the septal region below the corpus callosum on the medial wall of each hemisphere, the unfolded hippocampus, and the olfactory cortex and olfactory bulb (Figure 7c). In such sections, it is apparent that olfactory cortex is three or more times larger than neocortex, and that both the hippocampus and the olfactory bulb are nearly as large as neocortex. In a section stained for myelin, four regions stain darkly. One is primary visual cortex, V1 or area 17, another is primary somatosensory cortex, S1, or in primates, area 3b (Kaas, 1983). A third region is primary auditory cortex, and the fourth in frontal cortex may demarcate primary motor cortex, M1.

We find three of these areas of tenrecs, V1, S1, and A1, in opossums, hedgehogs, and rats (Figure 8), representing three other superorders. Indeed, these fields appear to exist in all examined mammals, with the possible exception for V1 of mammals with no functional object vision. Yet, even in the subterranean 'blind' mole rat, a small 'visual' area may be architectonically apparent (Cooper *et al.*, 1993), although involved in nonvisual functions. These three sensory areas may be not only early areas present in the first mammals, but also areas that emerge early in the development of cortex, possibly to organize the overall arrangement of other later developing areas in all mammals (Krubitzer and Kaas, 2005). Thus, V1, S1, and A1 may have been retained in extant mammals as necessary components of the developmental plan for neocortex. These three areas of cortex can be identified by a number of features, including direct

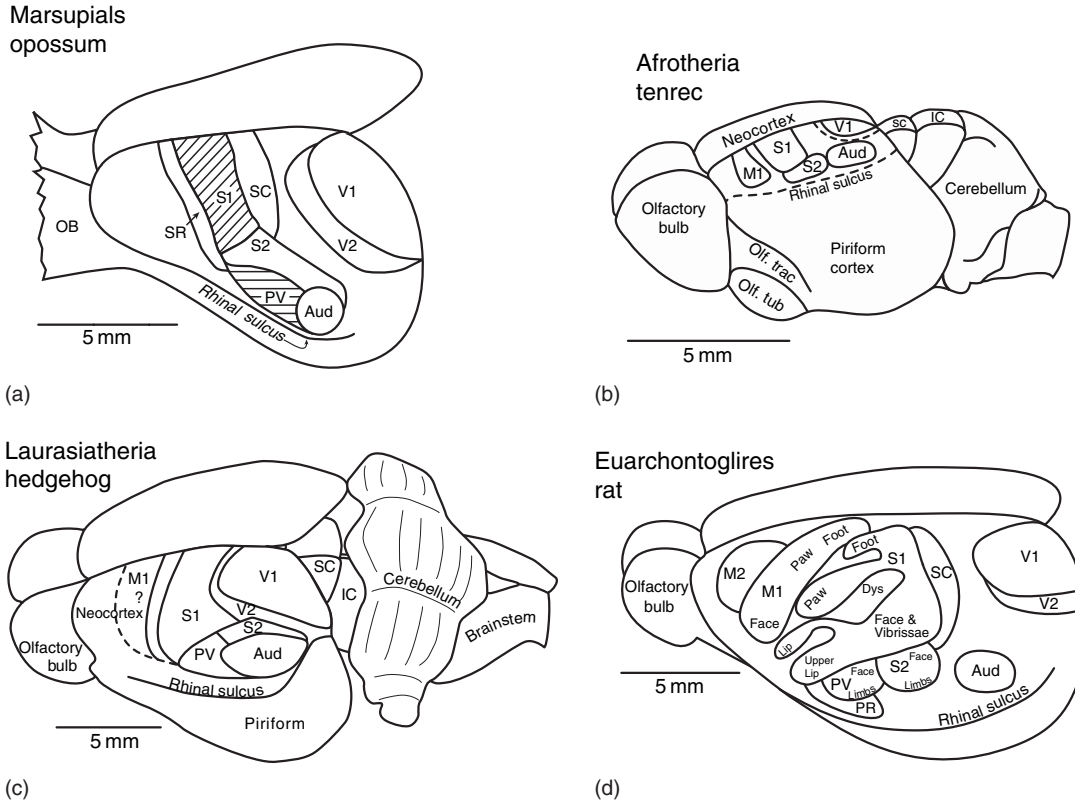

Figure 8 Dorsolateral views of the brains representing small-brained members of four of the major superclades of mammals. In each of these four brains, only a few areas of neocortex have been identified, and most of these are present in all four brains. All have primary and secondary visual (V1 and V2) and somatosensory (S1 and S2) areas, as well as a primary auditory area (Aud). The parietal ventral (PV) somatosensory area has been identified in three of these brains, but was not detected in tenrecs. Somatosensory fringe areas rostral (SR) and caudal (CR) to S1 may exist in all these mammals. A primary motor area, M1 has been identified in many placental mammals, but not in opossums. The presence of some areas in members of all four clades suggests that these areas were present in a common ancestor. a, The brain of a North American opossum (see Beck *et al.*, 1996, for details). b, The brain of a tenrec (see Krubitzer *et al.*, 1997, for details). c, The brain of a hedgehog (see Catania *et al.*, 2000). d, The brain of a rat (see Remple *et al.*, 2003). In (c) and (b), the brainstem is attached and the superior colliculus (SC) and the inferior colliculus (IC) both are apparent, as neocortex is so small that it fails to cover them. Adapted from Kaas, J. H. 2004. Evolution of Somatosensory and Motor Cortex in Primates. *Anat. Rec. Part A* 281A, 1148–1156, with permission from Wiley-Liss, Inc.

inputs from specific 'relay' nuclei of the thalamus, orderly representations of the corresponding receptor sheet in a characteristic manner, and a 'sensory' type of histological structure. At least one of these fields emerged in cortex well before the advent of mammals, as a dorsal lateral geniculate nucleus with inputs from the retina has been identified as projecting to a 'V1' of dorsal cortex of reptiles (Hall and Ebner, 1970). Auditory and somatosensory relay nuclei also exist in the thalamus of reptiles, but they appear to have largely subcortical (basal ganglia) targets. However, a small somatosensory region of dorsal cortex has been described in turtles (Figure 2). Cortical targets for the auditory thalamus apparently emerged with or directly before the first mammals (see Visual Cortex of Turtles).

The existence of a fourth area of neocortex of tenrecs, the primary motor area (M1), in the stem mammals remains unsettled. M1 has been identified in all placental (Eutherian) mammals that have been appropriately studied, so it is nearly certain that the common ancestor of all placental mammals had an M1. However, the evidence for M1 in marsupials and monotremes is uneven and a bit confusing. In placental mammals, M1 is located rostral to S1, with a narrow strip of somatosensory cortex separating the two (see below). In contrast, there is no convincing evidence of an M1 in marsupials (see Beck *et al*., 1996). M1 is usually identified by electrically stimulating cortex, as this will evoke movements that vary in type depending upon which part of M1 is stimulated. Hindlimb movements are evoked medially in the M1 strip; forelimb movements in the middle, and face, vibrissae, and tongue movements are evoked laterally in the M1 strip. In opossums (e.g., Beck *et al*., 1996; Frost *et al*., 2000), no movements could be evoked from the expected region of M1, although some movements were evoked by electrically stimulating S1, as in placental mammals. In addition, M1 can also be identified by architectonic characteristics that reflect the major function of M1 in motor control. Specifically, M1 does not have a distinct layer 4 of cortex, which is well developed in sensory areas, and it has at least a somewhat larger layer 5 containing large pyramidal neurons, as these are the motor output neurons to the brainstem and spinal cord. In opossums and other examined marsupials, there is no architectonic evidence for M1. Finally, patterns of connections can help identify M1. In Eutherian mammals, M1 receives somatosensory inputs from several somatosensory areas, as such sensory inputs are necessary to guide motor control. In opossums, such areas as S2 do not project to the expected location of M1. More significantly, M1 pyramidal cells project to motor neuron pools in the brainstem and spinal cord. In opossums, none of the cortex in the expected location of M1 projects to the spinal cord (Nudo and Masterton, 1990). While a small number of neurons in S1 and the second somatosensory area, S2, do project to the spinal cord, they terminate in the dorsal sensory part of the spinal cord, as do corticospinal projections from S1 in placental mammals, rather than in the motor neuron groups in the ventral spinal cord, as M1 projections do in placental mammals. Thus, it appears that opossums do not have a motor cortex, and whatever weak motor control cortex has on guiding motor behavior, it comes from somatosensory cortex. This lack of motor cortex is thought to account for the relative lack of skilled forelimb movements in opossums compared to rats (Ivanco *et al*., 1996).

A remaining puzzle is that opossums do appear to have a motor thalamus, a ventrolateral complex, VL, as defined by inputs from the deep cerebellar nuclei (Walsh and Ebner, 1973). VL projects to motor cortex in placental mammals, but its target in opossums appears to be S1 (Killackey and Ebner, 1973). One theory for the evolution of M1 is that M1 differentiated out of S1 (Frost *et al*., 2000; Lende, 1963), but there is no clear evidence for this, and M1 could have emerged in some other way. Another intriguing possibility that further study could rule out or verify, is that some of the marsupials with larger brains and better motor skills have independently evolved a motor cortex. In support of this speculation, the projections from the motor thalamus (VL) in some marsupials, appear to include some cortex rostral to S1 (e.g., Haight and Neylon, 1981).

Given that there is no evidence for a motor area in dorsal cortex of reptiles, and apparently no motor cortex in marsupials, together with the assumption that once evolved, a motor cortex would be too useful to abandon, a reasonable inference is that motor cortex evolved in the stem placental mammals after they diverged from marsupials. If so, monotremes should not have any motor cortex. However, this is presently uncertain. There is some evidence in both echidna and platypus that movements can be evoked in a region of cortex rostral to S1, and this cortex has architectonic features suggestive of M1 (see Krubitzer *et al*., 1995). It would be useful but probably difficult (because of limited opportunities for study) to obtain definitive evidence for M1 in monotremes. If M1 is present, we need to consider the possibility that M1 emerged with early mammals and was somehow lost in at least some marsupials. For now, the scenario that M1 evolved later with placental mammals seems more likely. If so, it is important to remember that neocortex still participated in motor control in

non-placental mammals. The somatosensory areas are more sensory than motor, but they do have motor functions.

3.03.4.2 Other Cortical Areas

What other subdivisions of neocortex were likely present in stem mammals? In all appropriately studied mammals, a second somatosensory area, S2, has been described as adjoining S1 on its lateral border (see Beck *et al*., 1996). S2 gets somatosensory inputs from S1, receives additional somatosensory inputs from the somatosensory thalamus, and projects to nearby sectors of somatosensory cortex and, in placentals, to motor cortex. S2 appears to be a component of neocortex in all mammals. An adjoining oval of cortex, the parietal ventral area, has been identified as present in a range of mammals, including many placentals (Krubitzer *et al*., 1986), marsupial opossums (Beck *et al*., 1996), and monotremes (Krubitzer *et al*., 1995). However, parietal ventral (PV) somatosensory area has not always been found, even after a detailed exploration of the appropriate region of cortex (Frost *et al*., 2000). PV closely resembles S2 in connections and architectonic features, so that it can be difficult to distinguish from S2. However, PV does have a separate representation of the contralateral body receptor sheet that mirrors that of S2, and evidence of two mirror-image representations provides clear evidence for PV. The evidence for PV in some species of the three major branches of mammalian evolution suggests that PV evolved in the early stem mammals, and has either been difficult to detect, or has been lost in some small-brained mammals (this is discussed in the following).

In mammals that have been tested, S1 projects to a narrow strip of cortex just rostral and just caudal to S1, as well as to S2 and PV (see Beck *et al*., 1996). These two strips of cortex appear by connections to be somatosensory in function, although they may be multisensory as well (Wallace *et al*., 2004). In either case, early mammals likely had four or five somatosensory areas, as well as perirhinal cortex with S2 inputs that would serve as a relay to the hippocampus.

In regard to visual cortex, most mammals appear to have several visual areas. With a few known exceptions, all carefully studied mammals have demonstrated a second visual area, V2 (Kaas and Krubitzer, 1991; Rosa and Krubitzer, 1999). V2 constitutes a visual area that borders V1 laterally (or rostrally in primates) along the representation of the vertical line of decussation of the retina (the vertical meridian through the center of gaze) of V1, and forms a smaller, mirror image of the representation of the contralateral visual hemifield that is in V1. Some investigators have interpreted the region of V2 of rats as consisting of a row of smaller visual areas (e.g., Montero, 1999), but this interpretation appears to stem from confusing modules within V2 as separate visual areas (Kaas *et al*. 1989). In many mammals, V2 is not homogeneous in function, but contains a row of patches or bands along its length that alternatively have somewhat different connections and functions. In a few other mammals, V2 appears to be absent. Thus, there is no evidence for V2 in the 'blind' mole rat (Cooper *et al*., 1993). In mammals with little visual function, V2 and other visual areas were likely lost in evolution. In addition, the least shrew (*Cryptotus parva*) has no V2 (Catania *et al*., 1999). Instead, V1 directly adjoins S1, leaving no space for V2. However, the 4–5 g adult least shrew is one of the smallest of mammals, probably near the limit in small size for mammals, and it may have lost V2 in evolution to allow other areas, such as V1 and S1, to remain large enough to preserve functions in its small brain. As cortical areas became smaller, they do so by having fewer neurons, and below some number of neurons, the functions of areas cannot be maintained. As the extremely wide distribution of V2 across mammalian taxa indicates that V2 evolved very early with or before the first mammals, the evidence for the absence of V2 in some mammals argues that cortical areas can be lost as well as gained.

By determining the connections of V1 and V2, other cortical areas with visual input can be identified. In a range of placental and marsupial mammals, V1 demonstrates connections with cortex just medial to V1 and just lateral to V2 (e.g., Martinich *et al*., 2000). Both the medial and lateral regions may contain one or more visual areas, and presumptive fields in these regions have been identified with different names by various investigators. A small region medial to V1 has been called prostriata in primates and the splenial visual region in other mammals (Rosa *et al*., 1997). Prostriata likely existed as a visual area in early mammals, as did one or more small visual areas lateral to V2. As a conservative estimate, early mammals had V1, V2, a prostriata, and a small visual area in temporal cortex for a minimum of four areas, but possibly five or six.

All mammals examined have a region of auditory cortex that is typically located in temporal cortex caudal to S1 and ventral to V2. Much of this auditory region has been characterized as primary auditory cortex, A1, on the basis of several criteria, including architectonic characteristics of sensory cortex,

auditory inputs from the principle (ventral) nucleus of the medial geniculate complex, and often, physiological evidence, not only for neurons responsive to auditory stimuli, but for a systematic representation of tone frequencies across the taxa (a tonotopic representation). A problem here in comparing species is that many taxa have more than one area that has some of the characteristics of primary auditory cortex. It is likely that across species different areas have sometimes been identified as the primary auditory area, A1, a field first identified in cats (see Kaas, 2005). A survey of studies on auditory cortex suggests that an area that represents tones from high to low frequencies in a rostrocaudal dimension across the field, as in cats, is present in a number of other studied placental mammals, and in opossums. This A1 may have been present in early mammals. But, the evidence for another primary-like anterior field, with a reversed order of tonotopic organization is also common, as well as for a bordering fringe of two or more secondary fields. From this evidence, it seems likely that early mammals had at least one primary auditory field, A1, and perhaps two, with a bordering belt of two or more secondary fields, for a total of three to five auditory areas. At least one of these fields may also have responded to somatosensory stimuli.

As a part of a system that is devoted to evaluating the quality of food, early mammals are expected to have one or more taste areas in neocortex. Comparative data are not extensive enough to allow this conclusion, but rodents (e.g., Sugita and Shiba, 2005) and monkeys (see Kaas *et al*., 2006) appear to involve both the tongue representation of primary somatosensory cortex, S1, and a laterally adjacent 'insular' region of cortex in processing gustatory information. This information in turn is relayed to a portion of orbital frontal cortex for an evaluation of the hedonistic properties of the ingested food. While there are great uncertainties, it seems reasonable to postulate that part of S1 as well as neurons lateral to S1 in dysgranular 'insular' cortex were involved in taste in early mammals, and the reward value of food objects was processed further in a orbital frontal area. In primates, the insula is an island of cortex in the depth of the lateral sulcus. The term is used here for the equivalent region of cortex in mammals without a lateral sulcus.

As with taste, there have been few comparative studies of the involvement of neocortex in nociception (pain) and temperature perception. Evidence from primates, cats, rabbits, and rodents implicates S1 and thus other somatosensory areas in at least the sensory-discriminative component of pain, while a region of anterior cingulate cortex on the medial wall of the cerebral hemisphere appears to be important in the affective-motivational component of pain (e.g., Johansen *et al*., 2001; Treede *et al*., 1999). A portion of 'insular' cortex lateral to S1 and S2 may be specialized for the affective component of pain. The same or a similarly located region of insular cortex may be important in processing thermal stimuli (e.g., Davis *et al*., 2004). What is not known is the extent to which pain and temperature depended on subcortical rather than cortical processing in early mammals, but the possibility or even likelihood of specialized cortical areas in insular cortex and/or cingulate cortex remains.

In regard to cingulate cortex of early mammals, current theory holds that this limbic cortex has perhaps four subdivisions, at least in some mammals (Vogt, 2005). Anterior cingulate cortex is involved in the important and basic functions of fear and avoidance behaviors, middle and posterior cingulate areas are sensory in some sense having to do with body and spatial orientation and somatosensory and visual functions. The retrosplenial region, adjacent to the splenium of the corpus callosum, is linked to the hippocampus and is thought to be involved in memory processing. Indeed, all of cingulate cortex appears to be involved in memory via associations with the hippocampus involving thalamic connections and cortical connections. Architectonic evidence for subdivisions of cingulate cortex have been described in a number of mammalian taxa, and nuclei of the anterior thalamus which project to these divisions have been recognized in the common laboratory mammals (Jones, 1985). Overall, more comparative study is needed, but the evidence supports the conclusion that the medial wall of the cerebral hemisphere of early mammals contained three to four, and possibly more, functionally distinct areas.

As noted above, cortex lateral to S1 includes S2 and other areas generally referred to as insular cortex. The insular region adjoins perirhinal cortex, the cortex along the rhinal sulcus. Perirhinal cortex appears to receive afferents from secondary somatosensory and visual areas, and have a role in fear-potentiated startle, via projections to the amygdala (Rosen *et al*., 1992), and memory via the hippocampus (Lin *et al*., 2000). A similar involvement of a perirhinal area in startle and memory may have characterized the neocortex of early mammals.

Finally, the significance of an orbital frontal area or region of cortex in taste has been mentioned, and this and other subdivisions of frontal cortex have been considered to be fundamental components of mammal brains (Preuss, 1995b; Uylings *et al*., 2003), although there are uncertainties about how to identify areas, and how to recognize them across species. As a

result, a broad comparative appreciation of the subdivisions of frontal cortex is lacking. Nevertheless, it seems reasonable to postulate that early mammals had two to four subdivisions of frontal cortex, including one or more divisions of orbital frontal cortex.

In summary, the fossil record indicates that early mammals had small brains with little neocortex. Comparative evidence from extant mammals suggests that this cortex was already subdivided into a considerable number of functionally distinct areas, including four or more visual areas, four or more somatosensory areas, two to three auditory areas, possibly a taste area separate from S1, two to four areas of frontal cortex, one or more perirhinal areas, and three or four cingulate areas. This produces an estimate of 17–21 cortical fields, and this could be somewhat of an underestimate. However, it seems unlikely that early mammals had more than 30 fields or many less than 17. As early mammals had little neocortex, perhaps 150–200 mm^2 per hemisphere, cortical areas would have been very small, possibly averaging about 10 mm^2 in surface area, with some areas being larger (e.g., S1) and others considerably smaller (e.g., S2). As areas were quite small, they may not have been subdivided into different classes of modules. Alternatively, a few areas such as V2 may have already been modular. However, more comparative evidence is needed to address these speculations. Finally, cortical areas differed in architecture (patterns of cell arrangements and other structural features), but the differences were not marked. The different functions of cortical areas depended more on connections, the inputs and outputs, than on specializations of areas in cellular and laminar structure.

3.03.5 What Happened to Neocortex in the Radiation of Mammals?

The short answer to the question above is 'different things' – but sometimes very little. As discussed above, the brains of some mammals appear not to have changed very much over the course of 230 million years. Opossums, hedgehogs, shrews, tenrecs, armadillos, and even rats and mice have retained small brains with relatively little neocortex. This cortex remains relatively undifferentiated in structure and cell types. Neocortex remains divided into a few areas, most or all of which were present in the first mammals. The placental mammals, hedgehogs, shrews, tenrecs, rats and mice, have added a cortical motor area, M1, rats and mice have differentiated a 'barrel field' of barrel-shaped modules representing individual whiskers of the face in primary somatosensory cortex, the smallest of shrews lost V2, and so on. But these are rather minor changes. Clearly, environmental niches can be found where brainpower is less important than reproductive capacity and other factors, and ancestral brains did not need to be changed very much.

3.03.5.1 Impressive Modifications of Small Brains

Even in small-brained mammals, several rather remarkable modifications of neocortex have occurred. To mention only a few, the brains of echolocating bats have specialized, without an expansion of cortex, to facilitate the tasks of flying and echolocating. The echolocating bats have an altered auditory system that over-represents the echo frequency and have specialized several auditory areas of cortex that perform computations based on echoes to locate and identify flying prey and avoid objects (Suga, 1995). The somatosensory system of bats has been modified (Calford *et al.*, 1985) for flying by representing the specialized sensory receptors, the Haarscheiben or touch domes with a protruding hair, on the wing and other parts of the body so that flight can be guided. Without these receptors, bats tumble and fall (J. M. Zook, personal communication). Some rodents and squirrels have emphasized vision by enlarging the visual midbrain structure, the superior colliculus, to 10 times its size in other rodents, and differentiating it structurally into more prominent layers and cell types (Kaas and Collins, 2001), while also adding, expanding, and differentiating subdivisions of visual cortex (Kaas *et al.*, 1989). The star-nosed mole has devoted much of its cortex to three large representations of the mechanosensory receptor structures, called Eimer's organs, that are tightly packed on the fleshy appendages of its nose (Catania and Kaas, 1997). This specialization allows the mole to detect and consume small prey at a rate that exceeds that of any other mammal (Catania and Remple, 2005). The duck-billed platypus blindly searches for prey with its eyes and ears shut in murky water by using sensitive tactile and electroreceptors on its rubbery bill (Krubitzer, 1998). To successfully occupy this niche, the platypus depends on a greatly modified neocortex that is dominated by several large representations of the receptors of its bill. The platypus devotes little cortical territory to visual or auditory cortex (Krubitzer *et al.*, 1995). Such specializations in even small-brained mammals indicate the great flexibility of neocortex as a computational structure. The vertical rows of neurons, modules composed of rows of neurons, and areas composed of modules in neocortex

can be reassigned in evolution to various tasks as needs arise. Similar modifications are seen in mammals that have also enlarged their brains, and modified them in other ways. For example, one can marvel at the great skill that raccoons have in blindly locating food items in water. Using their hands, they rapidly locate the source of tiny ripples and currents that are created by moving objects. This ability is made possible by extremely expanded representations in several areas of neocortex of the receptors of the hand (Welker and Seidenstein, 1959). Finally, it is hard not to be impressed with cebus monkeys, as we are members of a clade of primates without tails. Cebus monkeys use receptors on the glabrous pad of the tip of their tail to actively explore their environment and use their tail to retrieve objects of interest. This ability, of course, depends on devoting large portions of somatosensory areas of cortex to the tactile receptors of the tail (Felleman *et al*., 1983).

3.03.6 The Implications of Changes in Brain Size

Brains also change in other ways. One way that was obvious to early investigators such as Smith (1906) and Clark (1959) was that brains vary in size from very small to very large. Part of this variation, for uncertain reasons, is related to body size, so that brains tend to increase in size by an average factor of ~0.75 with increases in body size (Allman, 1999; Jerison, 1973). Such increases in brain size result typically in disproportionately large expansions of neocortex (Finlay *et al*., 2001), but such increases in neocortical size often do not seem to correlate with the acquisition of notably new abilities. For example, the behavior of lions, with much larger brains, do not seem to be remarkably different from those of domestic cats. Thus, we suspect that closely related mammals that are large or small might have brains with similar organizations, although the brains differ in size (this assumption needs careful evaluation). For example, the arrangement of sensory areas of neocortex seem to be roughly the same in the smaller brains of guinea pigs than the larger brains of capybaras (Campos and Welker, 1976), both related South American rodents. Yet, this cannot be completely true, as brains have a basic scaling problem. Large brains cannot simply be large versions of small brains, because the computational unit of the brain, the neuron, does not scale to large sizes with the brain, as the functions of neurons depend on their size (Bekkers and Stevens, 1970). Obviously, transmission times increase as the dendrites and axons of neurons get longer, unless they are modified by making them thicker, and in other ways. Neurons with longer, thicker axons result in brains that devote more of their volume to axons than the computational parts of axon terminations, dendrites, and cell bodies. As the cell bodies and dendrites of neurons do not vary much in size, larger brains are larger, in the main part, because they have more neurons and even more supporting glial cells. Having more neurons means that each neuron, while maintaining roughly the same number of contacts with other neurons, contacts a smaller proportion of the total number of neurons. This changes the organization of the processing network. To evolve large brains, these scaling problems can be reduced or solved in several ways, but mainly via increases in modular organization and local processing that decreases the need for long connections (Kaas, 2000b). To achieve this solution, large brains with much neocortex should have more cortical areas, and more functional subdivisions of areas into different types of modules, than small brains with little neocortex. There is much evidence to support this premise when species are considered across taxa, but not when one considers closely related members of a taxonomic group of different brain sizes. These later mammals may add neurons and disproportionately axon volume to neocortex with increases in brain size, while maintaining the basic cortical organization of the group. Without organizational adjustments, larger brains may just maintain brain functions, without appreciable gains in functions.

Another problem emerges if one considers the consequences of increasing the size of neocortex without adding modules or areas. If bigger brains are simply expanded versions of smaller brains, then the cortical areas must be bigger. Specific areas do vary greatly in size. V1, for example, is 700 times larger in surface area in a human than a mouse, and the cortex in humans is over twice as thick as in mice. This means that V1 in humans has many more neurons. The functions of cortical areas must, in part, depend on their sizes. Of course, one limit on any reduction in the size of a cortical area is that it must have enough neurons to perform its function. Cooper *et al*. (1993) have previously shown that the small sliver of primary visual cortex that is found in the 'blind' mole rat is too small to allow even a crude image. Thus, this cortex has become nonfunctional, at least for the purpose of object vision. Somewhat larger visual areas, with more neurons, may mediate object vision, but the pixels would be large and the image would not have much detail. This is largely due to the scope of the dendritic trees of neurons as they gather information over some limited portion of cortical area. As areas further increase in size, the

scope of the dendritic window gets proportionally smaller, and the area becomes more specialized for detailed vision at the cost of global vision. However, neurons are also influenced by thalamic inputs that terminate outside their dendritic arbors, via the horizontal intrinsic connections of neurons activated by those inputs. In V1 of a mouse, these short (~1 mm) intrinsic connections can tie all parts of V1 together so that the output of V1 can reflect global processing and directly mediate useful vision, but as V1 gets bigger and bigger, the outputs of individual neurons reflect less and less of what is happening in the total visual scene. Thus, the outputs of a large V1, as in macaque monkeys or humans, provide important details about a visual image, but not enough global information about the visual scene to guide most visual behavior. Other smaller visual areas would seem to need to make sense from the outputs of V1. A macaque monkey with V1 intact, but other visual areas missing, should be virtually blind (this has been difficult to test; see Nakamura and Mishkin, 1986). This line of reasoning suggests that in mammals with large amounts of cortex only a few cortical areas should be large. This general supposition seems well supported. Indeed, even V1 in large-brained mammals is not as large as it would be if it maintained a constant proportion of neocortex. Thus, V1 occupies proportionally more of neocortex in the smaller macaque brains than in the larger human brains (Kaas, 2000a).

The limited variability of neuron size and dendritic arbor size relative to the greater variability of brain size has the added implication that it is easier to change the functions of small cortical areas by adjusting dendritic arbor size than those of large cortical areas (Kaas, 2000b). In brief, increasing or decreasing the scope of the arbors in small areas has more impact on the sizes of receptive fields, and on the nature of processing from regional to global. Thus, pyramidal neurons in large areas may have smaller dendritic arbors than neurons in smaller areas (Elston *et al.*, 1996), as larger arbors would not enlarge receptive field sizes enough to alter functions in a significant way.

3.03.6.1 Larger Brains Often Have More Areas

We have discussed the possibility that in closely related species of different body size, the larger species with larger brains may not differ very much in terms of number of areas. However, when comparisons are made more extensively across mammalian taxa, brains do vary in number of areas, and larger brains tend to have more areas. While early investigators, such as Brodmann (1909), came to this same conclusion by studying cortical architecture in many different mammals, the evidence was not very strong as areas were subjectively defined and identified by subtle differences in histological appearance. More recently, it has been possible to define areas with more certainty by using the multiple criteria of architectonics, connectional, physiological, and gene expression differences. Unfortunately, such studies require a huge experimental effort, and results are more credible if verified by several research groups. It is fair to conclude that all of the cortical areas have not been defined with a high degree of certainty in any mammal, and for most taxa, very little is reliably known about how cortex is subdivided (see Kaas, 2005). Yet, it is clear from the results based on a few well-studied species that the number of areas is quite variable across species. For example, it is easy to see from only the shapes of the brains that squirrels and tree shrews have devoted proportionally more of their cortex to vision, as the visual occipital and temporal regions of neocortex are expanded over frontal motor, somatosensory, and prefrontal parts (Figure 9). Moreover, we know from previous studies that hedgehogs have few visual areas, perhaps four (Figure 8). While the full number of visual areas in either squirrels or tree shrews has not been determined, the number is certainly more than four. For squirrels, seven visual areas have been proposed, while nine have been described in tree shrews (Kaas, 2002). As these two mammals of about the same size are not closely related (Figure 1), more visual areas and proportionally more visual cortex

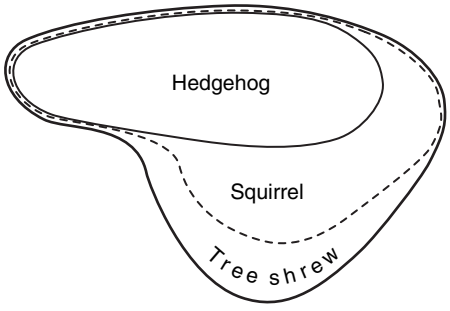

Figure 9 Differences in the shapes of brains suggest changes in function. Here, lateral views of the neocortex of a hedgehog, squirrel, and tree shrew are outlined. The brains have been scaled so that frontal cortex on the left is the same size to emphasize the differences in shape of temporal (ventral) and occipital (posterior) cortex. The highly visual squirrel and tree shrew have greatly expanded temporal and occipital regions of neocortex, and these regions are largely visual in functions. This trait is more pronounced in tree shrews than squirrels. These observations on extant mammals provide a rationale for deducing functional specialization of the brains of extinct mammals from the shapes revealed by skull endocasts.

evolved independently in both lines of descent. Domestic cats have been extensively studied, and they appear to have at least ten visual areas (see Grant and Shipp, 1991, for review). While there is uncertainty about how to divide visual cortex in macaque monkeys, recent proposals number over 30 visual areas (Felleman and Van Essen, 1991). The total number of visual areas in human brains is unknown, but clearly the number is large, as some estimates of total number of cortical areas place the number in the range of 150, possibly 10 times the number present in the first mammals.

Having more areas allows more sophisticated processing via the reiteration process, but also by increasing the number of parallel streams of processing (Kaas, 1989). Another important consequence is that the total amount of wiring (connections) in the cortex is decreased due to greater emphasis on regional processing (Mitchison, 1991). Finally, in large brains, the two hemispheres become less symmetrical in organization, so that areas in one hemisphere no longer have mirror-image counterparts in the other hemisphere. This effectively increases the number of areas while reducing the need for connections between the hemispheres via long axons that cross in the corpus callosum to connect matched pairs of areas (Ringo *et al*., 1994).

While we presently know far too little about how cortical areas are subdivided into classes of modules, we know that this does occur in some brain areas (see Purvis *et al*., 1992; Catania, 2002), and that the same area may be subdivided in different ways in different species. Monkeys have a V2 that is divided into repeating blocks of three types of band-like modules with different connections, architecture, and neural functions (Roe, 2003). In some mammals, including tree shrews and opossums, V2 is subdivided in another manner into modules with and without interhemispheric connections (Cusick and Kaas, 1986). Such modularity adds to the ways neocortex can vary across taxa, and provides a mechanism for grouping types of neurons that need to work together, thereby reducing the connection problem.

3.03.7 Summary and Conclusions

3.03.7.1 Neocortex Varies in Size and Complexity

An overview of the sizes and parts of brains of extant (living) mammals indicates that neocortex varies most in size and complexity relative to the rest of the brain. This indicates that neocortex is an important part of the brain for further study if one is interested in brain evolution. Such studies reveal that neocortex varies in many ways across taxonomic groups. Variations include those in morphological types of neurons, morphological specializations of the six layers that characterize cortex, types of modules that subdivide cortical areas into smaller functional units, proportions of cortical areas that are devoted to specific inputs, absolute size of areas, the size of areas relative to neocortex, intrinsic connections of areas, inputs and output targets of areas, number of areas, types and proportions of modulating inputs, the extent and distribution of interhemispheric connections, and so on. The great variability of these features suggests why neocortex has held center stage in studies of brain evolution in mammals. Neocortex varies in so many ways, allowing so many different adaptations to the environment.

3.03.7.2 Shared Features of Neocortex Organization Across Species Suggest Why Neocortex Is So Modifiable and So Important

The fundamental unit of computation is the vertical array or column of 100–200 neurons that are tightly interconnected, and driven by only a few specific inputs, while modulated and influenced by many other inputs. The grouping of neurons by functional role into layers, modules, and areas simplifies the process of modulating neuron responses by the responses of the most relevant other neurons via direct and indirect connections, as these neurons are nearby. The computations within each column transform the inputs to one or more different types of output, which can be sent to other cortical columns so that the computation process can be repeated with the addition of other inputs. Multiple cortical areas allow cortex to function in serial steps that transform simple computations into complex outcomes, and produce parallel streams that allow information to be used in many different ways.

3.03.7.3 The Fossil Record Indicates that Early Mammals Had Small Brains with Little Neocortex

The olfactory bulb and olfactory (piriform) cortex were relatively large, indicating olfaction was an important source of information about the external world. The small amount of neocortex suggested it played a modest role in regulating behavior. Some mammals with small brains and little neocortex have persisted up to present times, indicating that behavioral niches remain for mammals with limited brainpower. Perhaps because early mammals may have had brains close to the lower limit in size for mammals, few subsequent lines of evolution lead to

smaller brains. Instead, the fossil record indicates that increases in brain size, especially that of neocortex, occurred independently many times over, while some mammals with small brains continued to survive.

3.03.7.4 The Probable Organization of the Neocortex of Early Mammals Can Be Reconstructed, Using an Analysis That Identifies Brain Characters That Are Broadly Distribution Across Mammalian Taxa as Those Likely to Have Been Retained from a Common Ancestor

In a process known as a cladistic analysis, the emergence of novel brain features can be assigned to more or less distinct branching points in a phylogenetic tree (cladogram) for any group of mammals descendant from a specific common ancestor (recent or distant) based on parsimony. As identifying brain characters can be labor intensive and depend on costly experimental procedures, the process of reconstructing the organization of the neocortex of early mammals can be simplified by initially focusing on mammals with brains that resemble those of early mammals in size and proportions. The brains of hedgehogs, shrews, and other insectivores, together with those of tenrecs and opossums are strong candidates, but the brains of other mammals, such as rats, have only slightly increased the proportional size of neocortex, and thus they provide additional comparisons of clear value. Conclusions based on this limited sample can then be validated as consistent or challenged as inconsistent with observations from mammals with more derived brains, in terms of neocortex size and shape, so that all the major branches of the mammalian radiation are considered.

3.03.7.5 Early Mammals Had Poorly Differentiated Neocortex and Few Areas

A comparative analysis indicates that the neocortex of the first mammals was rather poorly differentiated into layers and different neuron types, although six layers of different types of connections and functions were present, as well as pyramidal cells, stellate calls, and two or more types of local circuit inhibitory neurons. Neocortex was divided into ~20 cortical areas, a small number in comparison to the 50–150 proposed for some extant mammals. More specifically, early mammals had a primary somatosensory area and 3–4 other somatosensory fields, primary and secondary visual fields and perhaps two other visual areas, a primary and one or more additional auditory fields, as well as areas of limbic, orbitofrontal, and endorhinal cortex. Motor functions depended on somatosensory areas until the advent of placental mammals, which were characterized by a primary motor area and possibly a secondary motor area. Thus, the neocortex of early mammals was dominated by areas devoted to analyzing sensory information. In several subsequent lines of descent, more sensory areas were added, increasing the complexity of the analysis of sensory information, and motor areas were sometimes added, increasing the sophistication of behavioral responses. Additional multisensory areas sometimes emerged that allowed computational outcomes to be more easily influenced by several sources of information.

3.03.7.6 Theories of the Subsequent Evolution of Neocortex in Mammals Can Be Guided by a Theoretical Consideration of the Implications of Increasing Brain Size

Larger brains with larger expanses of neocortex would not function efficiently without structural modifications. As neurons do not scale up very well with increases of brain size, large brains have more neurons. This generally means that neurons in large brains have connections with a smaller proportion of the total number of neurons than neurons in small brains. Thus, large and small brains function differently. In addition, larger brains require longer connections and thus more time for transmission unless axons are increased in thickness. In part, these connection problems can be addressed in evolution by devoting proportionally more of the larger brain to connections, resulting in decreases in neuron cell body densities in cortex, and having at least some longer, thicker axons. Connection problems can partially be addressed by evolving brains that are more modularly organized, as they became larger with an emphasis on local processing via short axons. Thus, mammals with larger brains and expansive neocortex are expected to have more cortical areas, more areas divided into more modules, more connections overall, and some connections over thick, long axons, but relatively fewer of the longer interhemispheric and subcortical connections. The functions of areas also depend on their size. As areas become larger, their intrinsic horizontal connections may become longer, but not in pace with the expansion of the cortical surface. Thus, neurons become less influenced by the activities of the increasingly distant other neurons in the area. This means that large areas transmit information to other parts of the brain only about a small subset of inputs to the areas. They provide detailed, but focused information. Neurons in smaller areas are influenced by neurons that are more widely distributed across the area, and the computation of local circuits of such neurons provides a more global (but

less detailed) picture of what is going on. Global views of sensory inputs are obviously more useful for directing behavior, but details can be valuable. Thus, a few large areas, and many small to moderately sized areas, would seem to provide the most useful system.

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3.04 Captured in the Net of Space and Time: Understanding Cortical Field Evolution

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Glossary

analogous	Having the same function.
Baldwin effect	The ability of an animal to respond optimally to a given environment.
cortical domain	The portion of cortex devoted to a given sensory system.
cortical field	The fundamental organizational feature of the cortex.
cortical field magnification	The amount of cortex within a cortical field devoted to processing inputs from a behaviorally relevant body part is enlarged.
evolvability	The ability of an organism to generate heritable, selectable phenotypic variation.
genetic assimilation	How an environmentally induced phenotypic characteristic becomes genetically coded in a population.
homologous	A characteristic inherited from a common ancestor.
homoplaseous	An independently evolved characteristic that looks the same across species.
module	Smaller units of organization within a defined cortical field.
pleiotropy	A single gene controls numerous activities during development resulting in various phenotypic effects in the adult organism.

3.04.1 Introduction

Examination of a number of different mammalian brains demonstrates that brain organization, particularly the neocortex, varies dramatically across species. This variation in neocortical organization is accompanied by a considerable degree of behavioral diversity. Specifically, differences in cortical sheet size, organization, number of cortical fields, and connections are associated with differences in sensory, perceptual, cognitive, and motor abilities. How these differences in neocortical organization in mammals arise in evolution and how these alterations generate variable behavioral repertoires are difficult questions to investigate directly because the evolutionary process is highly dynamic, and alterations to the brain occur over hundreds of thousands to millions of years. Despite the fact that evolution cannot be studied 'head on', we can circumvent the problems associated with studying evolution in two ways. First, we can examine the products of evolution, namely extant mammals, and compare their brain organization, to make inferences about the evolutionary process. Alternatively, we can study the developmental processes that generate different aspects of brain organization, since the evolution of the neocortex is the evolution of the developmental mechanisms that give rise to adult phenotypes. We can then postulate how developmental mechanisms may have been altered to produce different phenotypes (see The Origin of Neocortex: Lessons from Comparative Embryology).

The use of the comparative approach has led to number of important insights regarding brain evolution. Likewise, studies of development, particularly recent molecular studies, have provided much

in various aspects of cortical development and organization. However, utilizing the comparative or the developmental approach in isolation in an attempt to uncover principles of brain evolution is problematic. In terms of the comparative approach, examining any extant mammal allows us to observe only a static moment in the evolutionary process. In essence, we have captured, in our net of space and time, a number of individual phenotypes, or individual snapshots, in a process that is constantly in a state of flux. We take these snapshots out of our net, use a number of different tools to dissect and examine them, and then put them together to make an evolutionary moving picture. The problem is that each extant mammalian brain that we observe is a frozen frame or moment in its own moving picture; it has its own evolutionary history and will move in a unique future trajectory. Further, this approach tells us little about the transition between frames and how phenotypic transformations may occur. This is where studies of cortical development merge with comparative analyses.

Studies of the development of the nervous system can strengthen our inferences regarding how phenotypic transitions occur by providing a number of possible mechanisms for this process. However, like the use of the comparative approach, using a developmental approach in isolation to understand brain evolution is problematic. While a number of recent studies provide insight into potential mechanisms that could be involved in some aspect of cortical organization, such as regulating cortical sheet size, they do not demonstrate that such a mechanism is actually being employed in a naturally evolving system (see The Evolution of Neuron Types and Cortical Histology in Apes and Humans). Thus, only by combining both the comparative approach and developmental approach can we appreciate the types of changes that have occurred in different lineages, predict how these transitions may have happened, and validate these predictions by manipulating some aspect of development and determining if the resulting phenotype is consistent with a type of neocortical organization that would naturally occur, as validated through comparative studies.

In this article, we begin by exploring what constitutes a cortical field and discuss homologous features of cortical organization across mammals. Next, we discuss the importance of distinguishing homology from instances of homoplasy when making comparisons across species. Because the concepts regarding what constitutes a cortical field are changing in light of new studies on molecular development, in the second section of this article we discuss some of the molecular aspects of cortical field development, and describe both intrinsic and extrinsic contributions to cortical development, and the role of peripheral morphology and behavior in shaping the cortical field throughout the life of an individual. Then, we discuss the evolution of the neocortex and outline the types of systems level modifications that have been made to evolving brains. Finally, we speculate on the idea that the neocortex evolves to be flexible, and that genetically based adaptations of the brain and body may initially have been activity-dependent features of organization that were present only under unique and consistent environmental conditions.

3.04.2 What is a Cortical Field? Homology, Homoplasy, and Analogy

A cortical field is considered to be the principal organizational feature of the cortex, and most neuroscientists would contend that the addition of cortical fields to the neocortex is what endows greater degrees of neural and behavioral complexity to mammals. Indeed, most would agree that the neocortex, in general, and cortical fields, in particular, are the essence of the mammalian brain; the feature that distinguishes mammals from other vertebrates. We raise the question of what is a cortical field because this issue is particularly important for the study of cortical evolution. If one is interested in the evolution of the neocortex and the addition of cortical fields, then defining homologous cortical fields across mammals is critical. Specifically, it is important to determine which features of the cortical field are most usefully compared across species, and ultimately to appreciate how these features change during evolution.

Although concepts regarding what constitutes a cortical field are changing in light of new studies on the molecular development of the neocortex, in adult mammals, a cortical field is determined by a number of well-defined anatomical, histochemical, and electrophysiological criteria. These criteria were previously outlined by Kaas (1982), and although not exhaustive, have enabled investigators to subdivide the neocortex in a variety of mammals with a high degree of success. Some of these criteria include a complete representation of the contralateral sensory surface (or visual field for visual cortical areas), a unique architectonic appearance, and a distinctive pattern of connectivity. Other criteria include utilization of some subset of neurotransmitters, or the presence of particular behavioral deficits when the area is lesioned. Because errors can be made in subdividing the neocortex when any single criteria is used in isolation, using a combination of criteria to subdivide the

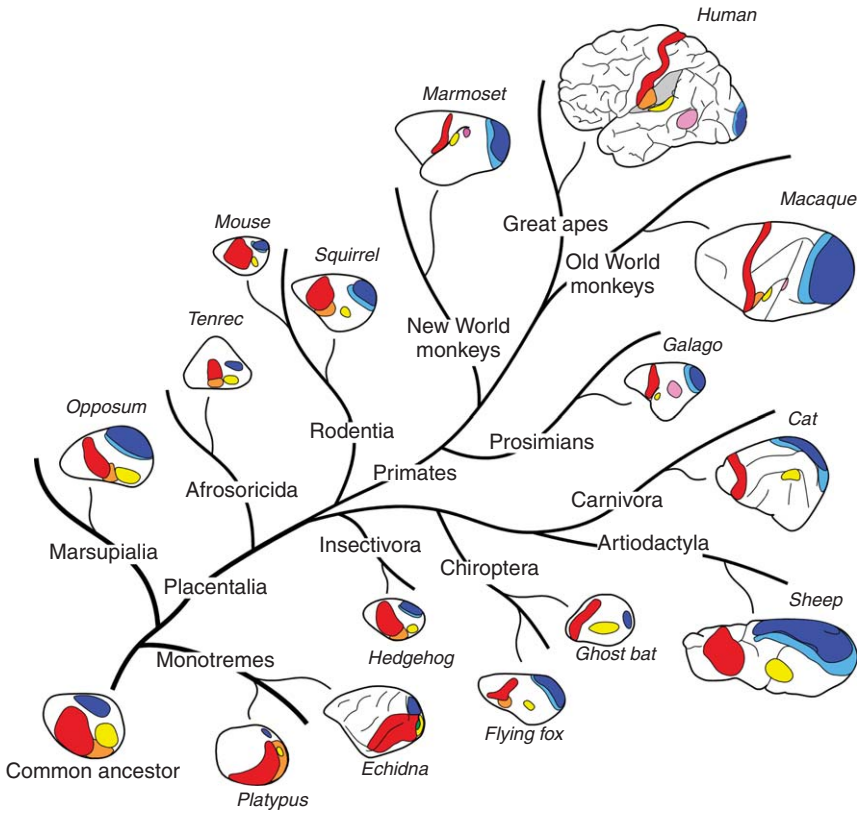

Figure 1 A phylogenetic tree depicting the relationships between major mammalian lineages. The cortex of each mammal contains a constellation of cortical fields that have been identified in all mammals examined. These cortical areas were likely inherited from a common ancestor, and therefore are homologous. Although the organization of the neocortex of the common ancestor is not known, a cladistic analysis allows one to infer the organization of unknown forms, such as the common ancestor. Dark blue = primary visual area; light blue = second visual area; red = primary somatosensory area; orange = second somatosensory area; yellow = primary auditory area; pink = middle temporal visual area. Redrawn from Krubitzer, L. and Kahn, D. 2003. Nature vs. nurture: An old idea with a new twist. *Prog. Neurobiol.* 70, 33–52.

neocortex allows for more accurate comparisons of cortical organization across mammals.

Using these criteria, it has been determined that in some mammals, such as mice, the number of areas that compose the neocortex is relatively small, on the order of 7–12 cortical fields. In other mammals, such as macaque monkeys, the number of cortical fields is larger, on the order of 30–50 cortical areas (see Kaas, 1988, 1993, for review). This increase in the number of cortical fields in some lineages, at least in part, is the neural basis of complex behaviors such as sophisticated communication (language in humans), learning, and cognition. While the number of cortical fields is highly variable in mammals, several cortical fields are common to all species (see Krubitzer, 1995; Krubitzer and Kahn, 2003; Krubitzer and Kaas, 2005). These fields include the primary sensory areas (primary visual area, V1; primary somatosensory area, S1; and primary auditory area, A1), second sensory areas (secondary visual area, V2; secondary somatosensory area, S2; secondary auditory area, A2, and rostral auditory area, R), as well as motor areas such as primary motor area, M1 (Figure 1). These fields are homologous because they have been identified in all mammals examined, and it is likely that these cortical areas arose early in mammalian evolution and were inherited from a common ancestor in all lineages, rather than having evolved independently in each group. As such, a number of features of organization are similar across groups of mammals including similarities in topographic organization, aspects of cortical architecture, and thalamocortical and corticocortical connections. Later in this article we will discuss the types of modifications made to this homologous plan of organization and how these modifications might have arisen in evolution.

A broad comparative analysis also indicates that some features of cortical organization look strikingly similar in different mammals, but this similarity is not due to inheritance from a common ancestor. Rather, these features are homoplaseous, and have independently evolved in each mammal.

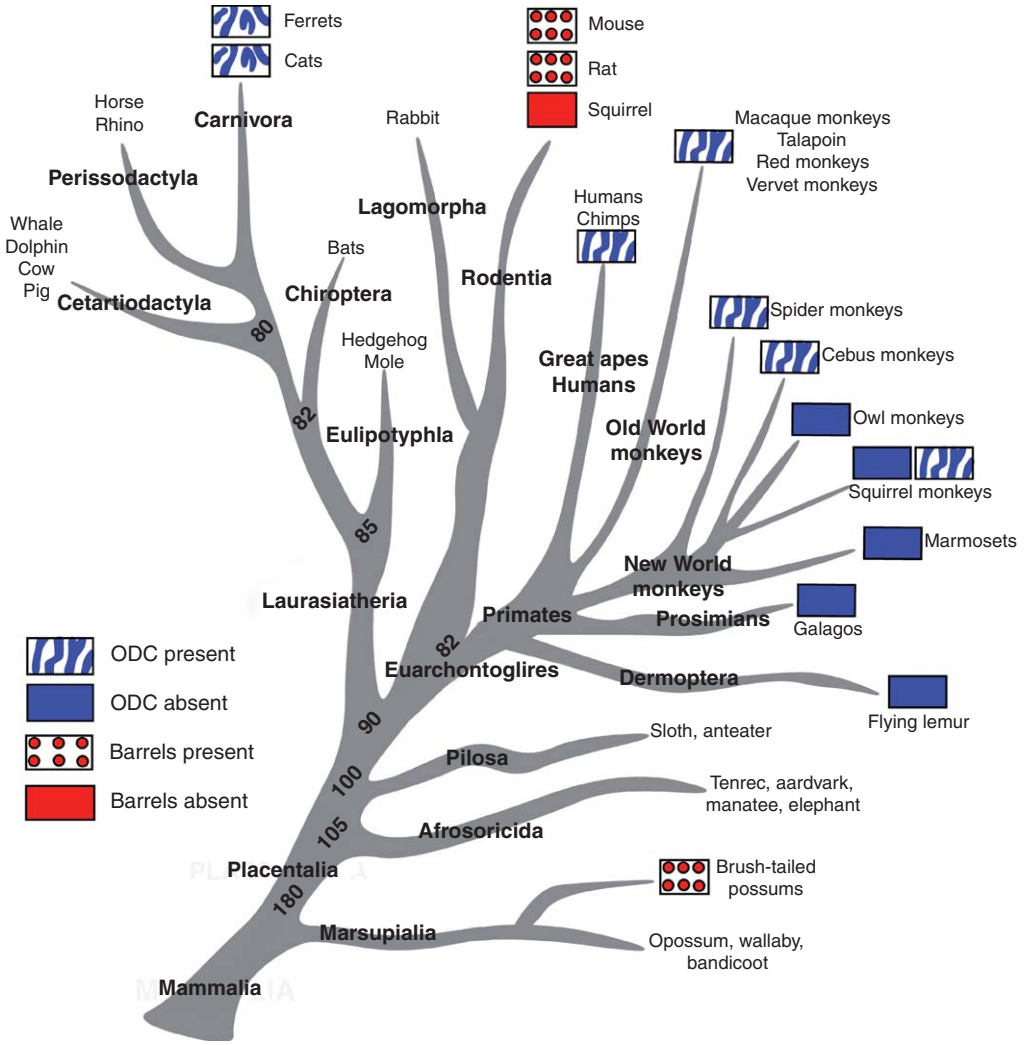

Figure 2 Homoplasy-independent evolution: a phylogenetic tree depicting the relationships between major mammalian lineages and the emergence of independently evolved features of cortical organization. Because the emergence of barrels in mice and rats arose independently from those in brush-tailed possums, they are considered as homoplaseous rather than homologous. Likewise, the presence of ODCs in ferrets and cats arose independently from those in some primate lineages. The fact that such similarities in organization emerge in different lineages despite over 90 million years of independent evolution indicates that the evolution of the neocortex is highly constrained. It also indicates that although the features themselves are homoplaseous, their presence could reflect the presence of homologous developmental mechanisms. Phylogenetic relationships based on Murphy, W. J., Pevzner, P. A., and O'Brien, S. J. 2005. Mammalian phylogenetics comes of age. *Trends Genet.* 20, 631–639.

An excellent example of a homoplaseous feature of the neocortex is the barrel field in the rat and mouse, and the brush-tailed possum (Figure 2; Weller and Haight, 1973; Weller, 1993). An out group comparison indicates that no intervening group of mammals has barrel cortex. Thus, the most parsimonious explanation for their presence in each group is that they have evolved independently in rodents and brush-tailed possums. Another example of homoplasy is the presence of ocular dominance columns (ODCs) in carnivores and some primates. ODCs are present in great apes and humans (Tigges and Tigges, 1979; Horton and Hedley-Whyte, 1984), Old World monkeys (e.g., LeVay *et al.*, 1975; Florence and Kaas, 1992), and a few species of New World monkeys (e.g., Florence *et al.*, 1986; Rosa *et al.*, 1992). They are absent in other New World monkeys, prosimians, and dermopotera (Figure 2), and in all other clades except carnivores (e.g., Löwel and Singer, 1987; Law *et al.*, 1988). This out-group comparison indicates that ODCs arose in primates after the divergence of New and Old World monkeys from prosimians (approximately 70 Mya), and that ODCs were lost in some New World species. The presence of ODCs in only two species of carnivores suggests that ODCs arose

independently in carnivores and primates, since the lineage that leads to carnivores diverged from that leading to primates over 90 Mya, and no intervening groups possess ODCs. What is remarkable about ODCs and the barrel cortex is that despite 90–180 million years of independent evolution, the arrangement of these modules looks very similar in carnivores and primates, and in rodents and brush-tailed possum respectively.

When making cross-species comparisons, there is often an assumption that homologous fields perform the same function or are analogous. However, this may not be the case. For example, over the years, a solid case for the presence of V1 in a variety of species has been established. All data indicate that V1 resides on the caudal pole of occipital cortex, contains a complete, first-order representation of the visual hemifield, receives connections from the dorsal division of the lateral geniculate nucleus (LGNd) of the thalamus, and has a striated appearance in tissue that has been sectioned perpendicular to the cortical layers and stained for Nissl substance. In cortex that has been sectioned tangentially and stained for myelin, V1 appears as a densely myelinated wedge at the caudal pole of the neocortex. Given these identifying features, V1 is proposed to be homologous across all mammals, and to form a basic component of a visual processing network in the mammalian neocortex. But what of analogy? Does it naturally follow that V1 as a homologous cortical area has a similar function or set of functions across groups of mammals?

The answer is 'no'. If we examine V1 in the mouse and compare it to V1 in the macaque monkey, several differences emerge. Most notable are the addition of modules to V1, such as orientation and ODCs, the addition of visual cortical fields, and the concomitant change in cortical connections in monkeys. Thus, V1 in monkeys and mice varies substantially in organization, and intrinsic and extrinsic connectivity. To illustrate this concept we have drawn a simple circuit containing three separate nodes (cortical fields A, B, and C in Figure 3). These nodes have a homologous pattern of interconnection across mammals (connections 1, 2, and 3 in Figure 3). In some groups of mammals, the nodes have been further subdivided to mimic the generation of modules (Figure 3). In addition, new nodes, representing new cortical areas, have been added to the network (D, Figure 3), which result in the addition of new connections and a potential re-weighting of existing connections between homologous nodes. This example shows that because of the emergence of new organizational features

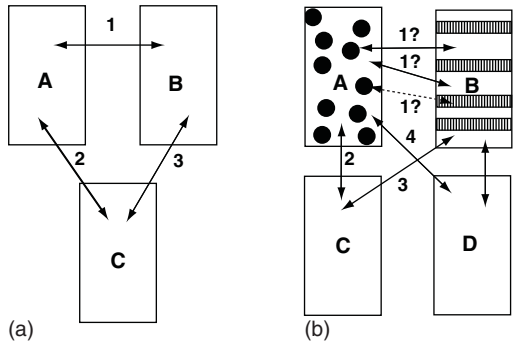

Figure 3 A hypothetical processing network (a) originally consisting of three cortical fields (A, B, and C) with a set of interconnections (1, 2, and 3). The evolution of this network (b) includes the addition of a new cortical field (D), the emergence of modules within existing cortical fields (circles in A and stripes in B), the emergence of new connections (4), and the re-weighting of existing connections (compare thick vs. thin line of connection 2 in (a) and (b)). These types of changes that naturally occur in evolution, indicate that homologous cortical fields may not be analogous since the interconnection relationships change and intrinsic processing modules emerge.

(modules), new inputs, and a re-weighting of retained connections, homologous cortical fields may not have the same function.

In answer to the question posed at the beginning of this section 'what is a cortical field?', we believe that it may be fruitful to consider cortical fields, at least in part, as homologous patterns of interconnection upon the cortical sheet. These patterns appear to be quite robust across species, and are associated with the emergence of specific architecture and neural properties in the developing nervous system. While maintaining their global relationships, these patterns shift, or 'float' upon the cortical sheet within the life of an individual (particularly during development), and to a greater extent, within and across species over time.

3.04.3 The Development of Cortical Fields

It has been appreciated for some time that both genes and the environment, as broadly defined, contribute to the development and the organization of the neocortex. How each of these factors contributes to development is couched in the long-standing 'nature vs. nurture' debate (see Krubitzer and Kahn, 2003 for review). Fortunately, the issue of the inherent, genetic contribution to the cortical phenotype has recently crystallized into hypotheses which are amenable to vigorous experimentation regarding the temporal and spatial distribution of genes and proteins that occur in development, and give rise to aspects of cortical organization including

cortical field location, size, and connectivity. The 'nurture' side of the debate has also become more experimentally tractable, and questions regarding the activity-dependent cellular mechanisms that alter aspects of development including the expression of genes, regulation of synaptic morphology and function, and dendritic and axon growth are now being examined. The problem is that in some instances it is difficult to draw a distinct line between genetic and epigenetic contributions to the phenotype, and the two become intricately intertwined.

3.04.3.1 Nature: The Contribution of Genes to Cortical Field Development

Understanding how genes control cortical field development can be broken into three broad categories. First, there are several genes that are intrinsic to the neocortex which control specific aspects of cortical development. The expression of these genes occurs in the normal developing system, and their action is independent of neural activity. Second, the expression of some genes in the central nervous system is induced by activity and requires feedback from the developing system to become activated. Finally, there are genes that regulate aspects of the body plan and peripheral morphology that contribute substantially to aspects of cortical organization.

3.04.3.1.1 Activity-independent genes intrinsic to the neocortex Recent work indicates that genes intrinsic to the neocortex, or the developing ventricular zone, control a number of aspects of cortical development, all of which have a large impact on the organization and function of the neocortex in the adult phenotype. Some examples include the regulation of the size of the cortical sheet, cortical field coordinates in the rostrocaudal and mediolateral axis, and thalamocortical connectivity.

In terms of the overall size of the cortical sheet, studies on cell cycle kinetics of neocortical progenitor cells in the ventricular zone indicate that the size of the cortical sheet is intrinsically regulated and that there are a number of plausible ways in which this regulation can occur. In general terms, the number of cells in the developing ventricular zone can be increased by extending the length of time that cells undergo symmetric divisions, and/or the rate at which cell divisions occur. A comparative analysis of small-brained mammals, such as mice, and large-brained mammals, such as macaque monkeys, indicates that cortical neurogenesis is both prolonged and accelerated in macaque monkeys compared to mice (Kornack and Rakic, 1998; Kornack, 2000). Several hypotheses regarding the specific genes and proteins involved in this process and the types of alterations to the kinetics of division have recently been proposed. For example, 'beta-catenin' is an intracellular protein that is expressed in neuroepithelial precursor cells during neurogenesis (Chenn and Walsh, 2002). In transgenic mice that over express a form of this protein, the size of the neocortex increases dramatically. This massive increase in the size of the cortical sheet is due to an increase in the proportion of progenitor cells that re-enter the cell cycle and continue mitotic division. Another gene proposed to alter cell cycle kinetics is *Brain Factor-1* (*BF-1* or *Foxg1*). This gene is expressed in telencephalic progenitor cells (Tao and Lai, 1992), and regulates cell proliferation and differentiation in the developing neocortex (Hanashima *et al*., 2002). *BF-1* is regulated by *FGF2*, which is also involved in regulating cortical sheet size by determining the number of cycles of division that progenitor cells undergo during cortical neurogenesis. For example, injections of FGF2 into the ventricle of embryonic rats results in a substantial increase in cortical volume (Vaccarino *et al*., 1999), and FGF2 knockouts have smaller neocorticies (Raballo *et al*., 2000). These studies indicate that the disproportionate size of the neocortex in different lineages could be regulated in several ways by different genes that affect the kinetics and timing of cell division in the ventricular zone.

Related studies of cell cycle kinetics in monkeys indicate that primary areas, such as V1, may be specified very early in development, during neurogenesis. For example, in primates, V1 is characterized by an increase in cell density and laminar complexity compared to other cortical areas, and compared to other mammals. In development, the rate of production cells in the ventricular zone is higher in the region where V1 will ultimately reside than in other regions (DeHay *et al*., 1993). Differences in laminar histogenesis for different regions of the ventricular zone have also been observed in mice (Polleux *et al*., 1997). These studies indicate that areal differences arise very early in neocortical development, well before thalamic innervation of the neocortex occurs.

In addition to intrinsic mechanisms that operate during cortical neurogenesis to specify cortical fields, recent work indicates that somewhat later in cortical development, the transcription factors *Emx2* and *Pax6* are involved in the expression and

patterning of downstream genes in the rostrocaudal axis of the neocortex, and potentially even cortical field size. For example, experiments in which these genes are deleted result in shifts of downstream genes such as *Cad8* and *Cad6* either rostrally (for *Emx2* deletion) or caudally (for *Pax6* deletion; Bishop *et al*., 2000). In addition to the observed changes in gene expression, *Emx2* and *Pax6* mutants also exhibit alterations in thalamocortical connectivity. In experiments in which *Emx2* is deleted and the neocortex is rostralized (e.g., rostral cortical fields are shifted caudally), cortex at the caudal pole that would normally receive thalamic input from the LGN receives inputs from the ventral posterior nucleus (VP) (which normally projects to somatosensory cortex rostral to this region; Bishop *et al*., 2000). Furthermore, mice in which *Emx2* is overexpressed have a significantly larger V1 than in normal animals (i.e., cortex has been caudalized; Hamasaki *et al*., 2004).

In terms of connectivity, some of the cadherins appear to regulate thalamocortical connectivity. For example, *Cad6*, *8*, and *11* are expressed in unique subsets of thalamic afferents (Suzuki *et al*., 1997; Korematsu and Redies, 1997). Further, *Cad6* is colocalized with the synaptic marker, synaptotagmin, and is correlated with the formation of synaptic connectivity between a source and its target in the developing nervous system (Inoue *et al*., 1998). The ephrins have also been proposed to play a role in thalamocortical development. While their presence in locations extrinsic to the neocortex, such as the ventral telencephalon, serves a role in gross topographic guidance, they appear to intrinsically mediate the refinement of thalamocortical connectivity within a cortical field (see Vanderhaeghen and Polleux, 2004 for review). For the development of cortical connections, recent work has demonstrated that FGF2, which may be regulated by *Emx2*, is involved in guiding (modulating) corticocortical connections (Huffman *et al*., 2004). Thus, the transcription factor *Emx2* controls a genetic cascade involved in structure formation, location, and connections.

It is important to note that evolutionarily, this type of regulation of events imposes formidable constraints on the developing and evolving nervous system. Given the constraints imposed by such a contingent system, it seems inevitable that very small changes in the timing and spatial distribution via base substitutions, recombination, and transposition, for example, of any one of the genes involved in these aspects of cortical field development can have a very large effect on the phenotype.

As mentioned earlier, a recent perspective on how cortical fields should be defined is to consider the subdivisions or areas of the neocortex from a spatiotemporal perspective. In this view, cortex is examined over time as a series of coordinated patterns of gene expression which are thought to be involved in generating features of the neocortex that will ultimately be realized in the adult, such as cortical layering, architecture, transmitter utilization, and connectivity. While this perspective is certainly important from both a developmental and evolutionary perspective, it may not be appropriate to define a cortical field in terms of the patterns of gene expression exhibited early in development for two reasons. First, the direct relationship between a functionally defined cortical field and some pattern or patterns of gene expression has yet to be established. Second, in the neocortex, early patterns of gene expression often represent potential, while the adult form directly generates the behavior that is the target of selection.

3.04.3.1.2 Activity-dependent regulation of genes that control aspects of cellular morphology, connection, and function In addition to the genes we described above, a number of studies describe intracellular, molecular mechanisms that are driven and regulated by neural activity, and generate changes in the temporal expression of genes within a cell employing these mechanisms. Altering the expression of genes can change aspects of synaptic morphology. For example, recent work demonstrates that increases in intracellular calcium, due to changes in neuronal activity, trigger a cascade of events, including the activation of the cAMP pathway and phosphorelation of CREB, which binds to the regulatory region of a gene and induces transcription of genes (see Finkbeiner and Greenberg, 1998; West *et al*., 2001 for review). There are several different types of molecules which are regulated by activity, and which in turn are involved in synaptic modeling during development. One of these is a class of proteins called neurotrophins. These proteins are relevant to the discussion above because their levels and secretion are regulated by activity, they are expressed in synapses, and they regulate morphological changes in both the pre- and postsynaptic elements (McAllister *et al*., 1995, 1999; Lein *et al*., 2000; McAllister, 2001 for review). Neurotrophins such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophic factor 4/5 (NT4/5) play a number of important roles in nervous system development

including mediation of rates of neuronal survival (see Levi-Montalcini, 1987; Miller and Kaplan, 2001 for review), induction of cell migration out of the ventricular zone (Borghesani *et al*., 2002), regulation of the extent of axon outgrowth (Segal *et al*., 1995), enhancement of dendritic outgrowth, and stimulation of protein synthesis in dendrites (Aakalu *et al*., 2001).

Another group of molecules recently identified by Shatz and colleagues (Corriveau *et al*., 1998; Huh *et al*., 2000) are the class I major histocompatibility complex (class I MHC) antigens. The expression of class I MHC is reduced in the developing cat LGN with the application of tetrototoxin (TTX) via intraocular injections given *in utero* (Corriveau *et al*., 1998). TTX blocks neural activity by deactivating sodium channels. In cats that are monocularly deprived during the critical period, class I MHC expression is reduced in the eye-specific layers of the LGN that were deprived. Further, in mice lacking class I MHC, refinement of retinogeniculate connections is incomplete (Huh *et al*., 2000). Thus, as in the above example for BDNF, activity controls the expression of these molecules, which in turn alters aspects of synaptic development.

While the above descriptions are brief and the intracellular processes that are modified by activity are not completely known, there are a number of potential intracellular mechanisms and molecules involved in nervous system construction whose action is modulated by activity. In the beginning of this section on development, we suggested that the boundary between genetic and activity-dependent contributions is somewhat blurred. This is the case for the scenario described above in which activity regulates gene expression, which in turn regulates aspects of nervous system construction and function. This type of activity-dependent regulation depends on calcium sensitive intracellular mechanisms that may be genetically determined and intrinsic to the composition of the cell. If this is the case, then the ability of the developing organism to respond to environmental fluctuations may be genetically specified and selected for in evolution, but the resulting phenotype would only be expressed in a particular environment (Krubitzer and Kahn, 2003; Krubitzer and Kaas, 2005). If the environment is stable, the specific phenotypic characteristic generated would be stable, and in essence would masquerade as an evolutionary (heritable) phenomenon.

3.04.3.1.3 Genes extrinsic to the neocortex but intrinsic to the organism contribute to aspects of cortical development and organization All mammals have a conserved body plan that includes forelimbs with distal appendages, hind limbs with distal appendages, a trunk, neck, head, face, snout, two eyes, two ears, one nose, and one mouth. Interestingly, this basic plan has been conserved in all vertebrates, due to genetic constraints, and like the neocortex, has been modified in a very limited fashion. Homeodomain genes, such as T-box genes and Hox genes, are involved in specification of the body plan; they arose early in the evolution of living organisms, and are highly conserved across taxa from arthropods to vertebrates (e.g., Patel, 2003; Boncinelli *et al*., 1994; Schilling and Knight, 2001; Banerjee-Basu and Baxevanis, 2001; Showell *et al*., 2004).

Despite the restrictions these genes place on the evolving body, morphological diversity of the limbs, head, and face abound. For example, limbs have been modified into wings (bats), flippers (dolphins), hoofs (ungulates), claws (cats), and hands (primates). For the head and face, alterations have been made to the location of the eyes on the head, the size, location, and mobility of the pinna, and the presence of vibrissae, follicles on a nose, or specialized oral structures. At a finer level of organization, the receptor arrays associated with a specialized morphology and behavior also undergoes modifications. However, like those of the body and brain, they are generally limited in number and include:

1. alterations in the location of receptors,
2. alterations in the density of receptors,
3. alterations in the number of receptors,
4. addition of new receptors, and
5. sensitivity of receptors.

Specific examples of some of these modifications would include the disproportionate amount and density of cutaneous receptors on the glabrous digit tips of the hands of primates, the concentration of cones at the fovea of primates and visual streak in rabbits (Hughes, 1977), the differential expansion of particular portions of the basilar membrane devoted to ultrasonic frequencies in echolocating bats (Ramprashad *et al*., 1979), and the addition of electrosensory receptors in the bill of a platypus (Scheich *et al*., 1986; Manger and Pettigrew, 1996), to name a few.

Not only does the actual structure of the body part contribute to features of cortical organization, but also how these body parts are utilized and modified for exploration is equally important. For example, for the somatosensory system, primates tactually explore objects with their glabrous hands, elephants with their distal trunk, muriad rodents with their vibrissae, the star nosed mole with the

many follicles of the nose, and the naked mole rat with their teeth (see Catania, 2005). Thus, body parts and associated receptor arrays that are used repeatedly and uniquely have large amounts of cortical space devoted to their representation in both sensory and motor cortex. Indeed, without exception, behaviorally relevant, specialized sensory receptor surfaces occupy a greater amount of cortical space than less relevant surfaces. This is observed at the sensory systems level in cortical domain allocation, and at the level of the individual cortical field (cortical magnification). A cortical domain is the amount of space allotted to a particular sensory system, and this differs for different mammals, even those with approximately the same size cortical sheet. For example the amount of cortical territory devoted to processing visual inputs is greater in the highly visual squirrel than in the mouse (Figure 4; Rosa and Krubitzer 1999). In terms of cortical field magnification, the amount of cortex devoted to processing inputs from the fovea is greatly enlarged in V1 of primates compared to the amount of cortex devoted to processing inputs from the rest of the eye. For the somatosensory cortex, in S1 and other

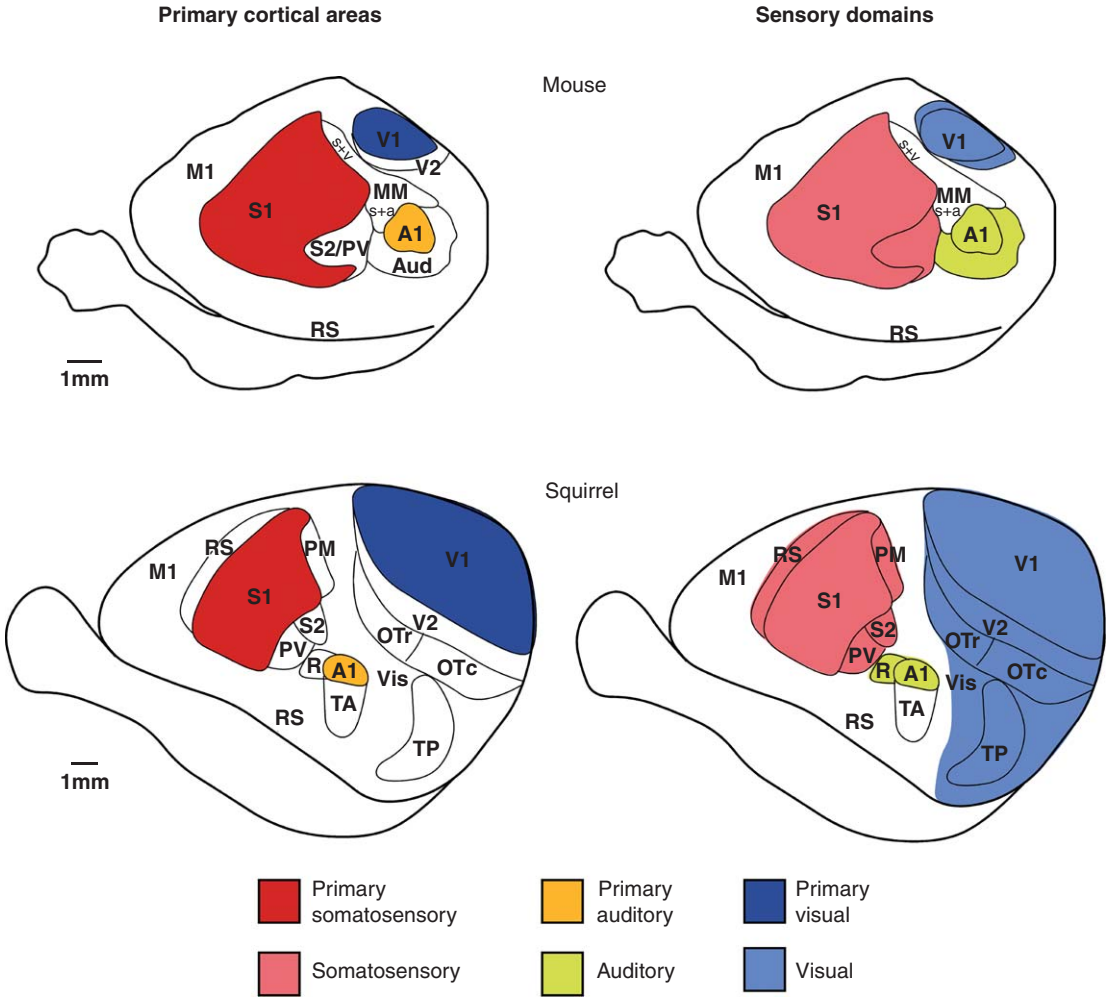

Figure 4 The organization of primary cortical areas and sensory domain allocation in the mouse (top) and squirrel (bottom). Each of these rodents occupies a particular niche and relies on different sensory systems for survival. The mouse is a terrestrial rodent that explores and navigates with its vibrissae, while the squirrel is an arboreal rodent that relies heavily on vision. Differences in cortical organization are observed at both the level of the cortical field and cortical domain. In the mouse, the primary somatosensory area is relatively large and occupies a good deal of cortex, while in the squirrel the primary visual area is relatively large compared to other primary sensory fields. Sensory domains, or the amount of cortex devoted to processing inputs from a particular sensory system, are also distributed differently in each species. In mice, the somatosensory domain is relatively large, while in the squirrel, the visual domain is extremely large and occupies at least one third of the entire cortical sheet. a, auditory; A1, primary auditory area; Aud, auditory; M1, primary motor area; MM, multimodal; OTc, caudal occipital-temporal cortex; OTr, rostral occipital-temporal cortex; PM, parietal medial area; PV, parietal ventral area; RS, rhinal sulcus; s, somatosensory; S1, primary somatosensory area; S2, secondary somatosensory area; v, visual; V1, primary visual area; V2, secondary visual area; Vis, visual.

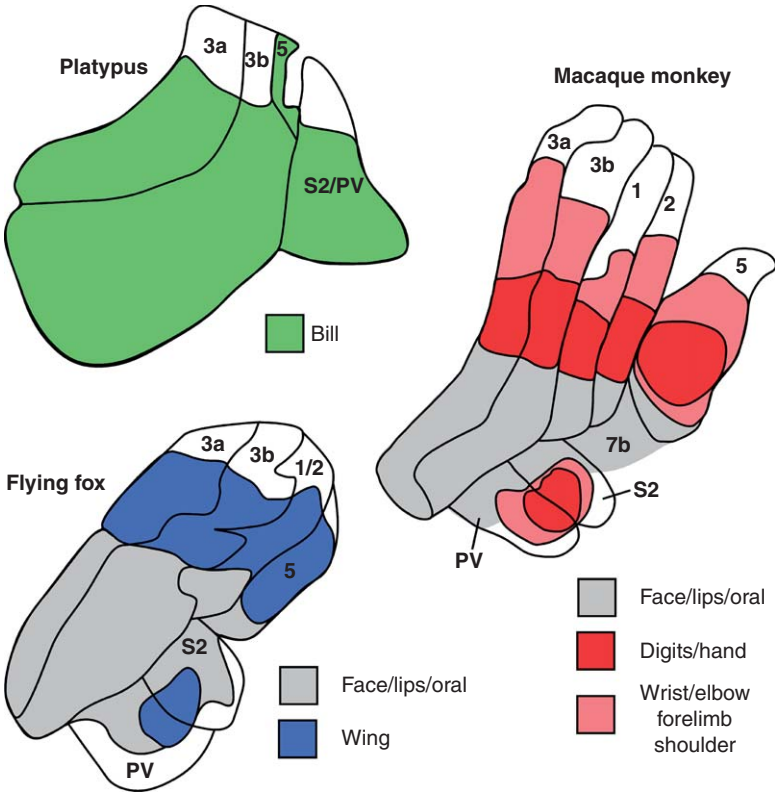

Figure 5 Cortical magnification of behaviorally relevant body parts within the somatosensory cortex of different mammals. In the duck-billed platypus, the bill representation (green) dominates all three somatosensory fields identified (R or area 3a, 3b, or S1, and S2/PV). In the highly dexterous macaque monkey, the representation of the glabrous digits (dark red), forelimb (light red), and oral structures (gray) dominate all somatosensory fields identified. In some fields, such as area 5, the magnification of the hand and forelimb dominates almost the entire field. Finally, in the flying fox, the wing (blue) and oral structures (gray) dominate all somatosensory areas identified.

cortical fields, the hand and mouth representations are magnified in primates, the wing and mouth representations are magnified in the flying fox, and the bill representation is magnified in the platypus (Figure 5; see Krubitzer and Disbrow, 2005 for review). As noted earlier, these specialized receptor surfaces are interfaced with the stimulus to be explored via specialized motor sequences. Thus, the motor system and the behaviors that allow for this interface are an integral part of sensory reception and cortical organization.

Since there is clearly an important relationship between cortical organization, peripheral morphology, and use, it is important to understand how body morphology evolves and how variability in body morphology is achieved in different lineages. Interestingly, the questions regarding diversification of the body plan in mammals are the same as those that arise when considering diversity in neocortical organization. Given the rather large constraints imposed on a basic plan of organization by these homeodomain genes, how can morphological diversity arise? It has been suggested that while the protein coding sequence of these homeodomain genes is relatively static across lineages, divergence in the regulatory portion of the gene can account for much of the morphological diversity observed in mammal body plans (Cretekos *et al.*, 2001). Thus, slight differences in the temporal and spatial patterning of genes generates large modifications in body plan organization. For example, the expression of a gene involved in the specification of the body plan (Hoxd9–13) was compared in two mammals with strikingly different forelimb morphology, the short-tailed fruit bat and the mouse (Figure 6; Chen *et al.*, 2005). Comparison of the distribution of Hoxd9–13 in bats and mice revealed that there were significant differences in the expression of this gene in the distal forelimb (dfl), but not the hindlimb, in later stages of limb development. Specifically, the anterior expression boundary of Hoxd9–13 in the bat is shifted posteriorly in the mouse (Figure 6). Thus, phenotypic diversity, or the transition from one phenotype to another that occurs in evolution, could be accomplished by subtle shifts in the expression of genes involved in

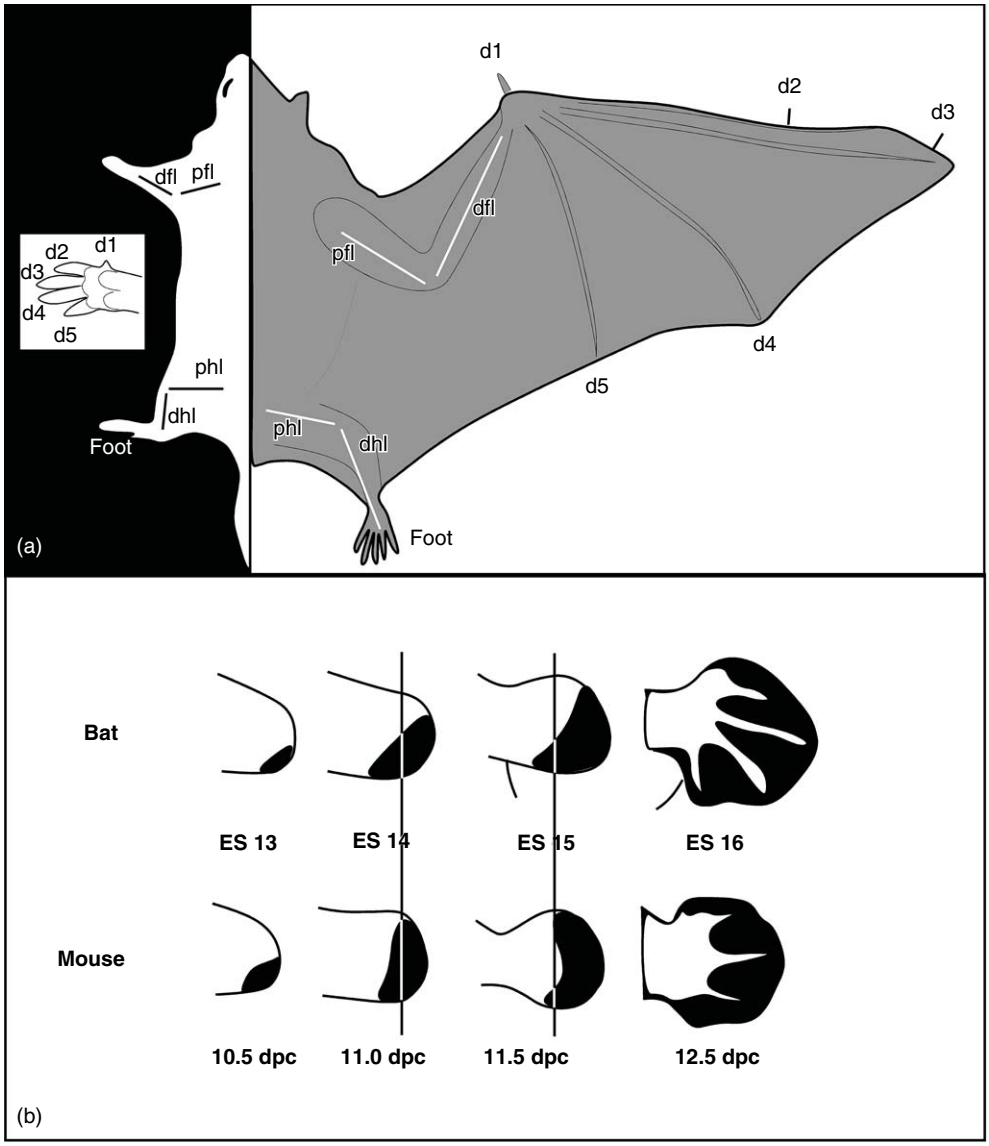

Figure 6 a, The body plan in mice and bats has a similar structural organization. Major body axis such as proximal and distal forelimbs and hind limbs (pfl, dfl, phl, and dhl), as well as individual digits (d1–d5), can be identified in both animals. However, modifications have evolved in each lineage in the form of the forepaw of a mouse and the wing of a bat. b, The expression pattern of Hoxd13 in the developing forelimb of the bat and mouse. The extent of the expression differences in bats and mice is evident during particular phases of limb development (bat ES 14, ES 15; mouse dpc 11, dpc 11.5), and such differences in homeodomain gene expression patterns could, at least in part, account for variations in forelimb morphology observed in each species. Such differences in expression are not noted for the hindlimb. dfl, distal forelimb; dhl, distal hindlimb; dpc, days post coitus; ES, embryonic stage; pfl, proximal forelimb; phl, proximal hindlimb. a, Modified from Cretekos, C. J., Rasweiler, J. J., and Behringer, R. R. 2001. Comparative studies on limb morphogenesis in mice and bats: A functional genetic approach towards a molecular understanding of diversity in organ formation. *Reprod. Fertil. Dev.* 13, 691–695. b, Modified from Chen, C. H., Cretekos, C. J., Rasweiler, J. J. T., and Behringer, R. R. 2005. Hoxd13 expression in the developing limbs of the short-tailed fruit bat, *Carollia perspicillata. Evol. Dev.* 7, 130–141.

major aspects of body and brain development. It should be noted that alterations in the temporal and spatial dynamics of gene expression have been known to account for variation of body segmentation in insects for some time (see Davis and Patel, 2002). It is only relatively recently that these well-established ideas from work on insects have been used to understand the evolution of the mammalian nervous system.

The case of body plan organization is another example where the boundary between intrinsic genetic contributions to the phenotype and activity dependent or environmental contributions are often difficult to draw. As Figures 4 and 5 illustrate,

specialized body morphology and use affect cortical domain allocation and sensory field magnification. The genes, which are involved in setting up the body plan organization, do not exclusively determine the final morphology of a particular body part, nor the resultant cortical organization. Indeed, several extrinsic factors related to the development of a body part contribute to the organization of the neocortex. For example, use directly affects the skeletal morphology, which in turn affects cortical organization. Several studies have shown that alterations in mastication behavior in development, often brought about by changes in diet, have a direct effect on craniofacial morphology (He, 2004), skull dimensions (Katsaros *et al*., 2002), mandibular morphology (Bresin, 2001), and bone density (Davies *et al*., 2005). The types of diet that produce such alterations during development are associated with hard versus soft food sources and the presence or absence of particular nutrients. Other extrinsic factors, which directly contribute to the development of body morphology and indirectly to cortical organization, are factors such as temperature, humidity, salinity, diet (see Johnston and Gottlieb, 1990 for review) and even gravity (e.g., Singh *et al*., 2005). The observation that body plan morphology can be altered by epigenetic factors is analogous to the observations made for the neocortex. That is, despite the very large constraints imposed by regulatory genes on fundamental aspects of body morphology or cortical organization, a large degree of phenotypic variability is still possible, and alterations to the body plan can indirectly alter cortical organization.

3.04.3.2 Nurture: How Activity Contributes to the System Level Aspects of Cortical Development and Organization

The relationship between the cortical domain, cortical field magnification, peripheral morphology, and use in the adult mammalian neocortex has important implications for developmental and adult plasticity, and evolution. In terms of development, it seems clear that peripheral morphology, sensory receptor organization, and the specialized motor programs that are part of efficient sensory reception, play a very large role in determining a number of aspects of cortical organization that are observed in adult mammals. Several series of recent experiments in our laboratory in which peripheral sensory receptor arrays have been physically excised or activity has been modified throughout development underscore this point. For example, in a recent study *Monodelphis domestica* were bilaterally enucleated well before the retinal ganglion cells reached the diencephalon and before the thalamocortical afferents reached the neocortex (Kahn and Krubitzer, 2002). Using electrophysiological, anatomical, and architectonic analyses in these animals after they reached adulthood, we found large shifts in sensory domain allocation, in that all of cortex that would normally be occupied by the visual system was occupied by the auditory and somatosensory system (Figures 7a and 7b). Interestingly, architectonically defined area 17 was still present, although reduced in size, and major thalamic projections from the LGN were preserved. However, there were also alterations in thalamic projections in that area 17 or 'V1' received additional input from the VP nucleus, the medial geniculate (MG) nucleus, and nuclei in the anterior group (Kahn *et al*., 2006). Further, corticocortical connections were altered in that area 17 received inputs from S1, A1, and frontal cortex. These patterns of thalamocortical and corticocortical connections are not observed in normal *Monodelphis* (Kahn *et al*., 2000).

Related experiments in congenitally deaf mice revealed much the same results (Hunt *et al*., 2005, 2006). These experiments were somewhat more subtle in that the sensory receptor array was not removed, but the ability to transduce auditory stimuli was eliminated in these animals throughout development. As with the blinded animals, congenitally deaf mice had large alterations in sensory domain allocation and alterations in cortical and thalamocortical connections (Figures 7c and 7d). All of cortex that would normally process auditory inputs contained neurons responsive to visual and somatic stimulation (Hunt *et al*. 2006). A surprising observation was that this lack of sensory driven activity resulted in alterations in connectivity at very early stages of sensory processing. In addition to its normal targets, the retina also projected to the MG nucleus and middle layers of the superior colliculus, structures generally associated with auditory processing (Hunt *et al*., 2005).

In adult mammals, plasticity within cortical fields has been observed, but the magnitude of the reorganization is much less pronounced than that observed in developing animals. The studies that examined the relationship between sensory experience and cortical map reorganization detailed the precise conditions under which plasticity will occur and described the map changes that were generated under those conditions. For example, studies in which monkeys were trained on digit discrimination tasks demonstrated a direct relationship between increased discrimination performance and an increase in the cortical space in

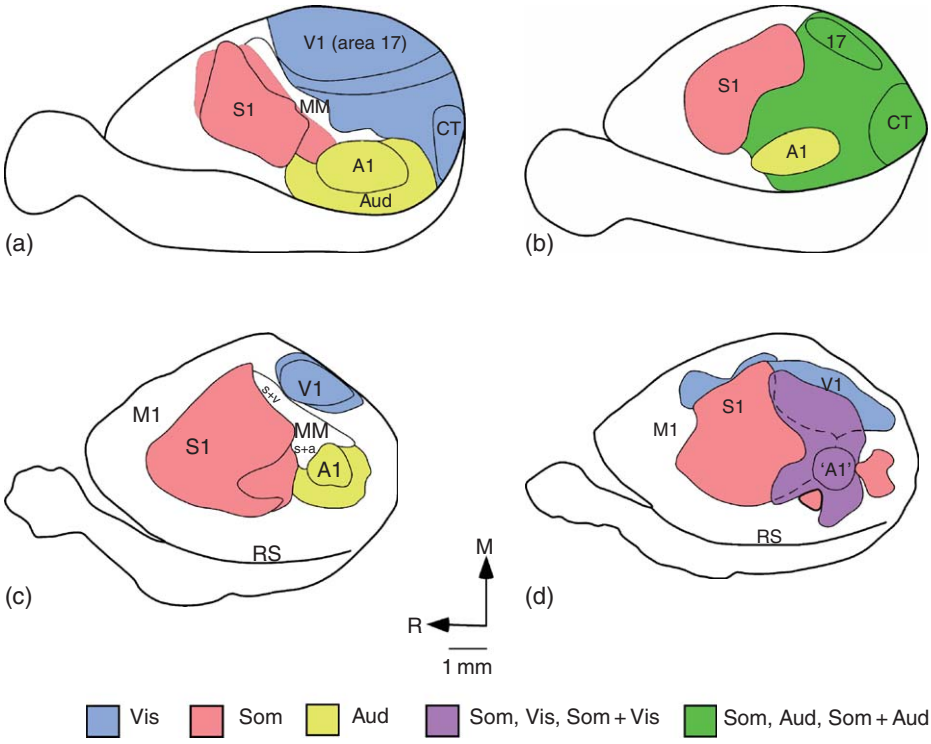

Figure 7 The organization of neocortex in normal opossums (a), normal mice (c), opossums bilaterally enucleated very early in development (b), and congenitally deaf mice (d). In the normal animals, both cortical fields and cortical domains are illustrated. In the bilaterally enucleated opossum, all of cortex that would normally be involved in visual processing, contains neurons responsive to somatic, auditory, or both somatic and auditory stimulation (green). In the congenitally deaf mouse, the cochlea is still present and a reduced eighth nerve exists, but no auditory driven activity is present. In this mouse all of cortex that would normally be devoted to processing auditory inputs contains neurons responsive to somatic, visual, or both somatic + visual stimulation. In both of these animals, the cross modal plasticity is extremely large such that all of cortex that is deprived of normal inputs is responsive to new types of sensory stimulation. In both mice and opossums, the cortical areas deprived of their normal inputs can still be identified architectonically, but at least in the opossum, the fields are smaller than in normal animals. a, auditory; A1, primary auditory area; Aud, auditory; M1, primary motor area; MM, multimodal; RS, rhinal sulcus; s, somatosensory; S1, primary somatosensory area; Som, somatosensory; v, visual; V1, primary visual area; Vis, visual. b, Modified from Kahn, D. M. and Krubitzer, L. 2002. Massive cross-modal cortical plasticity and the emergence of a new cortical area in developmentally blind mammals. *Proc. Natl. Acad. Sci. USA* 99, 11429–11434. d, Data from Hunt, D. L., Yamoah, E. N., and Krubitzer, L. 2006. Multisensory plasticity in congenitally deaf mice: How are cortical areas specified? *Neuroscience* 139, 1507–1524.

S1 (area 3b) devoted to the trained digit, while no expansion of adjacent nontrained digits was observed (Figure 8a; Recanzone *et al.*, 1992a, 1992b). Further, a requisite of the expansion was that the animal must attend to the task; repeated passive stimulation of the digit alone did not result in an expansion. Similar results have been observed for the auditory and motor cortex. In the auditory system, discrimination training of particular frequencies leads to an expansion of the cortical space devoted to that frequency (Figure 8b; Recanzone *et al.*, 1993). Likewise, training in a motor control task that involves particular hand movements, results in an expansion of those movement representations in motor cortex (Nudo *et al.*, 1996). These studies are important because they are the first to demonstrate a direct relationship between alterations in the neocortex with learning, and thus, the neural substrate for behavioral fluidity within the life of the individual.

The studies of developmental and adult plasticity demonstrate that peripheral morphology, sensory driven activity, and in normal circumstances, the behaviors associated with sensory reception play a large role in generating aspects of cortical organization including sensory domain assignment, cortical field size, the amount of space devoted to representing a particular body part or sensory receptor surface, and cortical and subcortical connectivity. These alterations are independent of the genes intrinsically expressed in the neocortex, which restricts the avenues along which evolution can travel. Thus, despite these restrictions, a fair amount of functional and anatomical fluidity is possible both within the life of an individual and in species over the course of evolution.

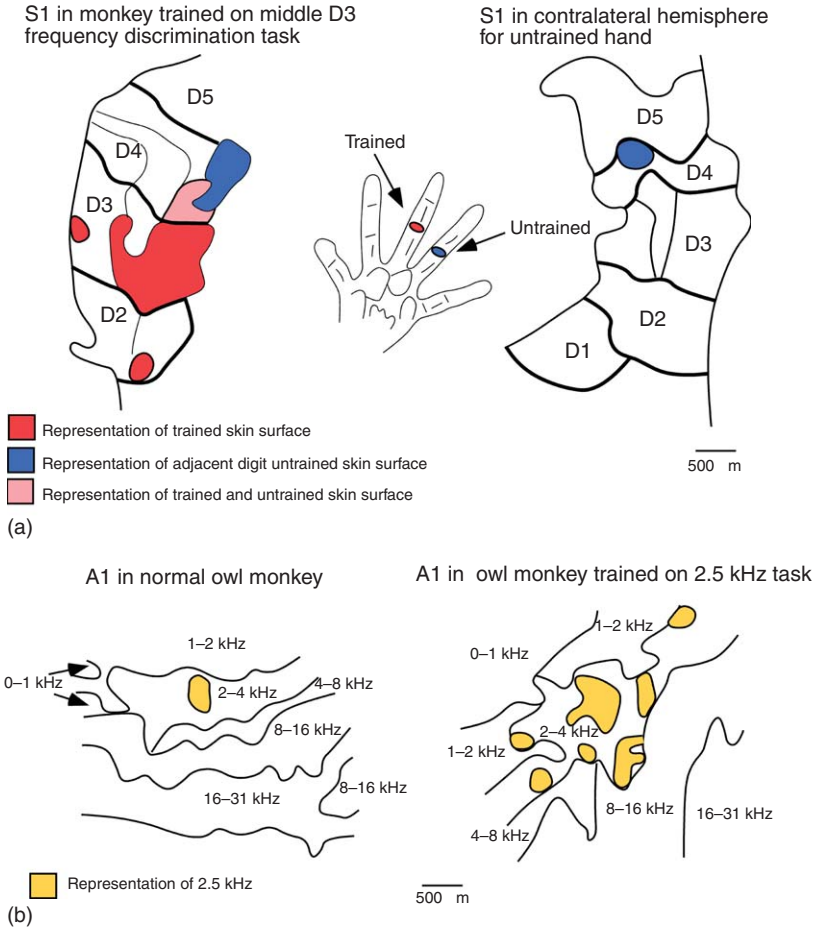

Figure 8 Cortical plasticity in adult owl monkeys following: a, somatosensory and b, auditory training. In the somatosensory cortex, training on somatosensory discrimination tasks increases the animal's ability to detect differences between two different stimuli, and this improvement in discriminatory ability is associated with an increase in the amount of neocortex devoted to representing the skin of the trained digit (red). In this case, the middle glabrous D3 was trained, and the contralateral S1 representing that portion of D3 (red) had an expanded representation compared to nontrained digits (blue). This plasticity was not observed in the hemisphere ipsilateral to the trained hand. Indeed, the portion of the cortex that represents the same location on the skin of the hand opposite to that trained was so small it was not found. A similar result was observed for the primary auditory cortex (A1). In owl monkeys trained on a 2.5 kHz discrimination task, the amount of cortex devoted to representing this frequency was expanded (b, yellow in left panel). A1, primary auditory area; S1, primary somatosensory area. a, Modified from Recanzone *et al.* (1992a, 1992b). b, Modified from Recanzone, G. H., Schreiner, C. E., and Merzenich, M. M. 1993. Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J. Neurosci.* 13, 87–103.

3.04.4 The Evolution of Cortical Fields

Earlier in this article we described the basic plan of cortical organization that all mammals possess, likely due to inheritance from a common ancestor (homology). Despite the large alterations that can occur in peripheral morphology, use, and lifestyle, the basic aspects of organization and connectivity of these fields are highly stable across lineages. However, there are modifications to this plan of organization, and a comparative analysis reveals that, at least at the systems level, these modifications take a similar form. In this section, we will describe some of the alterations that have been made to the cortical sheet in general, and to cortical fields in particular. We then postulate how some of these changes may have arisen in evolution, based in part on the information we have gained regarding the developmental mechanisms that construct cortical fields and their connectivities.

3.04.4.1 Changes in the Size of the Cortical Sheet

In addition to considering the cortical field in isolation, it is also necessary to consider general features of the brain as a whole that vary in predictable ways across species, which in turn have a large impact on the internal organization of the neocortex and the cortical field. The most

obvious feature is a change in the size of the brain and the size of the cortical sheet. Observations in a variety of mammalian brains indicate that there are two distinct types of changes in cortical sheet size, one in which the entire brain and its parts, including the neocortex, increase in size proportionately, and one in which there is a disproportionate expansion of the neocortex relative to the size of the rest of the brain.

Proportional changes in the overall size of the brain can result in an absolute increase in the size of the cortical sheet and the size of cortical fields. For instance, marsupials range in size from 4g to 67kg. Like the body, the range in brain size in marsupials is extreme. The marsupials we have examined in our laboratory include the dunnart (marsupial mouse, *Sminthopsis crassicaudata*), striped possum (*Dactylopsila trivirgata*), quoll (*Dasyurus hallucatus*), and short-tailed opossum (*Monodelphis domestica*; see Huffman *et al.*, 1999). In all but the striped possum, the most remarkable difference in the brains of these animals is that of absolute size. For example, the quoll and dunnart are both Polyprotononts from the family *Dasyuridae*. They differ substantially in body size with the dunnart weighing an average of 10g, and the quoll weighing an average of 750g. However, both are terrestrial hunters, occupy a similar niche, and have similar sensory specializations related to their predatory lifestyles (i.e., well-developed visual system). Examination of the neocortex of each animal demonstrates a clear difference in absolute size. However, much of the organization in terms of relative location and size of primary cortical fields are remarkably similar. This is best illustrated when the quoll brain is scaled to that of the dunnart. This scaling of brain size to body size and neocortex size relative to the rest of the brain is observed in other orders of mammals as well. For example, in a wonderful comparative analysis by Campos and Welker (1976), the neocortex of the capybara and guinea pig were compared. These investigators demonstrated that the size and relative location of primary cortical fields in the very large capybara compared to the much smaller guinea pig scales with the size of the body and the size of the brain as a whole (Figure 9).

The idea that the size of a cortical field scales linearly with brain size must be qualified. Comparative analysis has also shown that with dramatic specializations in the sensory epithelium, concomitant changes occur in the amount of neocortex devoted to that specialized sensory system, and the sizes of primary areas associated with that sensory system increase. Thus, if cortical sheet size is held constant and the internal organization of two highly derived species is compared, then differences in the allotment of neocortex and cortical field size can be readily observed.

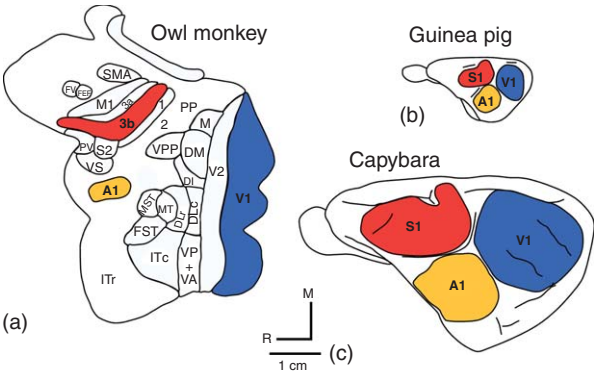

Figure 9 The organization of primary cortical fields in the: a, owl monkey; b, guinea pig; and c, capybara drawn to scale. In some species, the size of the brain has increased with body size, and the neocortex has increased in size proportionately with the rest of the brain (capybara). In this case, cortical field size has scaled linearly and the organization of the neocortex is much like that of other smaller rodents such as the guinea pig. In mammals, such as the owl monkey, whose body size is about ten times smaller than that of the capybara, the neocortex has enlarged disproportionately to the rest of the brain, although its absolute size approximates that of the guinea pig. With this disproportionate increase, the size of primary fields is reduced and more cortical fields are present. A1, primary auditory area; DLc, caudal division of dorsolateral visual complex; DLr, rostral division of dorsolateral visual complex; DM, dorsomedial visual area; FEF, frontal eye field; FST, fundal superior temporal area; FV, frontal ventral eye movement field; ITc, caudal division of inferotemporal cortex; ITr, rostral division of inferotemporal cortex; M, medial visual area; M1, primary motor area; MST, medial superior temporal area; MT, middle temporal visual area; PP, posterior parietal cortex; PV, parietal ventral area; S1, primary somatosensory area; SMA, supplementary motor area; V1, primary visual area; V2, secondary visual area; VA, ventral anterior area; VP, ventral posterior nucleus; VPP, ventral posterior parietal area; VS, ventral somatosensory area. a, Adapted from Krubitzer, L. and Kaas, J. H. 1993. The dorsomedial visual area of owl monkeys: Connections, myeloarchitecture, and homologies in other primates. *J. Comp. Neurol.* 334, 497–528. b and c, Modified from Campos, G. B. and Welker, W. I. 1976. Comparisons between brains of a large and a small hystricomorph rodent: Capybara, Hydrochoerus and guinea pig, Cavia; neocortical projection regions and measurements of brain subdivisions. *Brain Behav. Evol.* 13, 243–266.

The second type of size change that can occur is a disproportionate increase in the size of the neocortex compared to the rest of the brain. This results in a change in the pattern of neocortical organization. As in proportional increases in brain size, a disproportionate increase results in an absolute increase in the size of homologous cortical fields; however, the increase is less extreme than in the former type of size change. Furthermore, with a disproportionate

increase an additional organizational change to the neocortex is observed in that the number of cortical fields increases (Figure 9). This is nicely illustrated by comparing species that have different sized bodies, a similar absolute neocortical size, but a different neocortical size relative to brain and body size. For instance, although the capybara is well over 50 times the size of the owl monkey (50–70 kg vs. 1 kg), the neocortex of the owl monkey is disproportionately expanded, and its absolute size approximates that of the capybara. Examination of the neocortex of both species reveals very different types of organization. In the capybara, V1, A1, and S1 are large and compose much of the neocortex. In the owl monkey, V1, A1, and S1 are smaller than in the capybara, but many more cortical fields are present (Figure 9).

The question of how a disproportionate increase in neocortical size results in an increase in cortical field number is difficult to answer. It is possible that an increase in cortical field number, with an increase in the size of the neocortex relative to the rest of the brain, is due to a physical mismatch in the target (cortical sheet) and the projection zone (dorsal thalamus), or to a mismatch in the molecular coordinates between the thalamus and the cortex. This mismatch may result in new combinations of thalamocortical connections projecting to the expanded cortical sheet, in addition to the retained, highly restricted thalmocortical patterns of the primary and second sensory fields.

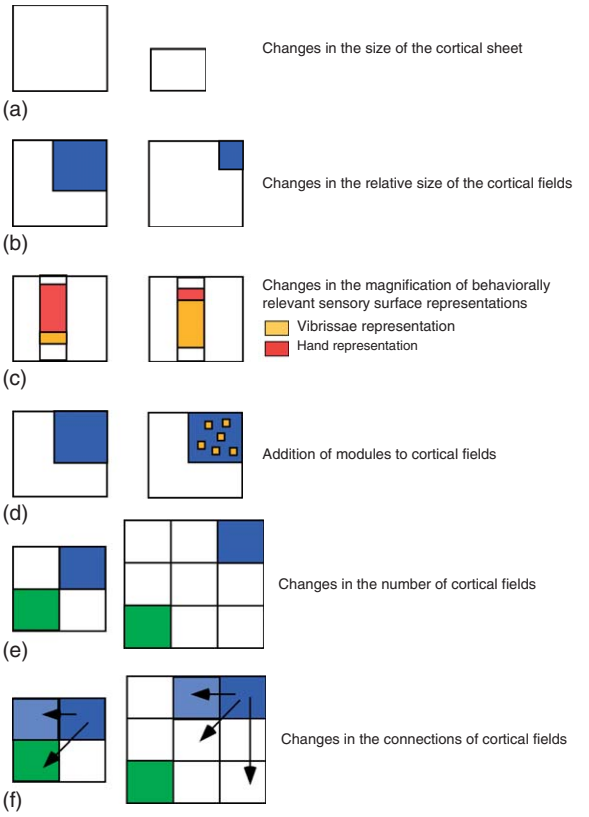

Figure 10 Modifications to the neocortex: a schematic representing the types of systems level changes that have evolved in different mammals. These changes, although few in number, presumably account for the wide range of behavioral differences observed in different lineages. Modified from Krubitzer, L. and Kaas, J. 2005. The evolution of the neocortex in mammals: How is phenotypic diversity generated? *Curr. Opin. Neurobiol.* 15, 444–453.

3.04.4.2 What Features of the Cortical Field Have Changed during Evolution?

In addition to changes in the size of the cortical sheet, several types of modifications have been made to the evolving neocortex (Figure 10). These modifications have been well documented (Krubitzer, 1995; Krubitzer and Kahn, 2003; Krubitzer and Kaas, 2005) and include:

1. changes in the relative size and internal organization of cortical fields,
2. changes in lamination of cortical fields,
3. changes in cell types,
4. changes in cortical thickness,
5. changes in the connections of cortical fields,
6. changes in the number of cortical fields,
7. the addition of modules to cortical fields, and
8. changes in the size of the cortical sheet (see above).

Interestingly, the brevity of this list of possible systems level modifications that brains have undergone or potentially could undergo suggests that it must be extremely difficult to modify the neocortex in evolution. Indeed, while we cannot predict the exact changes that may occur in future brains, we could predict with a fair amount of certainty what would not happen, and the types of changes that one would likely see. The observation that the types of modifications that have been made to the brain are limited indicates that these systems level modifications can generate a tremendous amount of phenotypic variability in terms of behavior.

3.04.4.3 The Module and Cortical Field Evolution

The module has been described in sensory cortex for a variety of different mammals (Figure 11). Modules are smaller units of organization that reside within a classically defined cortical field, and they have a long and dynamic history. Mountcastle (1957) described the first module, termed the cortical

column, almost 50 years ago (also see Mountcastle, 1978). He described the cortical column as a fundamental unit of cortical organization composed of a vertical group of cells extending through all of the cortical layers. This unit should not be considered as a fixed structure, but as a continuum with set dimensions, and no absolute boundaries. The modern concept of the module is different than its original conception in that it refers to different configurations of horizontal or tangential cell groups that do have fixed boundaries, and do not necessarily traverse all cortical layers. We have defined modules as "small architectonic, neuroanatomical, and physiological territories that can be distinguished from other tissue within the classically defined cortical field" (Manger *et al*., 1998).

Modules have been observed in a number of different cortical fields in different mammals and examples include barrels in rodent S1, blobs in V1 of primates, stripes in S1 of the star-nosed mole, ocular dominance bands in V1 of primates, and cytochrome oxidase (CO) bands in V2 of primates, to name a few (Figure 11). Although modules are a common feature of cortical organization that most mammals share, in most instances they are homoplaseous. The similarity of size and structure of modules across mammals argues that large constraints must be placed on evolving nervous systems. While evolution has been likened to a 'tinkerer', the bag of tools used to generate new phenotypes and the genetic material available for construction is highly limited. Thus, while the particular module itself may be homoplaseous, its presence may be due to homologous developmental programs (coordinated patterns of genetic interactions) that unravel in a particular molecular, neural, and sensory environment.

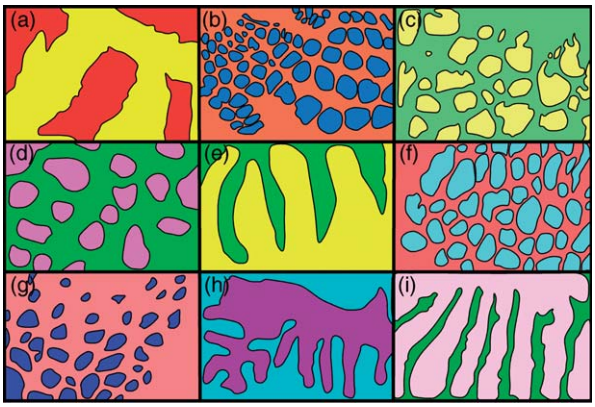

Figure 11 A schematic representing the many types of modules that have been identified in different sensory cortical areas in different mammals. While independently evolved or homoplaseous, the similarity in structure, shape, and size indicates that there are similar constraints imposed on the evolving and developing nervous system. a, Myelin bands in V2 of squirrel monkeys; b, barrel cortex in S1 of rats; c, modules in insular cortex of dolphins; d, clusters in entorhinal cortex in macaque monkeys; e, ODCs in V1 of talapoin monkeys; f, clusters in entorhinal cortex of humans; g, barrel cortex in S1 of brush-tailed possums; h, electrosensory/mechanosensory bands in S1 of platypus; i, rhinarium bands in S1 of the star-nosed moles. Modified from Manger, P., Sum, M., Szymanski, M., Ridgway, S., and Krubitzer, L. 1998. Modular subdivisions of dolphin insular cortex: Does evolutionary history repeat itself. *J. Cog. Neurosci.* 10, 153–156.

The identification of modules within cortical fields has implications for how a cortical field is defined. The traditional, and still dominant, view of cortical organization holds that the neocortex is compartmentalized into highly discrete cortical areas. However, the evidence for modular organization in cortical fields calls into question the traditional view of neocortical compartmentalization. Modules meet most of the criteria that generally are used to define a cortical field in that they are architectonically or histochemically distinct, have a unique set of connections, and contain neurons that are functionally distinct. When considered together, they form a complete representation of the sensory epithelium. An apt comparison between traditional and modern views of cortical fields is illustrated well for V1 and V2 of squirrel monkey neocortex (Figure 12). Until relatively recently, V1 and V2 were described as discrete, homogeneous representations of the visual hemifield with a distinct architectonic appearance and pattern of connectivity. The use of new histochemical staining techniques, optical imaging techniques, and fine-grained electrophysiological exploration of these fields has provided a very different view compared to traditional views. Rather than appearing as homogenous regions of cortex, both V1 and V2 have been further divided into modules. V1 is composed of blobs, interblobs, orientation columns, and ODCs. V2 is composed of thick and thin CO dense bands as well as interbands, and contains multiple representations of the visual hemifield.

Electrophysiological recording experiments of V2 in cebus monkeys and optical imaging experiments in macaque monkeys indicate that there is a re-representation of the same portions of the visual hemifield in these different bands (Rosa *et al*., 1988; Roe and Ts'o, 1995). Therefore, there is more than one map of the visual field in V2, and the separate maps are architectonically, histochemically, and connectionally distinct. These results suggest that 'chunking' V2 into one large, coherent field may

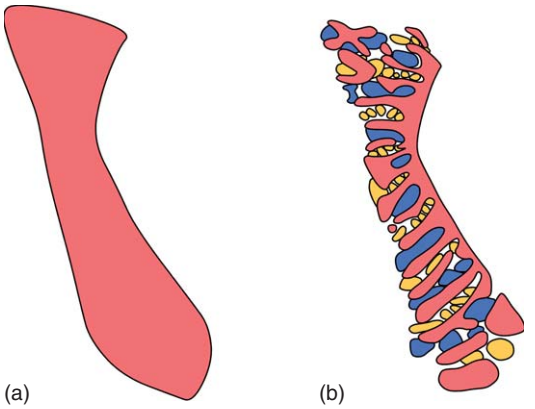

Figure 12 A schematic representing the: a, traditional and b, modern view of the organization of V2 in monkeys. Traditionally, V2 was considered to be a single, homogenous field adjacent to the rostral border of V1. Anatomical and functional studies (Rosa *et al.*, 1988; Roe and Ts'o, 1995) of the organization of V2 have since determined that it is modularly organized, and that there appear to be three independent representations of the visual field within this traditional area, associated with different histochemically identified stripes. These different strips, or bands in V2, have different patterns of connectivity. Thus, a new interpretation of this region of cortex is that three separate, completely interdigitated fields exist within the traditional V2. Adapted from Krubitzer, L. and Kaas, J. H. 1990. Convergence of processing channels in the extrastriate cortex of monkeys. *Vis. Neurosci.* 5, 609–613.

not be appropriate. Rather, V2 in primates could be considered as three separate, interdigitated fields (Figure 12).

In terms of modular organization and the evolution of cortical fields, we have proposed previously (Krubitzer, 1995; Krubitzer and Kahn, 2003) that modules reflect a stage in cortical field evolution within a lineage; that 'snapshot' alluded to in the introduction of this article. As noted earlier, we believe that a cortical field represents, at least in part, some patterns of connectivity on the cortical sheet. Within the life of an individual (particularly during development), and across species over time, this pattern of connectivity can shift such that the position of homologous fields is geographically displaced (Figure 13). Further, there are discontinuities within a cortical field (modules) that may represent an invasion of new inputs, discorrelated with existing inputs. This could represent fields completely embedded within other fields, as we believe is the case for V2. Over time, if selected for, these inputs coalesce and form partially invaginated regions, which may ultimately completely coalesce to form a new cortical field (Figure 13; see Krubitzer, 1995; Krubitzer and Kahn, 2003 for full explanation). Thus, the different modular and nonmodular organization of cortical fields within different sensory systems in different mammals represents different stages of this process in each lineage.

3.04.4.4 What Constrains Cortical Evolution?

There are three observations from comparative studies which indicate that neocortical evolution must be highly constrained. The first is the very presence of a common constellation of cortical fields, which was outlined in Section 3.04.2. That these fields and aspects of their connectivity and function can be modified substantially is without question. However, what is notable is that they have never been completely lost, even in highly derived mammals, such as the blind mole rat, which has micro-ophthalmic eyes covered by skin and a highly degraded retinofugal pathway (Klauer *et al.*, 1997; David-Gray *et al.*, 1998). The reduced visual system in blind mole rats is only involved in the circadian system. Yet, despite the lack of use of this system for visual functions, the geniculo-cortical pathway is still intact, and area 17 or V1, as architectonically defined, is still present and resides in the far rostral pole of the neocortex. The second observation is the very limited types of systems level changes that have been made to the brain, as outlined above. This suggests that the neocortex is not altered in a random fashion. The final, related observation is the instance of homoplasy. The fact that remarkably similar modules have formed, despite hundreds of millions of years of independent evolution, indicates that considerable constraints are placed on evolving nervous systems and that modularity is a part of this process.

What imposes constraints of the evolving neocortex? Primarily, genes constrain evolution and limit the types of phenotypic modifications that are possible, and these constraints are due to both pleitropy and contingency. Genetic pleitropy, or the fact that a single gene controls a number of activities in development, leads to functional integration, and as a result, it exerts a restriction on the number of possible changes that could be effected by any particular gene. Genetic contingencies restrict neural development and evolution in that any genetically mediated event is most often dependent on one or more prior genetic events and in turn may instruct some combination of downstream genetic events. Thus, it is rather difficult to substantially modify an organism by extreme genetic manipulations. This suggests that small genetic alterations can generate large phenotypic

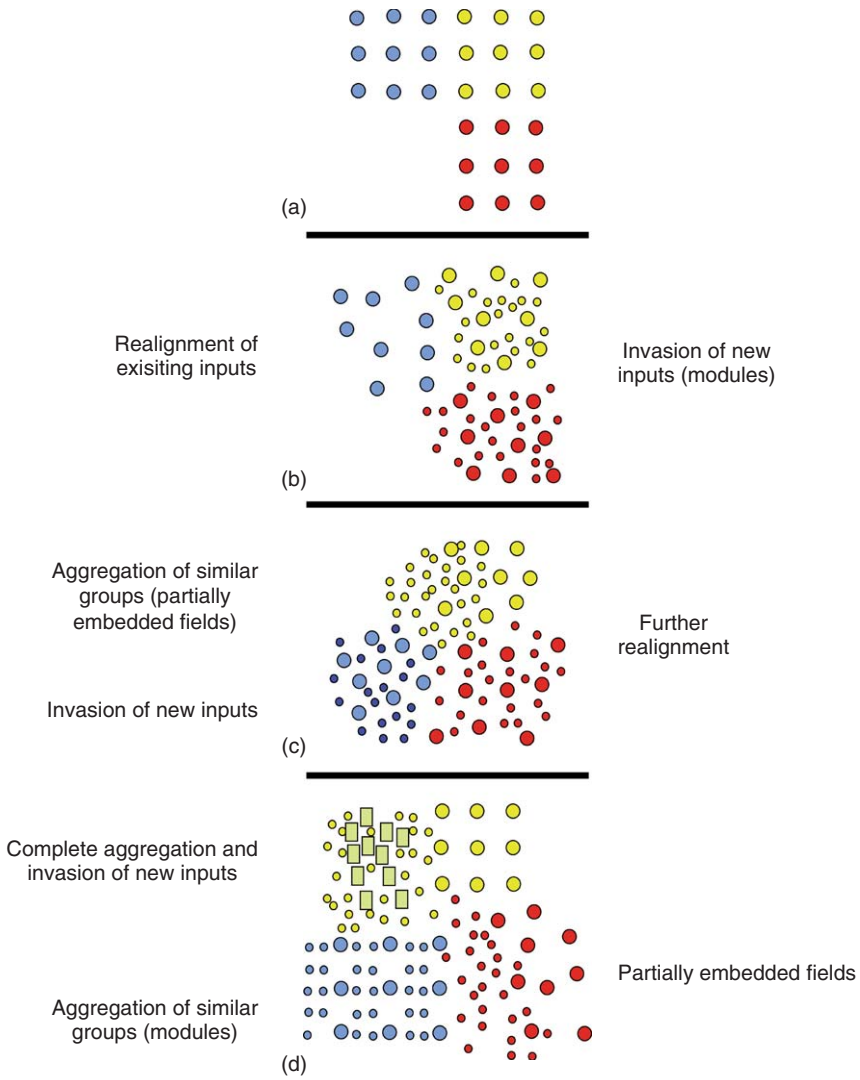

Figure 13 A theory representing the relationship between modules and the evolution of cortical fields. a, represents a hypothetical state of the neocortex with different colored circles representing a cortical field, or some pattern of thalmocortical interconnections within a field. An invasion of new inputs to existing fields (b, small red and yellow dots) results in a modular organization within these fields and a realignment of existing inputs. Modularly organized inputs may aggregate to form a partially embedded field (small yellow dots in (c)), causing a further realignment of fields, and new inputs may invade existing fields (small blue dots). Inputs that initiated within a cortical field and formed a modular arrangement (yellow dots), may completely aggregate to form a new field, and new inputs may invade this field (yellow squares). We propose that this is how cortical fields evolve and that each figure (a–d) illustrates snapshots or frozen frames that we observe in extant mammals. Modified from Krubitzer, L. 1995. The organization of neocortex in mammals: Are species differences really so different? *Trends Neurosci.* 18, 408–417.

modifications and that phenotypic change can be accomplished in the absence of nonactivity-dependent genetic change.

In addition to genetic forces, there are also substantial constraints imposed on evolving nervous systems by the environment in which an animal operates. When we discuss the nervous system, we rarely talk about physics, but the physical parameters of any environment are set and quantifiable. For example, nervous systems must contend with gravity, self-movement, and the movement of objects and other animals in time and in the three dimensions of our universe. The physical parameters of a stimulus are also important, and include the presence or absence of photons, the rate at which a stimulus travels and bends through space, the diffusion of molecules through different media, and the perturbations of molecules in different media, such as changes in air pressure. Although the amount and patterns of a physical stimulus that impinge on any given mammalian sensory receptor array may be distributed

differently in different terrestrial and aquatic environments, and in diurnal versus nocturnal mammals, the actual physical unit that is transduced, such as a photon, is invariant and therefore serves to anchor the evolutionary boat. While it seems clear that genes and their highly coordinated activities constrain a system, it is important to keep in mind that within a population of individuals, both the spatial and temporal expression of genes involved in the processes described above are normally distributed. This natural variability allows for some degree of flexibility within a relatively fixed genetic environment. Energy, while absolute, is variably distributed within any environment such that the amount and pattern of photons falling on a retina, for example, is different in different ecospheres. While we have noted above that both genes and the physical parameters of the environment constrain the development and evolution of mammalian neocortex, and ultimately behavior, it should be noted that the combinatorial possibilities of these two fixed parameters can generate a high number of degrees of freedom for potential phenotypic outcomes despite these constraints.

Despite these constraints, it is clear that sensory driven activity and the animal's own movement within an environment can generate a large amount of phenotypic variability. We have discussed the types of systems level changes that can occur with variable use and under particular environmental conditions in the developing and adult nervous system. But, how do such alterations become genetically encoded within a population and ultimately evolve?

At first reading, the idea that acquired traits can somehow evolve seems to smack of Lamarkianism. However, the notion that a living organism's ability to respond to environmental fluctuations has a genetic basis is relatively well established and compatible with Darwinian selection. This idea was formulated over a century ago by Baldwin (1886, 1902), and termed the Baldwin effect. The Baldwin effect is the ability of an animal to respond optimally to a particular environment. This effect could hold true for behaviors as well as anatomical features or aspects of functional organization of the neocortex. Thus, the Baldwin effect is the idea that genes for plasticity evolve, and that the phenotype that is optimal for a given environment could become genetically encoded and evolve if the genes that encode for plasticity and those for the actual phenotypic feature in question covary (Figure 14). This characteristic would then be selected for and be displayed even in the absence of the original environmental stimulus that induced it. This phenomenon was experimentally tested by Waddington and termed genetic assimilation (Waddington, 1959, 1961).

A related process has recently been described as 'evolvability'. Evolvability is the ability of an organism to generate heritable, selectable phenotypic variation (Kirschner and Gerhart, 1998). These authors propose that selection for evolvability has occurred and has three components. At the level of the individual, the ability to be flexible would contribute directly to physiological fitness. At a group level, individuals within the group would be buffered against the lethal effects of mutation. Finally, at the level of the clade, such an ability would allow the clade to radiate into new (emptied) environments. Recently, experimental support for the notion that evolvability is a selected trait has been put forward by Earl and Deems (2004). They find evidence that the rate at which genetic change in the form of recombination, substitutions, and transpositions occurs is variable in different lineages and is genetically encoded.

Taken together, it appears that activity can regulate gene expression which, in turn, can regulate anatomical and functional characteristics of the developing nervous system within an individual lifetime. This process, or the ability to respond to some external stimulus, is optimal in some individuals and can be selected for (the Baldwin effect). In a particular environment, an optimal trait can become genetically encoded in a population and evolve if there is a strong correlation between phenotypic and genotypic space (genetic assimilation). Finally, the ability to respond optimally and to assimilate, while maintaining a fundamental plan of organization, is a variable trait itself, and is the target of selection (evolvability).

3.04.5 Conclusions

How should we view the evolution of the cortical field? While a cortical field has been previously proposed to be a fixed, genetically determined structure that occupies some area on the cortical sheet, a comparative analysis highlights the dynamic nature of a cortical field within the life of an individual and over generations within and across lineages. We believe that the cortical field is an event or a process, not an entity that is easily captured. While genes and the physical environment impose severe constraints on this process, neural activity within the developing organism generated by the highly constrained physical

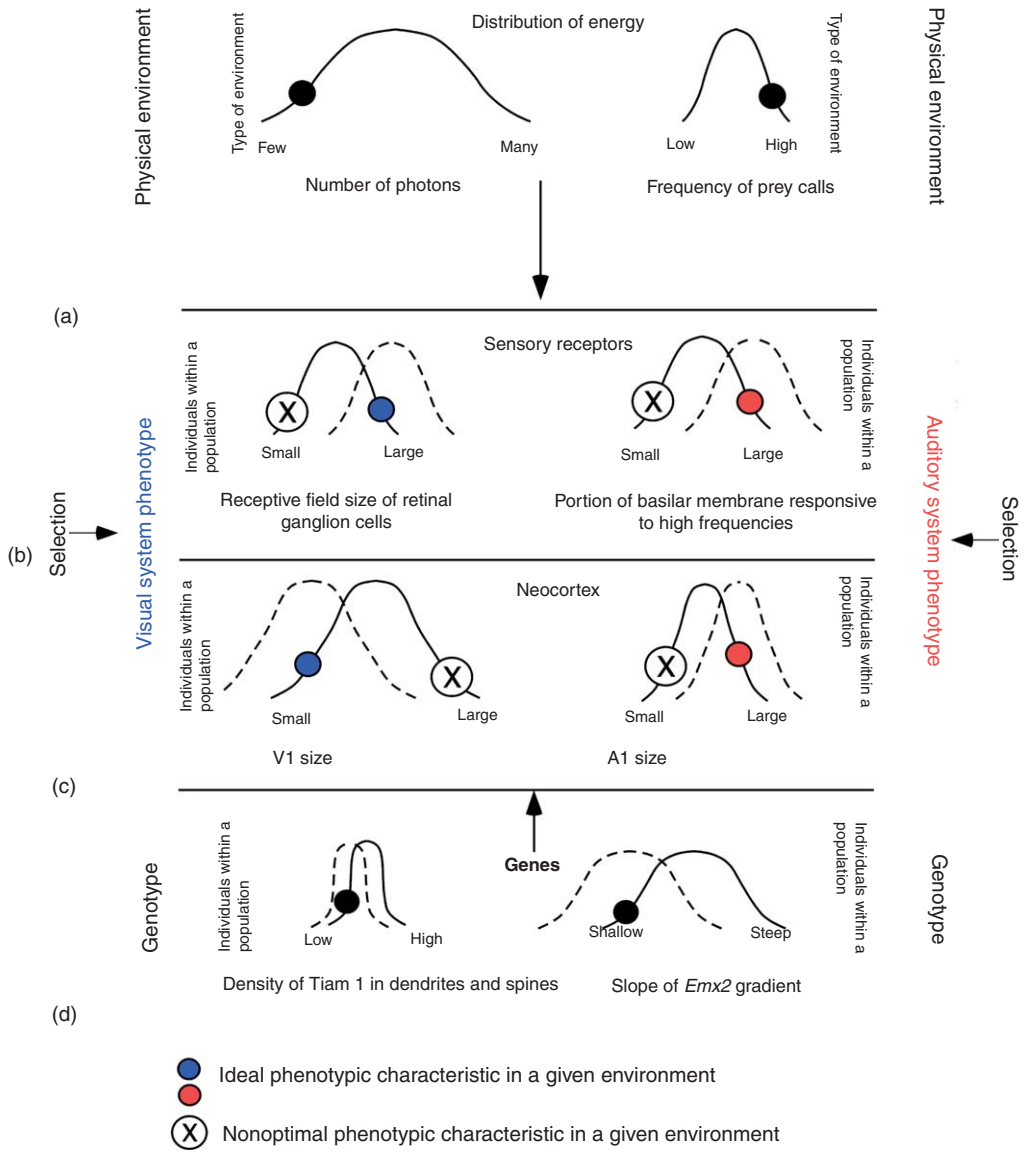

Figure 14 A schematic illustrating the Baldwin effect and genetic assimilation, and how features of cortical organization that are initially activity dependent, become encoded by genes and evolve. Within a particular environment (a), light levels may be low, and prey call frequency may be high (black dots on the distributions in a). The optimal sensory receptor phenotype (b), receptive fields size of ganglion cells distribution of frequency on the basilar membrane (blue and red dots respectively) are normally distributed within a population. For the neocortex (c), the optimal phenotype for this environment would be a small V1 and a large A1 (blue and red dots respectively). These size differences of cortical fields are normally distributed within a population. Finally, particular genes which are normally distributed in a population (d) control aspects of cortical field organization either directly via *Emx2*, or indirectly through activity-dependent mechanism (e.g., Tiam 1). Although natural selection acts on the phenotype, the genes that control for the particular phenotype in question as well as plasticity may co-vary, and thus allow activity-dependent contributions to the phenotype to become genetically encoded and evolve. This type of selection could shift the distribution (dashed lines) of genes that both enable plasticity (activity dependent), as well as those directly determine the characteristic (e.g., Emx2 and size of cortical fields). A1, primary auditory area; V1, primary visual area. Modified from Krubitzer, L. and Kaas, J. 2005. The evolution of the neocortex in mammals: How is phenotypic diversity generated? *Curr. Opin. Neurobiol.* 15, 444–453.

parameters of the environment, and the movement of the organism itself in time and space, serves to loosen these constraints. An extant mammal represents only a snapshot in this process. This snapshot may give the impression that a cortical field is static, when, in reality, we have simply caught a frozen moment in the continually moving picture of life.

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3.05 Cortical Evolution as the Expression of a Program for Disproportionate Growth and the Proliferation of Areas

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Glossary

allometry	The study of alterations in form causally correlated with changes in size.
cortical area	A proposed unit of the cortex, typically containing a topographically mapped representation of a sensory, motor, or computed surface, and a characteristic set of thalamic inputs and subcortical outputs.
module	In computer science and cognitive science, a functionally encapsulated unit performing a particular computation. In neurobiology, often used to refer to any repeating unit of structure.
pleiotropy	The case where a single gene contributes to the execution of many functions, preventing its optimization for any single function.
prosomere	Hypothesized embryonic segmental structure of the forebrain.
radial unit hypothesis	The hypothesis that the essential unit of mature cortical function, the cortical column, and also the unit of evolutionary selection and replication is the assembly of neuroblasts migrating to the cortical plate on a single radial glial guide in development.
sensory exploitation	The idea that in the evolution of communication systems, the sender evolves to maximally activate the generic sensory system of the receiver.
subnet	An assembly of neurons performing a logical unit of computation.
symmetric and asymmetric cell division	Symmetric cell divisions give rise to identical daughter cells, both capable of further division; asymmetric division produces a cell that will become a differentiated mature form.
topographic map	The feature of retention of nearest-neighbor relationships when one array of neurons projects upon another.

3.05.1 General Introduction to Developmental Structure in Brain Evolution

Evolved structures are the result of successful adaptation to the environment, and evolution occurs by the variation and selection of genetic programs, as they are expressed in the development of the organism and in mature phenotype. In this article, we will discuss the evolution of the cortex in a developmental context, focusing on how the various versions of cortex we see in different mammalian radiations are expressions of a generally conserved developmental program, produced over variable lengths of time and variable scales. In classical evolutionary biology (Gould, 1977, 1980), conserved developmental programs are often viewed as constraints, limiting the range of variation offered for selection (see Principles of Brain Scaling). Possible reasons for stabilization of developmental programs are multiple. Very early events in development may become fixed, as they may contribute to the structure of so many mature systems no single system can be caused to vary independently. Or, independent of development, single genes may contribute to disparate functions (termed pleiotropy) so that variation in the gene cannot be linked to the optimization of a single system. More recently, however, the extent to which fundamental developmental programs are conserved across phyla has proved to be quite breathtaking and caused a substantive rethinking of the significance of conservation, away from the negative-to-neutral interpretations of constraints just described. Rather, the explanatory focus has shifted to the structures of genetic systems that allow robustness and stability to coexist with variability (Gerhart and Kirschner, 1997; Radman *et al.*, 1999; Wilkins, 2001).

Conserved features include the polarity, symmetry, and segmentation of the fundamental invertebrate and vertebrate body plan (Duboule and Dollé, 1989; Graham *et al.*, 1989); the designation of areas for special senses and limbs (Callaerts *et al.*, 1997); the control of the cell cycle and symmetry-breaking events that control cell cycle entry and exit; and other features of cell specification (Gerhart and Kirschner, 1997). Many particular mechanisms central to nervous system construction are similarly stable across taxa, such as mechanisms for axon extension and inhibition (Dickson, 2002), mechanisms for approaching and crossing the neural midline (Stein and Tessier-Lavigne, 2001), and activity-dependent stabilization of synaptic connections (Greenough and Bailey, 1988; see Scaling the Brain and Its Connections). Though highly conserved, such a set of fundamental mechanisms seems ill-described as constraints. If these fundamental mechanisms represent optimal or near-optimal solutions to repeatedly encountered developmental problems, the sense of the word 'constraint' as limit is poorly applied to them.

A longer view of the definition of 'adaptation' and 'environment' than each particular animal's interaction with its immediate physical setting will provide some of the appropriate context for understanding why developmental programs are likely to be conserved along with the conserved neural circuitry that the developmental programs will eventually represent. In addition, a more sophisticated understanding of how complex behavioral functions might be distributed over the essentially conserved architecture but widely varying size of the cortical surface is absolutely essential. We will discuss the evolution of complex sensory and behavioral functions first.

An example from current neuroethology of a phenomenon called sensory exploitation throws better light on what kinds of possible adaptations are good solutions to conflicting adaptive demands (Ryan, 1998). In the first days of neuroethology, the observation that an anolis lizard might have a bright orange dewlap that it extends for aggressive displays, or a male frog a particular croak with acoustic features that attracts females of its own species were hypothesized to be roads to insight into neural coding in both sensory and motor systems (and they were, but not in the way anticipated). Researchers imagined isomorphic specializations in the nervous systems of both the senders and the receivers, from the sensory periphery on into the central nervous system.

While there must be some committed circuitry for conspecific recognition, even after years of work neuroethologists generally failed to find specific adaptations in receivers, especially in the sensory periphery, when comparing closely related species using different signaling systems – that is, there were no orange dewlap detectors in the retina, or anywhere else in early visual processing. Instead, they found that the signaler had evolved to produce a maximally perceivable signal for the receiver's sensory system or a maximally contrastive signal for the immediate environment, thus exploiting the generic visual or auditory system of the receiver (Ryan and Rand, 1995; Persons *et al.*, 1999). This makes sense when the multifunctional nature of sensory systems is understood: the same visual system that must respond to aggressive social signals must also recognize food, recognize threat, navigate terrain, and respond to a wide range of other social signals,

which simply cannot be realized computationally with any efficiency as a collection of committed detectors (Field, 1994).

The style of the initial steps of neuroethology has unfortunately traveled unmodified into some current theorizing about cortical organization, sometimes replacing detectors with modules. One example is the discussion of the evolution of primate vision and the neural representation of trichromacy in the cortex (Barton, 1998). In this case, the differentiation of a photopigment capable of improving discrimination in the red–green end of the visual spectrum is attributed to the single function of fruit detection with associated central nervous system alterations rather than a neatly placed transducer employing generic contrastive processing, which can measurably improve not only the discrimination of particular fruits, but their ripeness, leaf maturity, other edible prey, assignment of boundaries and edges to improve navigation through cluttered forest environments, social signaling, and so forth (Moller and Hurlbert, 1996; Nickle and Heymann, 1996; Regan *et al.*, 2001; Dominy and Lucas, 2001; Finlay *et al.*, 2005b). Views of how behavioral adaptations are translated into neural specializations will be important when we examine hypotheses about how cortical areas might proliferate.

What environment are organisms adapted to? In classic adaptation scenarios, individuals compete for reproductive success in a stable environment, improving their perceptual capacities, signaling of reproductive quality, and physiological and behavioral abilities in general, producing an adaptive walk through a universe of potential adaptive states responsive both to environmental pressures and the nature of the particular competition they have engaged (Dawkins, 1976, 1986). This type of scenario often generates greater and greater specialization, particularly arms races in the means of sexual selection. However, most individuals in their own lifetimes or in their immediate ancestors' lifetimes have faced environments of great disturbance: climatic shifts and ecological catastrophes both local and global (Alvarez *et al.*, 1980; Albritton, 1989). Those individuals that survive have the robust and stable genomes and nervous systems suitable for both classes of environments, the stable and the catastrophic. Our genomes and nervous systems contain histories both of adaptive specialization in stable environments and successful survival through massive environmental changes (Gerhart and Kirschner, 1997).

Finally, as our sophistication grows both in understanding nervous systems (e.g., Schüz and Miller, 2002) and generalized computer and network structures (Watts and Strogatz, 1998; Nolfi and Floreano, 2002; Newman, 2003), we are beginning to get a better idea of what kinds of modifications might be necessary to scale up brains to larger bodies, adapt brains to new behavioral niches, and produce intelligent behavior. Quite analogously to the first-pass detector guess for how to evolve sensory systems, often the first guess about how to generate a new behavior is to propose a new committed module for the brain (Chomsky, 1975; Barkow *et al.*, 1992; Fodor, 1992), which has rarely proved to be the actual case; for many of the same types of reasons, committed detectors are poor solutions to sensory problems. In addition, it proves that aspects of modular construction (to be discussed) pose difficult challenges for nervous system scaling.

The allometry of scaling of brains with bodies (Jerison, 1973; Schmidt-Nielsen, 1984) and the coscaling of sensory systems are phenomena that have never been satisfactorily understood (Finlay *et al.*, 2001). Why brains should scale regularly in size with bodies at all is unclear, as we know that in many nonbiological systems there is no need for the size of a control system to match the physical size of the entity controlled. Why visual acuity should scale roughly with body size (Kiltie, 2000) and why the ratio of brain size to body size is somewhat better correlated with behavioral complexity than absolute brain size alone (Jerison, 1973; but see Gibson, 2002) are similar puzzles (see Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals). Our available explanatory schemes have not coped well with these questions, but the application of new work in the properties of network architectures may begin to help.

In the following pages we will review some of the basic generalizations of how mammalian nervous systems tend to scale and adapt, and how those are linked to conserved developmental programs. When possible, we will try to evaluate whether we are looking at conserved programs that exist because of some constraint limiting the range of evolutionary solutions, or conserved programs that represent selected optimal solutions. Finally, we will propose and evaluate some exploratory models of network scaling in this evolutionary and developmental context.

3.05.1.1 A Conserved Order of Neurogenesis across Mammals and Its Relationship to Brain Allometry

In 1995, in an initial analysis of the relationship of variations in the order and relative duration of

neurogenesis to variations in the size of mammalian brain regions, Darlington and Finlay compared two large sets of data on both phenomena and found an unusually close and predictable relationship (Finlay and Darlington, 1995). Using the information collected for primates, insectivores, and bats for comparable brain regions by Stephan *et al.* (1981), a data set that has been the subject of numerous analyses, we first reiterated a finding that had been known before, but not usually highlighted: approximately 97% of the variance in the sizes of brain parts was predicted by the size of the whole brain, and 99% if a second limbic or olfactory factor was added (Gould, 1975; Jolicoeur *et al.*, 1984; Barton *et al.*, 1995; Figure 1a). This was an unusual emphasis, because most investigators, interested in mapping the 'differences' in size of brain parts to 'differences' in animal's behavior and niche, disposed of the shared variance and examined the residual variance, using various statistical approaches (Barton and Harvey, 2000; Clark *et al.*, 2001; de Winter and Oxnard, 2001). Since we were interested in the relationship of absolute volumes of brain or neuron numbers to neurogenesis, it was necessary, as well as fortuitous, to focus our attention on shared variance.

In addition to the predictability of brain component scaling from brain size, a second important feature was disproportionality – different brain components enlarge with markedly different slopes, such that the mammalian brain comes to be dominated in volume by different structures as it enlarges, notably the cortex. The large human cortex is just the size it should be for a primate brain our size (Hofman, 1989) and for absolutely larger brains, like the elephant's, the cortex is an even greater proportion of total brain volume.

The proximate cause of the disproportionality in the enlargement of brain parts can be understood by looking at neurogenesis, how neurons are generated in early development across mammalian species (Figure 1b). The ordinal position of the peak day that neurogenesis ceases for each cell group and structure in the brain is very highly conserved in the mammals studied (this end of neurogenesis is called the cell group or structure's birthday), although the total duration of neurogenesis varies from approximately 10 days in the mouse to over 100 days in monkeys. A two-factor equation can be written that captures 99% of the variance in this species/structure matrix (Clancy *et al.*, 1999, 2001).

Underlying the conservation of ordinal position, however, is a very strong nonlinearity: the numbers of cells generated do not increase linearly with extended developmental time, but exponentially over time, reflecting the doubling and redoubling

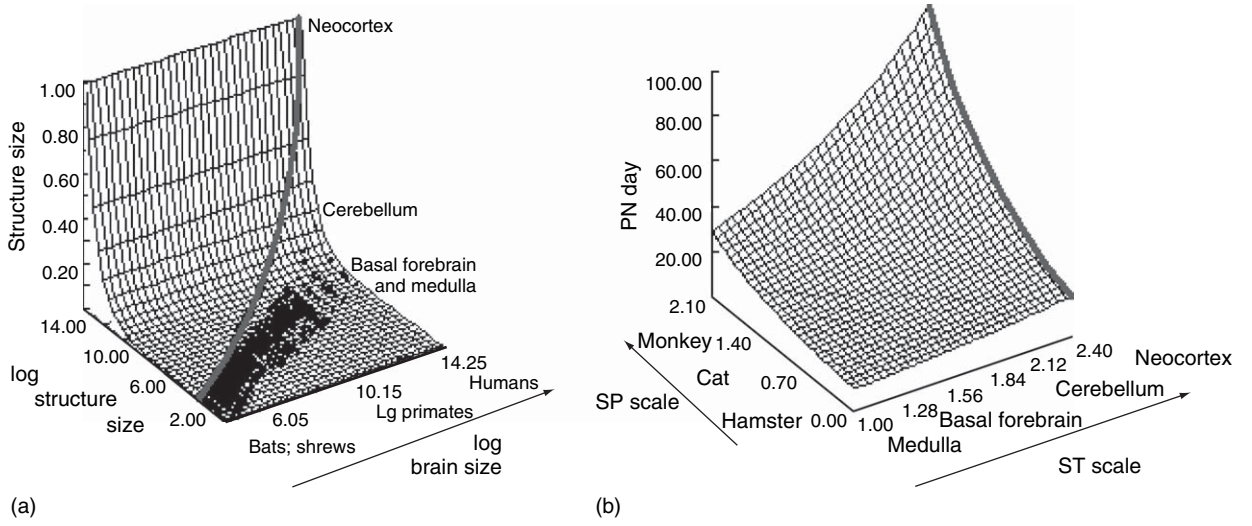

Figure 1 a, A combined log (*y*-axis) and nonlog (*z*-axis) plot of brain structure volumes versus log brain volume (*x*-axis) for the 131 primates, bats, and insectivores of the Stephan data set. This style of graphing is chosen to highlight both the predictability and disproportionality of those structures scaling at the steepest slopes with respect to brain size, particularly the neocortex. b, Model of the predictability of the birth date of a structure in a species given the ordinal position of each structure's birth date across animals (structure scale, ST) and the relative duration of neurogenesis in a species compared to others (species scale, SP). The seven species modeled in this analysis are hamsters, mouse, rat, spiny mouse, possum, cat, and monkey; 51 neural structures are modeled, from motor nuclei of the medulla to cortical layers. Those structures with high ST values are the latest-generated ones and are the same that become disproportionately large as brain volume enlarges. Lg primates, large primates such as rhesus macaque and the anthropoid apes; PN day, number of days postconception. a, Redrawn from Finlay, B. L. and Darlington, R. B. 1995. Linked regularities in the development and evolution of mammalian brains. *Science* 268, 1578–1584.

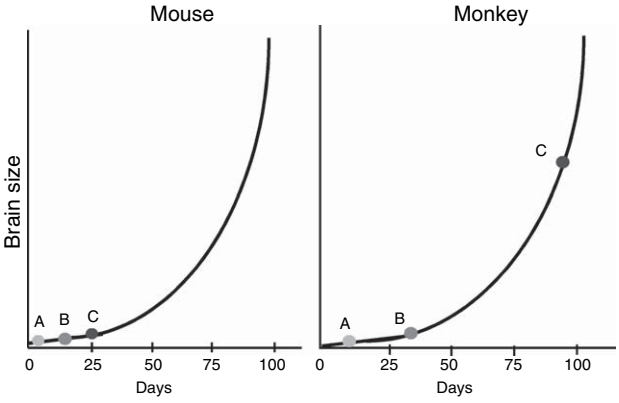

Figure 2 Late equals large. A schematic of the consequences for eventual size of a structure generated early (A), intermediate (B), or late (C) in the order of neurogenesis for a species with a short period of neurogenesis and a small brain, like a mouse, versus one with a long period of neurogenesis and a large brain. In the long-development species, the precursor pool for late-generated structures has a longer time to multiply and becomes disproportionately large.

nature of the symmetric phase of cell division in early embryogenesis (Figure 2). The consequences of exponential growth for lengthening the period of neurogenesis by roughly a factor of 10, the ratio difference from mouse to monkey, are quite different for the end neuron number in structures with early birth dates (like the medulla), middle birth dates (like the midbrain), and late ones (like the cortex). Our shorthand term for this relationship is late equals large.

Thus, particular parts of the brain increase disproportionately by a 'developmental rule'. This was quite a disturbing finding in that most previous accounts of disproportionate enlargement of some parts of the brain were cast as special adaptations due to the virtues of those parts. Particularly, some special organization or adaptive advantage was usually ascribed to the cortex to produce its disproportionate size in primates and ourselves: its efficient layering, the columnar structure, its hierarchical/parallel associative connectivity. However, the developmental rule that will produce a disproportionately large cortex in a large animal is already present in the much smaller stem primates with their small cortices, who presumably had no particular plans of their own for generating a useful structure to house a language cortex or any other kind of elaborated cognition. In addition, after a decision to allocate more tissue volume to olfactory-limbic structures or to cortex, the cortex scales disproportionately in all mammalian radiations we have studied (Reep *et al.*, 2007).

Before we go on to discuss this perplexing observation and the relationship of brain structure and function in evolution, we will go into a little more detail about just what the developmental rule is. We have published a number of further analyses and reviews to which we refer the reader for detail about such subjects as differences in neurogenesis in eutherian and noneutherian mammals (Darlington *et al.*, 1999), closer structural analyses including scaling of the thalamus (Finlay *et al.*, 1998, 2001), the special case of the limbic system concentrating on olfactory bulb and hippocampus (Kaskan and Finlay, 2001; Reep *et al.*, 2007), the scaling of the visual system (Kaskan *et al.*, 2005; Finlay *et al.*, 2005b), and the relationship of other developmental events to neurogenesis (Clancy *et al.*, 1999, 2001).

3.05.1.2 Prosomeres Are the Developmental Units Organizing the Duration of Neuron Proliferation in the Forebrain

Mammals differ from most other vertebrates by confining most of their neurogenesis to early development, rather than generating brain throughout life (there are exceptions to this generalization, of much current interest; Scharff, 2000). Does the conserved order of neurogenesis we see reflect a random pattern that happened to crystallize at the time of divergence of mammals, or some more fundamental organization? It proves that the conserved pattern of early neurogenesis we see, as well as where ongoing neurogenesis can be found in adult mammalian brains, can be explained by reference to an organizational scheme defined by patterns of expression of regulatory genes and transcription factors in early neurogenesis, the prosomere model (Rubenstein *et al.*, 1994). The basic axes that define this structure are common to the entire brain, which begins as an extended plate, the neural plate, which subsequently rounds up and connects its lateral-most edges to become the neural tube. The neural tube, whose original form is most obviously visible in the spinal cord, consists of repeating segments of similar fundamental structure with local variations. The part of the plate (and later tube) near the midline is called basal for its embryonic position, and in the spinal cord, this basal plate gives rise to motor neurons. The lateral part is called alar, Latin for wing, again for embryonic position, and in the spinal cord will produce secondary sensory neurons. The topology of the embryonic tube is maintained in the adult brain, with generative areas that initially neighbored each other, producing neighboring but translocated adult neurons. The embryonic relationships of cell groups in the adult brain can be recovered using classical neuroanatomical methods combined with painstaking developmental observations up to

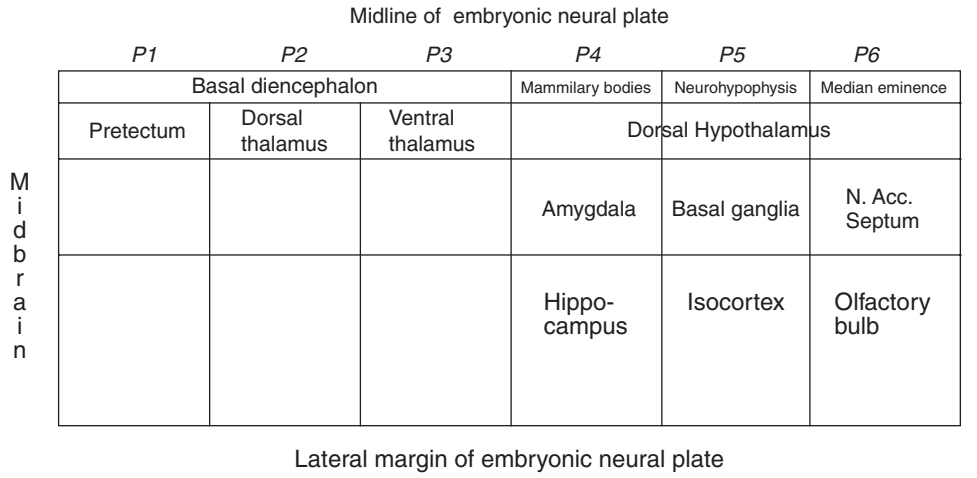

Figure 3 Components of prosomeres, the embryonic segments of the telencephalon, described by Rubenstein *et al.* (1994). The lateral-most part of the early neural plate is the part that undergoes the most extended cell division and becomes disproportionately large in large brains. N. Acc., nucleus accumbens.

about the level of the midbrain. The extended and convoluted pattern of neurogenesis in the forebrain, however, makes it impossible to track unlabeled cell groups from their place of origin. The ability to visualize gene expression gradients was required in order to trace each cell group back to its position of origin on the two axes of the embryonic brain, anterior–posterior and basal–alar.

An assignment of the traditionally named brain parts to this axial system of 'prosomeres' is given in Figure 3. For the most part (with some exceptions), as adult brain divisions reflect embryonic neural tube positions, these assignments can be made unambiguously. Hypothalamic and some basal forebrain structures are in the basal prosomeres; the large cellular masses of the forebrain, for example, the basal ganglia, are intermediate, and the cortical structures – olfactory bulb, hippocampus – most lateral or alar. If embryonic axial position is correlated with birth date (Finlay *et al.*, 1998), both axes contribute to the solution, but the alar–basal axis predominates, with late birth dates for cell groups associated with alar positions, early birth dates with basal. Our shorthand for the relationship of timing of neurogenesis to brain part size 'late equals large' can now be extended to a spatial axis of gene expression 'lateral equals late equals large'. Note that for two of the most anterior structures in the most lateral–alar position, the olfactory bulb and hippocampus, there is in fact no terminal birth date and neurogenesis continues throughout life, even in mammals (Bayer, 1980, 1983). For the cortex, at the same alar position, neurogenesis does appear to stop (Rakic, 2002; there is debate on the issue; Gould *et al.*, 1999).

Therefore, the conserved pattern of neurogenesis is not the crystallization of an arbitrary order that happened at some point in mammalian evolution, but is an expression of an axial pattern that at least in part is common to all vertebrates. The fact that the telencephalon is a likely division (but not the only one) for disproportionate enlargement across all vertebrates, and the cortex specifically enlarges in mammals appears to find its roots in this aspect of embryonic structure.

3.05.1.3 Brain Adaptations, Specializations, and Residual Variance

It is important to understand the actual physical characteristics of brain evolution to understand the nature of the shared and residual variance. In the Stephan data set (Stephan *et al.*, 1981), brain weights vary from a fraction of a gram to over a kilogram, a factor of about 20 000. At any particular brain weight, the residual variance of individual structures is approximately 2.5; that is, two species similar on the two factors (whole brain and limbic) might commonly have individual structures varying by over a factor of 2, occasional pairs considerably larger, which would be very conspicuous to an investigator looking for individual or species differences in the sizes of brain components. It also proves that the distribution of variance in volume across structures is quite uneven (Glendenning and Masterton, 1998).

This residual variation is an interesting aspect of species variation, and we do not discount it as an important window into brain structure, but we have set our job to understanding the significance of the factor of 2 in the context of the factor of 20 000, not the factor of 2 alone. Should we expect all adaptation-relevant increases in brain size to be carved out of the residual variation? The answer is

no, but it requires that our assumption of structure–function identity be loosened somewhat, particularly in the case of potentially multimodal regions like the cortex. There is no doubt that the sensory periphery is usually committed to its particular tasks – olfactory bulbs are for chemoreception, the retina for vision, and later on we will list large differences in the sensory periphery for animals in particular environmental niches that require different types of competence. A useful analogy for understanding brain evolution is the two-hit model of cancer initiation (Knudsen, 2001). In order for tumor genesis to begin, both proliferation 'and' mutation must occur. Rather than selecting for increased size for a function bound to a particular structure, a structure that has accessible variation in its size (first hit) may be well placed to acquire new functions, either by genetic specification (second hit) or through the epigenetic route of experience.

3.05.2 The Particular Case of Proliferation of the Cortex

The proliferation of the cortex, considering either total volume or general structure, is not identical across all mammals, and we would note a few of the interesting differences before concentrating on some commonalities. First, different radiations of mammals appear to allocate proliferation preferentially between limbic forebrain (olfactory bulb and hippocampus) and iso- or neocortex, with carnivores and primates showing more neocortical proliferation and insectivores, rodents, and anteaters greater olfactory bulb and hippocampus proliferation (Gould, 1975; Jolicoeur *et al.*, 1984; Reep *et al.*, 2007). As is well known, the cortex increases in area as it enlarges, but not exclusively – the cortex also increases in depth, as measured in number of neurons and particularly those concentrated in the upper cortical layers – in the smallest brains, a differentiated layer 4, the thalamic input layer, cannot often be detected, while in primate brains, often layer 4 has obvious sublamination (Valverde, 1990). Interestingly, the cetacean brain does not show an increase in depth with increasing size, suggesting an altered pattern of proliferation (Hof *et al.*, 2000). Finally, there is a great amount of local variation in such features as periodic expression of cytochrome oxidase, and various neurotransmitter receptors and neuromodulators both within the cortex, which have not been assessed systematically across species. Here we will discuss the proliferation of cortical areas, the size of cortical areas in relationship to niche, and some aspects of connectivity.

3.05.2.1 What Is a Unit of Cortex in Developmental Terms?

3.05.2.1.1 Radial units and cortical areas These two concepts, the first a developmental process and the second a characteristic of the mature cortex, are the best-known hypotheses about the structure of evolutionary variability in the cortex. Rakic has proposed that the radial unit, which is a region in the embryonic neural tube containing the precursor cells that will give rise to almost all of the cell types in an adult cortical column, named after the radial glia that provide the highway from the ventricular surface, is the fundamental unit of cortical proliferation (Rakic, 1990). By extending development, as measured in cell cycles producing the precursor cells in the cortical ventricular zone, more radial units are generated, generating more cortical columns and more cortical areas (Takahashi *et al.*, 1997). Rakic has characterized the generation of new cortical areas as a developmental negotiation between the cortex and the thalamus between their prespecified arrays of regions, initial protomaps (Rakic, 1988).

Cortical areas were first described as features of adult cortical organization: within the general uniformity of cortical layering, discontinuities in cell size, density, and total number and differences in axon distribution were noted (Brodmann, 1909). These regions of cytoarchitectonic discontinuities correspond to areas receiving particular thalamic input and thus correspond to the representation of modalities, which are usually topographically mapped, and a host of other features (Kaas, 1987; Krubitzer, 1995). At least the primary sensory and motor areas (V1, A1, and S/M1) are specified in their relative locations in the embryonic cortex by early polarizing events (Ragsdale and Grove, 2001). Thus, a cortical area could be an addressable unit of cortex variation and evolution: perhaps the whole set of instructions for producing a new area could be duplicated, as Kaas has suggested, much as the number of segments in a vertebrate whole body plan may increase or decrease under selection. In addition, if a cortical area is a unit analogous to a body plan segment, secondary modifications might be able to be attached to it, just as the segments of an insect body or vertebrate spinal cord have pronounced local specializations. Thus, new features could be attached to specific regions, like ultrahigh temporal resolution in cortical areas used for echolocation in bats (Suga *et al.*, 1981; see Somatosensory

Adaptations of Flying Mammals). Similarly, cortical areas could reasonably be increased or reduced in size, depending upon the relationship of their modality to each species' particular adaptive needs.

3.05.2.1.2 Multidimensional models The physical nature of scaling of biological tissue over the range of cortical surface areas that go from millimeters to meters requires some amplification of the first two hypotheses. A large cortex, or cortical area, appears to have much more internal detail than a smaller cortex. By this we mean that a large cortex contains not only more cortical columns and identifiable areas, but also more anatomical features such as stripes, puffs, and blobs in transmitter expression, and aspects of activity, interleaved ordered thalamic, intracortical, and callosal connectivity, as well as the elaborated functional maps correlated with the anatomical differences. They neither appear to be scaled-up versions of a stem insectivore cortex, nor the stem cortex replicated over and over across the cortical surface. All mammalian cortices, with the exception of those of monotremes, which differ in topology, appear to share three fundamental sensory and motor regions, and elaborate themselves along the same general lines, but with substantial variation in the details of all the features of cortical organization described above (Krubitzer, 1995).

Various hybrid models have been proposed, therefore, beginning from the assumption of a cortex that has specification of the rules of connectivity and general organization for three primary sensory areas, and then also for elaborating the cortical structure as it expands in size and functionality. One aspect of several of these models is the idea that cortical areas and other features arise in a combinatorial fashion by the interplay of genetically specified and environmental sources of variation. Because of some unusual aspects of gene expression in cortical neurons, cortical neurons themselves may generate an unusual variety of phenotypes (Kaushal *et al.*, 2003). Maturational gradients in cell generation and innervation in combination with intrinsic periodicities in the expression of various neurotransmitters and neuromodulators and the core set of specified cortical regions may also cause emerging arealization and segmentation, realized at different scales (Kingsbury and Finlay, 2001). Finally, intrinsically produced and environmentally specified activity is a powerful organizer of cortical areas in experimentally rewired cortices, and presumably must also be in development when it is unperturbed (Pallas, 2001).

Other than the very general feature of the allometric scaling of the cortex and some of its areas previously described (for example, Frahm *et al.*, 1984), and some systematic features of changes in the pattern of cortical cytoarchitecture with cortical size (Valverde, 1990), systematic comparisons of cortical organization at different scales that might allow us to better discriminate hypotheses about the process of cortical evolution have not been done. We undertook to quantify the manner in which the cortex proliferates, in the number of areas, and in the size of identifiable cortical areas, using primarily the extensive cortical mapping work in a variety of species of Kaas and Krubitzer as our sources for these analyses (Finlay *et al.*, 2005a). Both of our investigations using measurements taken from published studies have as their fundamental question, what the units of cortical expansion and differentiation might be and their developmental mechanisms.

3.05.2.2 Proliferation of the Number of Cortical Areas

The number of cortical areas increases generally with brain size, but the regularity of the increase was not known. We examined the proliferation of the number of cortical areas with respect to brain size in 24 mammals representing six orders, comparing visual, somatosensory, and total areal proliferation (Finlay *et al.*, 2005a). For each species, we ascertained or measured overall brain weight and overall cortical surface area; for the details of the studies employed, how a cortical area should be defined, the method of measurement and statistical analysis, we refer the reader to the original study. However, we will briefly describe our central choices and tactics.

Not all researchers agree on the definition or the number of cortical areas. Some make significantly fewer subdivisions (for example, Zilles, 1985), while others argue for the existence of a large number of smaller areas even in small brains (for example, Olavarria and Montero, 1990). Essentially, we chose to remain agnostic on the true definition of a cortical area and to rely instead on the pragmatic consideration of which explicit criteria allow us to examine the most species. The arguments of Kaas and Krubitzer on what constitutes an area are, however, compelling (for example, Kaas, 1987; Krubitzer, 1995). Their criteria for identification of an area are multidimensional, and include the presence of a fully mapped visuotopic, somatotopic, or other computed dimension, internally consistent patterns of thalamic, intracortical, and callosal input and output, and in some cases identification of the features of cortical cytoarchitecture or neurotransmitter or modulator expression.

The number of cortical areas might be best predicted by one of several independent variables,

including cortical surface area, or the weight of the whole brain, or one of the various formulas for encephalization, the ratio of brain to body weight. There was (and still is) good reason to suspect that in the case of two animals with equal absolute brain sizes, the more encephalized one might have a greater number of cortical areas. Unfortunately, in this data set overall, the largest-bodied animals and most encephalized are all primates, and the smallest, and least encephalized are all insectivores. Thus, the two measures of cortical area and encephalization are highly correlated ($r = 0.98$, $R^2 = 0.96$, $n = 19$). In addition, brain weight also correlates highly with cortical surface area, ($r = 0.95$, $R^2 = 0.91$, $n = 19$). Since total cortical surface area is the most proximate variable to the dependent variables we measured (number of cortical areas, ocular dominance column width, and axonal spread in the cortex), we have done our statistical analyses with respect to total cortical surface area, but in explanation of these data, the co-variation of cortical area with other brain measures should not be forgotten. Finally, because species may share traits through common descent rather than through independent adaptation, we employed the method of the comparison of independent contrasts (Purvis and Rambaut, 1995) in order to correct for the effects of phylogenetic relatedness.

Figure 4 shows the predictability of the 'number' of cortical areas overall, somatomotor areas only, and visual areas only from total surface cortical area (note we compare the number of areas, as a name of a unit of cortex, to the surface area, a measure of the cortex, an unfortunate ambiguity arising from their normal terminology). Though all three relationships were regular and highly statistically significant, surface area captured less of the variance when predicting the number of visual areas. The unusual scaling of striate cortex, which particularly in the primate lineage does not divide into subareas as the somatomotor regions do, may account for some of this variation.

The observation that the log of cortical area strongly predicts the log of the number of cortical areas shows that the relationship is predictable, but a logarithmic graph is not directly instructive about the kind or number of developmental mechanisms underlying this pattern of proliferation. As most biological developmental mechanisms bear some relationship to cell size, they operate over finite physical distances and not their ratios. To better visualize the change in cortex size for comparison to developmental mechanisms, the number of cortical areas is plotted against cortical surface area 'without' logarithmic transformation in Figure 5. For increases from the smallest cortices (from 10 to 400 mm^2),

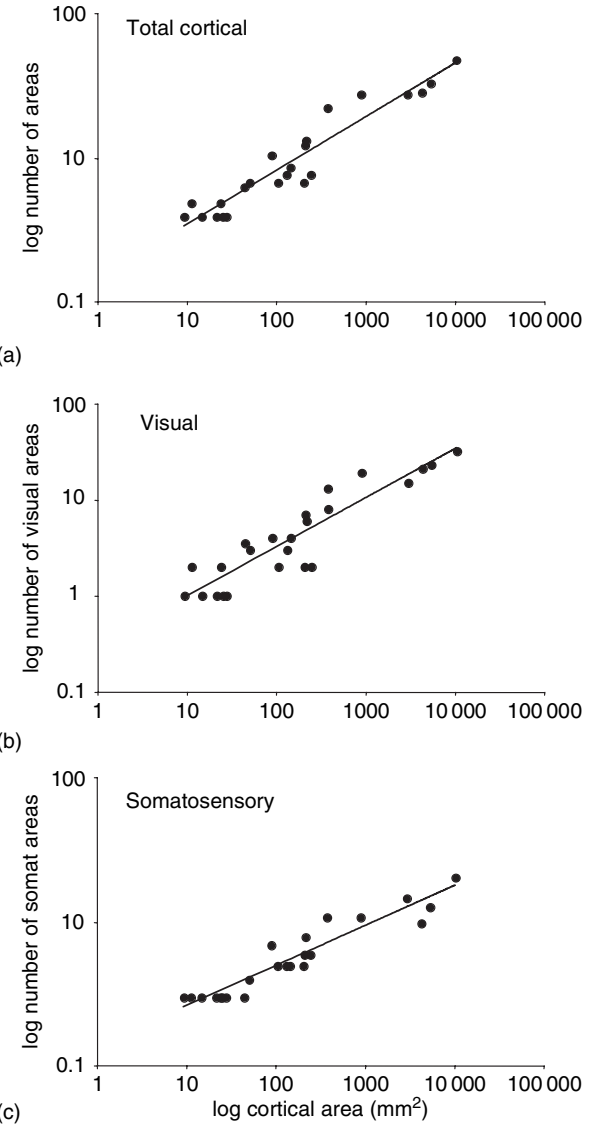

Figure 4 Simple regression of a, log total cortical area proliferation on log cortex surface area (log total areas = 0.172 + 0.379 log area, $R^2 = 0.89$, $n = 23$); b, visual area proliferation (log visual areas = –0.529 + 0.509 log area, $R^2 = 0.82$, $n = 24$); and c, somatomotor area proliferation (log somatomotor areas = 0.154 + 0.287 log area, $R^2 = 0.87$, $n = 23$).

cortical area proliferation is rapid. Thereafter, however, only massive increases in cortical area produce new cortical areas. It is also important to understand the absolute range of cortical surface expansion: in the approximately 500-fold range of the cortical areas graphed, the entire cortex of the least shrew (*Cryptotis parva*) could fit comfortably within a small fraction of the striate cortex alone of the rhesus monkey (see Finlay *et al.*, 2005a for the data employed and list of sources).

With the exception of the primary cortical areas, whose approximate position and boundaries appear to be fixed by early genetic specification, the

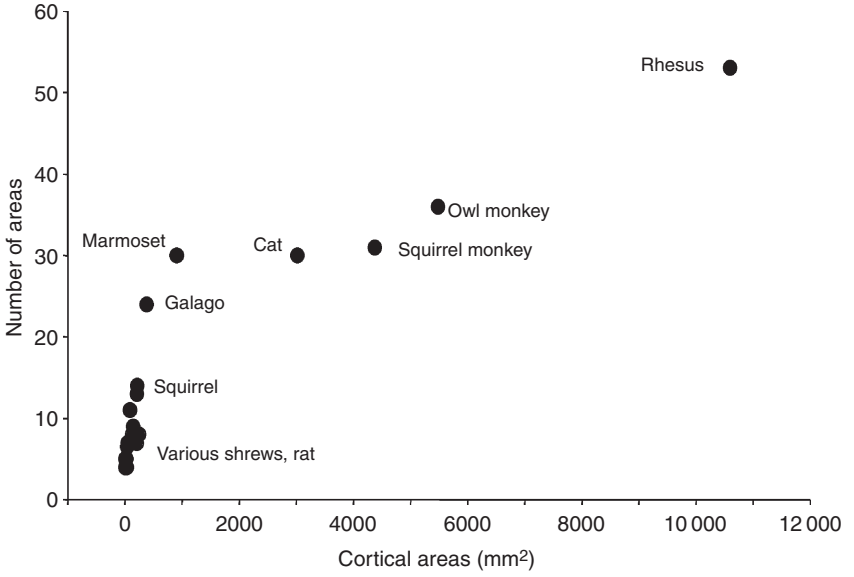

Figure 5 The number of cortical areas plotted as a function of cortex surface area, without logarithmic transformation.

mechanism by which other cortical areas emerge is not understood, but the size ranges over which the rate of proliferation changes, suggests two separate mechanisms. As cortical area increases from approximately 20 (the smallest shrews) to 400 mm^2 (galago) the number of cortical areas increases rapidly, from 4 to 24. Thereafter, another 400–4000 mm^2 of cortical area nets only another six cortical areas (those animals with 30 cortical areas enumerated are the cat, the marmoset, and the squirrel monkey). However, it is at the brain size of cats and small monkeys that the well-studied substructure of visual cortical areas begins to emerge, the ocular dominance columns, puffs, and blobs of primary visual cortex (Hubel and Wiesel, 1962, 1968), and we suggest that the cortical areas of small brains and the morphological specializations such as ocular dominance columns within cortical areas of large brains may both be manifestations of the same underlying developmental mechanism, activity-dependent axonal (and dendritic) sorting. The spatial extents of single axon arbors, dendritic trees, and ocular dominance columns in the cortex essentially do not scale with brain size (Kaas, 2000; Finlay *et al.*, 2005a; see also Manger *et al.*, 1998). Most models of stripe-in-topographic-map formation in brain tissue essentially pit axon–axon affinities against axon–substrate affinities (for example, Swindale, 1980). We hypothesize that as brains get bigger, more specific aspects of sensory stimuli may provide the correlational structure necessary (that is, the increased axon affinities) to allow the segregation of new, functionally specific cortical areas once additional volume of cortical tissue (diluting axon–substrate affinities) is made available. We have no model, as yet, for the increase in cortical areas in the largest brains.

Therefore, though the nature of peripheral sensory specializations may have a direct effect on the nature of the maps formed in the cortex, we are proposing that cortical areas also proliferate by a developmental rule, at least over the smaller ranges of brain size. This hypothesis is distinct from the idea of a cortical area as a specially selected, modular processing region. While a cortical area might be heavily involved in the processing of a particular submodality important to a species, we propose it emerges due to developmental rules present in all species (axon–substrate affinities and Hebbian fire-together, wire-together) influenced by its peripheral specialization and not the selection of new mapping and processing rules.

3.05.2.3 The Size of Cortical Areas, Sensory Specializations, and Behavioral Niche

The relative size of cortical areas in animals specialized for different niches can also inform us whether it is possible to select for the size of a cortical area and allocate resources to a particular modality differentially by changing developmental rules. Using essentially the same data sources and methods of the prior analysis, we quantified instead the surface area of primary sensory cortices (visual, somatomotor, and auditory), and the total cortical surface area devoted to the same modalities in either nocturnal or diurnal animals (Kaskan *et al.*, 2005). The nocturnal animals of this analysis all make use of their eyes for nocturnal vision, such as the rat, possum, or

owl monkey, and are not fossorial animals whose eyes have degenerated (for this contrast, see Cooper *et al.*, 1995). The fundamental assumption to be tested is that nocturnal animals should allocate fewer resources to vision and more to somesthesis and audition, and thus, across taxa, nocturnal animals should show a grade shift with a smaller primary, or total visual cortex with respect to total cortical area, and relatively larger somatosensory and auditory regions.

Prior to comparing animals in different niches, we first examined the scaling of cortical areas independent of niche and replicated a finding that has already been described in various studies with completely different species – that the volumes of identifiable cortical areas or regions each have specific, statistically discriminable allometric scaling with total cortical area, with primary visual cortex scaling at a steeper slope than other primary sensory regions (Frahm *et al.*, 1984; Stevens, 2001). The observation that frontal cortex scales at a steeper slope than other cortical regions and that humans have a larger but predictable volume of frontal cortex (Jerison, 1997; Semendeferi *et al.*, 2002) is analogous to this analysis.

Figure 6 plots the regression slopes of V1 (a) and total visual cortex, including all secondary visual areas of the parietal cortex (b) for 20 nocturnal and 8 diurnal species against total neocortex. Again, as measured by the method of independent contrasts, no significant difference appears between nocturnal and diurnal mammals in their visual cortical scaling – either in V1 or in all visual cortical areas. The regression plots themselves are notable

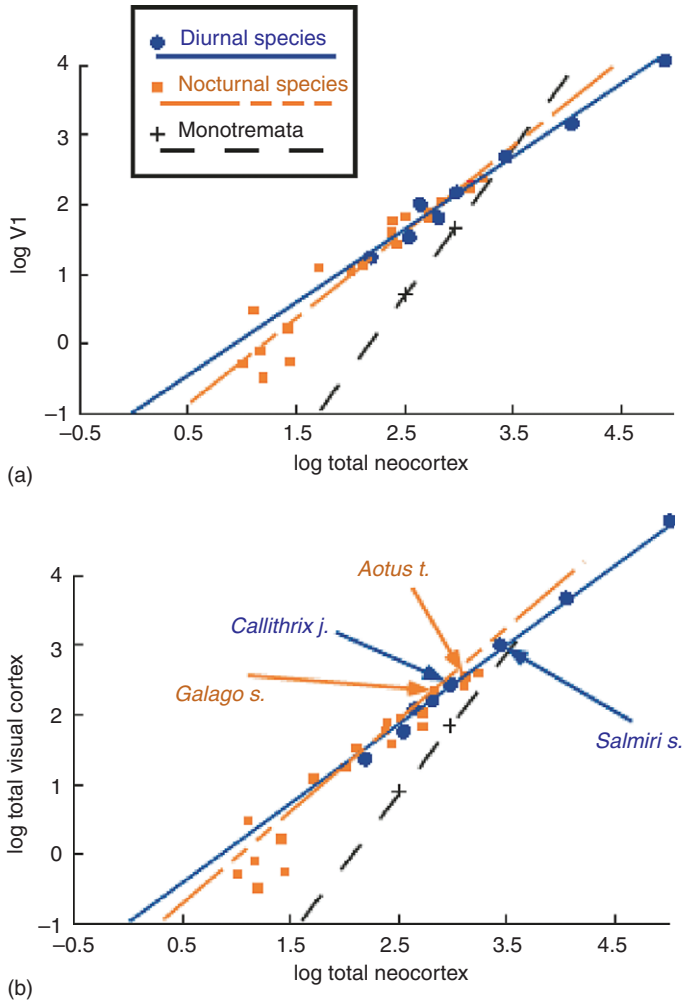

Figure 6 The volume of primary visual cortex (a) or all mapped visual cortical areas (b) versus total cortex volume in nocturnal (orange points) and diurnal animals (blue points). There is no statistically significant difference between these groups for visual cortex or motor or somatosensory cortex (not shown). Reproduced from Kaskan, P., Franco, C., Yamada, E., Silveira, L. C. L., Darlington, R., and Finlay, B. L. 2005. Peripheral variability and central constancy in mammalian visual system evolution. *Proc. R. Soc. Biol. Sci.* 272, 91–100.

for their complete overlap. If diurnal animals devoted more cortex to vision, we would expect to see a grade shift in these plots, with the diurnal regression line displaced upward but parallel to the nocturnal. Neither did nocturnal animals possess more somatosensory or auditory cortex – primary nor total – as might be expected if they could designate more anatomically defined cortical areas to modalities important in the dark. The retinas of these animals are quite different, as would be expected, with nocturnal animals possessing many more rods. Since we were able to capture statistically significant differences in the allometry of V1 and total visual cortex compared to other cortical areas independent of niche, our confidence that we could detect a difference between nocturnal and diurnal species in cortical organization is reasonable.

Should a nocturnal visual system require less neural volume than a diurnal visual system? If the nocturnal computational problem of vision could be demonstrated to be harder, it could be argued that it should require more. Our central observation is that it appears to require neither less nor more (as defined by cortical area volume) in the face of greatly different photoreceptor distributions. Nevertheless, the interpretation that greater usage should have a direct correlation with the size of corresponding neural systems is prevalent in the literature (Barton *et al.*, 1995; Barton, 1998; Barton and Harvey, 2000). These observations range from large tactical allocations of brain mass between the limbic system and neocortex, attributed to preference for the visual modality, to very specific differences in cell types associated with fugivory versus folivory. In the latter case, it is perplexing that subtle differences in visual requirements for frugivory versus folivory should be associated with differences in visual system organization, while major differences to accommodate nocturnality and diurnality should not. Niche, brain size, and cortex size are difficult to dissociate in primates. It may be, therefore, that the observations are confounded by conserved scaling phenomena unrelated to function. Alternatively, frugivory and a certain visual cortical organization might be necessarily related, but with selection on gross brain size as the only means of inducing the required detail in brain organization. For example, in a study of dexterity in animals whose dexterity ranged from hooves to hands, brain area devoted to control of the forelimbs was highly positively correlated with dexterity, but the area devoted to the forelimbs was in turn accounted for entirely by total neocortex size (Nudo and Masterton, 1990).

The hypothesis that visual cortex differs between nocturnal and diurnal mammals may yet be true, in two different ways: first, other aspects of cortex than size must surely be important, and second, our measurement of size may inaccurately assess true functional allocation. Evolution might well act on variables such as dendritic structure or density, receptive field size, axonal arborization, myelination or basic cell physiology; for example, consider the intriguing case of dyslexia, now associated in some of its forms with difficulties in detecting rapid transients in more than one modality (Merzenich *et al.*, 1993). Differential expression of some feature of synaptic transmission in the cortex may allow some individuals to represent information in rapid stimulus transients, producing ordered phonemic representations (Petersen and Fiez, 1992), while others without it do not. Details of basic cortical processing could well be different in animals of different niches.

Considering the second point, how well do the anatomically defined cortical areas reflect actual functional allocation in the cortex? A number of new imaging and other functional studies suggest that we may have been overimpressed by the major thalamic input to an area when cortical regions were named, and we thus assess true functional allocation inaccurately. Functions may play more freely over the cortical matrix specified early in development than we have imagined, with the most likely substrate through long-range intracortical connectivity. We find violations of modality specificity of cortical areas in evolution, where, for example, the visual cortex comes to respond to auditory stimulation in the blind mole rat (Bronchti *et al.*, 2002), in essentially normal function, where the auditory cortex responds to the visual stimulation of lip-reading (Calvert *et al.*, 1997), in experimental rewiring of visual information into auditory areas (Gao and Pallas, 1999), and in cases of sensory loss in humans where visual cortex becomes critical to tactile Braille reading in the early and late blind (Sadato *et al.*, 1996). In the adult, our understanding of corticocortical connectivity is limited, but recent work shows that connections may be widespread and fail to conform to traditional hierarchies and notions of connectivity (Falchier *et al.*, 2001; Rockland, 2001). Thalamocortical connections also show a distributed nature with a matrix of superficially projecting cells not confined to the intralaminar nuclei, which may serve to bind sensory experiences by connecting multiple cortical and thalamic areas (Jones, 1998). On the whole, the findings that such broad structure–function matches in the cortex exist imply that the neocortex is not a piecemeal collection

of areas, each with its own discrete function, but a generalized processing device.

3.05.2.4 Integration of Individual Variability and Developmental Plasticity into an Evolutionary Account

A final perplexing feature in the understanding of brain size regulation is the report of remarkable individual variability in the size of cortical areas, which would seem to be in stark contradiction to the very regular scaling we have just described in allometric studies. We will assume that published allometric studies have managed to determine representative mean structure volumes for scaling work and that the scaling results are accurate at the species level for which they are intended. Here we address the question of how we are to understand the importance of structure sizes if individual members of a species may occasionally but substantially differ from one another in the relative sizes of brain parts.

What kinds of variations are reported at the individual level, within species? The best information comes from a number of studies of the primate visual system, particularly the rhesus macaque. Van Essen *et al.* (1984) have found individual animals whose primary visual cortex differed by a factor of 2 or more. Similarly, the variability of the human visual cortex exceeds substantially the variability of the entire cortex (Gilissen and Zilles, 1995). There are only a few studies, to our knowledge, of the variability at the individual level of the number and arrangement of cortical areas (Qi and Kaas, 2004; Airey *et al.*, 2005), but comprehensive imaging of individuals may soon allow this kind of comparison to be made. Few of these observations have as yet been tracked onto individual variation in visual capacity, and it would be interesting to do so. However, there is reason to believe that with the exception of variations in cell density in the visual periphery that directly affect acuity, the basic processing of the visual system is robust to wide variations in number of neurons in interconnecting populations, due to the equilibrating effect of processes such as activity-dependent stabilization in early development and compensatory norming in adulthood, producing the remappings described in the previous section.

Preferential allocation of space to various kinds of sensory specializations is commonplace 'within' particular cortical areas (Suga *et al.*, 1981; Silveira *et al.*, 1989; Catania and Kaas, 1997). In addition, complete loss of a sensory system either phylogenetically (Cooper *et al.*, 1995) or very early in development reduces the volume and area of a cortical area through the epigenetic route of degeneration of the intermediate thalamic nucleus (Finlay and Pallas, 1989; Rakic *et al.*, 1991; Kahn and Krubitzer, 2002). Both of these kinds of alterations, within-modality specializations, and sensory system loss, seem likely to use the epigenetic pathway of mapping a new thalamic organization onto the cortex rather than alteration of cortical specification.

All evidence reviewed so far – regular allometric scaling of the entire cortex (Finlay *et al.*, 1998, 2001), of particular cortical areas (Jerison, 1997; Stevens, 2001; Kaskan *et al.*, 2005), and of the number of cortical areas (Finlay *et al.*, 2005a); independence of specific cortical area volume from niche (Kaskan *et al.*, 2005); individual variability (Van Essen *et al.*, 1984); and developmental plasticity (Pallas, 2001) – converges on the same interpretation. The regularity of major components of neural allometric scaling, best predicted by cross-mammalian developmental constraints, suggests that mismatches of neural ratios or of typical structure/function allocations must be a regular, compensated phenomenon in mammalian evolution (Xiong and Finlay, 1996). The independence of the relative size of primary sensory areas from niche (Kaskan *et al.*, 2005) and the coupling of dexterity to whole cortex size rather than the relative size of the somatomotor cortex (Nudo and Masterton, 1990) suggest that the relative size of a cortical area with respect to the whole cortex is either very difficult to change or unimportant to function. Large individual differences in the sizes of particular brain areas unaccompanied by flagrant disabilities tell the same story about individual development, as do innumerable instances of developmental plasticity. Thus, particularly for intrinsically cross-modal structures like the cortex, structure and function may not be uniquely linked at neurogenesis, and neural resources may be allocated to new functions as necessary. The fact that we have named a structure visual cortex (because that is typically what it does) does not prevent it from becoming Braille cortex when circumstance permits (Sadato *et al.*, 1996).

3.05.3 Computational Considerations in Cortex Proliferation

The description of the evolution of brain size, cortex size, and the size and number of cortical areas is qualitatively and quantitatively consistent, but fundamentally unsatisfying. With the exception of very broad, tactical allocations of space to different classes of neural processing (such as cortex vs. hippocampus; McClelland *et al.*, 1995) either for

whole vertebrate radiations (Gould, 1975; Finlay *et al.*, 1998) or for very broad behavioral niche (Clark *et al.*, 2001; de Winter and Oxnard, 2001), we have not found much to tie features of brain organization to environmental niche, nor to any aspect of behavior. All we have done is to speak negatively about premature allocation of function to structure and speak vaguely and positively about the cortex as a general-purpose processing device. Yet the brain, a very expensive tissue to maintain, increases with body size at an extremely predictable rate for parts that have a direct relationship to body size, and for parts that do not. Sensory systems, particularly in what appears to be their central allocation of neural volume, do not economically scale their size to their apparent usage. Cortical areas proliferate in a fairly direct relationship to overall cortical area, and there is really no direct evidence of any kind as yet linking any explicit feature of behavioral adaptation to genetically produced changes in cortical architecture. We will argue now that the missing component of our understanding may be the nature of network architecture in the brain, particularly the cortex, and an understanding of how networks scale.

We now approach the question of brain scaling and resource allocation in a different manner, considering candidates for the class of network architecture that the cortex might possess (Milo *et al.*, 2004), with a brief review of what is known about their scaling properties. We will then lay out one toy model of a network whose properties we can perturb to see the nature of its scaling. It is important to understand here that we are making no claim that the cortex necessarily resembles the model that we are exploring here. Rather, we employ models to make hypotheses about how networks might scale in an explicit and testable manner, and in so doing, perhaps recognize in the behavior of the models some aspects of cortical scaling and brain–body allometry that have been determined empirically.

3.05.3.1 What Are the Dynamics of Brain Scaling?

As brains get bigger, it is important that they do so efficiently. Brains face the problem of fitting into necessarily limited spatial and metabolic budgets (for example, Aiello and Wheeler, 1995), placing a premium on brains that minimize the amount of tissue not contributing to the organism's fitness. While pruning unnecessary cells is a relatively simple matter, decreasing average axon length is a considerably more complex problem. A longer axon is just as good as a shorter one computationally, but the longer one squanders the brain's spatial and metabolic budgets (Swindale, 2001). The ratio of somas to axons and dendritic trees decreases with scale, requiring new wiring strategies (Zhang and Sejnowski, 2000). Moreover, brain size is limited to the amount of space the head can provide, given that the head has more functions than enclosing the brain, and by the sheer expense of hauling a heavier body part. In mammals, head size itself is under some constraint by the female reproductive organs (Leutenegger, 1982).

Given the brain's limited spatial and metabolic budgets, a wide range of fundamental questions in brain network architecture must be addressed. Does the same network architecture apply to the scaling of large and small brains? What are the properties of various classes of proposed modules in the brain, from the sense of repeating, open units such as a cortical column, to encapsulated, closed processing units in the sense of Fodor (1983, 2000)? What happens when new functions are introduced into new networks, or the size of components is altered? In the following section, we outline a few of the issues arising in our first explorations of these computational questions.

There are several immediate consequences of body scaling. Different body types and sizes require different motor programs to maintain a reasonable level of energy efficiency. Additionally, changes in body type and size also change the relative muscle mass and pH buffering capacity, which in turn directly affects what motor programs will maximize the aerobic to anaerobic ratio. Typically, the larger and more complex the body type, the greater the required level of motor sophistication and physics modeling (Keimel and Roth, 1992).

While receptive field sizes and higher-level convergence vary not only across species but even across the body of a single animal, there appear to be minimum tactile acuity levels beyond which larger body size requires a larger somatosensory region in the brain. In specialized systems of various well-studied mammals, from the star-nosed mole, to the echolocation systems of bats, to the primate fovea, an increase in sensory organ acuity is closely related to the relative volume of primary sensory cortex devoted to the high-acuity region (Suga *et al.*, 1981; Silveira *et al.*, 1989; Catania and Kaas, 1997). For instance, in the acoustic fovea of bats, a hypertrophied sensitivity to a particular frequency in the cochlea is paralleled by a highly disproportionate amount of auditory cortex devoted to that frequency. Since such sensory foveas are not simply increases in receptor density, but also interrelated with unusual motor and perceptual processing demand, the subsequent within-area cortical

gerrymandering may be the result not only of an increase in afferents but of a need for more computational power. Any necessary relationship between the number of afferent units and the amount of cortical area necessary to process their signals has not, to our knowledge, been documented. Thus, the dynamics that couple afferent numbers with total brain size is an unexplored question. We will argue that a critical component of understanding brain–body allometry lies in the governing dynamics of wiring strategies employed by the brain.

3.05.3.2 Classes of Wiring Strategies

A wiring strategy is a plan by which neurons, or groups of neurons, interconnect efficiently (that is, minimize metabolic and spatial expense) while taking into account the settings of several parameters, perhaps the most important of which is overall brain size. For the purposes of this paper, we will focus our discussion on synaptic interconnections at the level of cortical columns, nuclei, or lobes; or more generally, groups of neurons, which we refer to as subnets. Ultimately, to be efficient, a wiring strategy must attain good performance on two axes: reuse and intersubnet communication.

1. *Interconnecting subnets for the purpose of reuse*. To increase efficiency, subnets should combine and produce behavior not implemented by any one subnet. The recombination of subnets results in a reuse of existing resources, conserving the number of neurons needed to accomplish any given set of tasks. Such reuse of cortical resources is very commonly observed in current imaging work. Increased executive demand and task difficulty, for instance, causes activation of the frontal cortex for a variety of different tasks across different modalities (Duncan and Owen, 2000). How subnet reusability might be achieved is a question we will address later.
2. *Interconnecting subnets for the purpose of communication*. The fundamental purpose of the brain is to control the body. No matter how elaborate the internal architecture, on output, the brain must act as a whole and cannot execute contradictory commands. Different subnets, participating in the planning and execution of any given task, must maintain some level of communication to ensure that eventually subnets work in concert with each other. In order to avoid the kind of dysfunctional situations seen following spinal cord or corpus callosum transection, which essentially produces two brains in one body (Sauerwein and Lassonde, 1994), every neuron in the brain must be connected to every other neuron in the brain either directly or indirectly.

Both subnet reusability and subnet interconnectivity can be regarded as two sides of the same problem. The average distance – measured by the number of synapses, or the number of hops, any neuron is from any other – directly dictates:

1. the extent to which that neuron can be reused in different neural circuits;
2. the recombinatorial power of the subnets, to which that neuron belongs; and
3. the ability of those subnets to communicate with other subnets.

To maximize 1, 2, and 3, a connection strategy must minimize average hop count. There are various ways this can be accomplished, depending upon computational goals and constraints.

3.05.3.2.1 Small worlds The problem of minimizing the average hop count has been extensively studied and modeled (Watts, 1999). Consider a daisy-chained group of nodes arranged in a circle. The maximum (worst case) internode distance is the number of nodes in the chain minus one; the average is the number of nodes in the circle divided by two. Provided there are two or more nodes in the circle, the constant formula for the average hop count remains fixed irrespective of the number of nodes. However, with just one projection, spanning the diameter of the circle, both the worst case as well as the average internode distance is dramatically reduced. By adding just a few long-distance projections, the average distance between neurons could be decreased in just the same way.

However, small worlds has been primarily used to model connections between computers on the Internet or the connections between people in a social network. While it has been argued (Manev and Manev, 2004) that a small world network could be a reasonable description of the brain, there are important aspects to small world networks that make them unsuitable as a model for synaptic connectivity. Unlike computers, humans, or mail hubs, neurons are not routers that are capable of directing a message to its appropriate destination. They have only one axon and any message a neuron sends reaches every dendrite on which the axon terminates. Moreover, individual neurons are virtually never mere communication relays: they process and mutate incoming data in the process of transmission.

Furthermore, single-neuron-to-single-neuron communication is not the essential wiring challenge. In most mammalian neural systems, an individual

neuron has very little computational weight. Rather, parcels of processed information are sent in parallel from one subnet on to other subnets. Neural subnets of even very modest complexity would be unlikely to communicate with each other via a single synapse; the process of decoding a binary serial stream requires many more resources than sending the data in parallel. It is important to recognize that maintaining a certain number of degrees of separation between neurons (or average hop distance) is not a wiring strategy in and of itself, but the result of a wiring strategy. Random projections, for example, could easily reduce the degrees of separation between subnets and yet be useless in actually transmitting the relevant information.

3.05.3.2.2 Nearest neighbor If the small world model does not seem to be a good model for the mammalian brain, what other models are there? Arguably, an alternative approach to efficiency would involve physically moving related subnets closer to each other, decreasing the average synapse length, although the average node distance (hops) would remain unchanged. If, for instance, two subnets are both responsible for processing information from the same modality, it might make sense to move them as close to each other as possible. The principle of minimizing axon length might be driving sensory systems to stay together in the cortex and may also be behind the ubiquitous feature of topographic maps (Kaas, 1997). On the other hand, intramodal processing of various kinds is central to cortical processing at every stage, which will necessarily disrupt wiring strategies dependent on single-modality topography. The nature of the topographic dimension to be represented often varies with the processing stage, for example, moving from cochleotopic to spatiotopic representations within the auditory system. Overall, the Hebbian fire-together, wire-together strategy cannot produce a single nearest-neighbor solution based on modality, though it may contribute to aspects of the wiring strategy.

3.05.3.2.3 Modules So far this paper has not attempted to define subnets in any other way than as simply a group of neurons working in tandem. Sometimes, such constellations of neurons have been called modules: self-contained components that are used in combination with other modules. In terms of neural circuitry, a module is a cluster of interconnected neurons that have a set of input neurons and a set of output neurons – collectively called an interface – with any number of interconnected neurons in between. Additionally, a module provides a particular functionality to other modules without requiring other modules to know how the functionality is implemented.

Modules, as a concept borrowed from computer science, strive for opacity and encapsulation, such that their implementation is hidden behind an interface that defines the scope and nature of the set of inputs and outputs the module can accept and produce (Parnas, 1972). Software-like modules have been argued to exist in the brain (Chomsky, 1975; Fodor, 1983). There are several reasons why software-like modules might make sense in a neural context. Modules are extensible – that is, modules place no requirements on the internal wiring of other modules nor connect to internal components, and thus can be added *ad lib*. Moreover, modularity could provide a level of insulation from conflicts and cross-talk that can arise in complex multifunctional systems, especially ones that have been extended by accretion (like evolution). Often, a degree of modularity is required to prevent clusters of neurons from being reused in different circuits. For example, fail-safe critical systems such as those controlling heart rate and respiration should function as autonomous (and potentially redundant) modules, but leave open a command interface that can accept high-level input. Neuroethologists have uncovered a number of systems that rely on such command neurons (Nolen and Hoy, 1984).

On the other hand, modules are not fault tolerant. A modular system requires massive redundancy to cope with the complete or even partial failure of any one module. Most significant to our discussion, though, is that modules increase the average hop distance: the bigger the module, the greater the hop distance. In other words, a smaller ratio of interface neurons to hidden neurons results in a greater average distance between any two neurons in different modules, subsequently decreasing the reusability of that module's neurons. Additionally, larger modules tend to implement more complex and therefore less abstracted units of computation, which leads to a decreased reusability of the module as a whole. Because of these wasteful features, the brain is not likely to pursue a strategy of using more than a few large modules.

3.05.3.3 Exploring the Effects of Uncoordinated Scaling in a Neural Net

Because neither nearest-neighbor nor modular wiring strategies seem likely to give a full account of the wiring of the mammalian brain, from the arguments above, and because the units of small-world networks have properties quite unlike neurons, we have chosen to employ a standard,

three-layered neural net architecture to explore the effects of scaling on network configuration. We address the effect of scaling up any part of a network architecture on the properties of the rest of the network; our interest is to gain some insight as to why the sizes of the various sensory surfaces, motor interfaces, and brain parts are so closely correlated as species scale in evolution when energetic efficiency would suggest part-by-part independence should be preferred. Prefacing with our conclusion, we find that scaling any kind of subnet within a neural net also causes a scaling response in all neighboring subnets, and arguably in all the subnets within a system.

3.05.3.3.1 The model employed We used a standard neural net architecture of either 64 or 128 nodes, limiting the number of connections to a unit in the net to 10. First, we trained a 64-node neural net on either one of two arbitrary tasks chosen not because of their similarity to any known neural process, but because they have been well studied. These two tasks are character recognition and graph fragment completion, two of the first tasks to be successfully implemented by artificial neural networks and still in use today (i.e., the US post office uses neural nets to read zip codes). No interesting differences between these two tasks emerged in net configuration and we will not address this aspect further. In order to understand the allocation of function within the neural net, we made lesions in one node at a time and measured the decrease in firing rate across all other nodes.

The absolute value in the change of firing rate across all nodes has very high contrast consequent to this lesion, with a small group of nodes significantly more affected by the lesion than all the others (Figure 7), revealing subnet contours (Figure 8).

When a subnet node is in a feedforward position, the nodes afferent to it are not affected by the lesion. Nodes affected by a lesion are fewer in number than would be expected by random, proportional interconnections.

Through single-node lesion testing, we found nine subnets in the 64-node network. We repeated the experiment with a 128-node network performing the same character recognition task (which had a proportionally scaled input layer) and found 13 subnets. Though not surprising, the first point to take note of is that average subnet size increased in response to an increase in the number of nodes in the hidden layer. With the task remaining constant, while the number of nodes doubled (128:64), the number of subnets did not double (13:9). Average subnet size went from 7.1 nodes to 9.8 nodes.

Suppose that the body becomes bigger, generating a larger S1 to maintain sensory acuity (Figure 8). Does this have consequences for the rest of the brain (or net) that the larger S1 is patched into? To explore the effects an enlarged S1 required by an enlarged body on the structure of the rest of the network, we artificially enlarged sections of the 64-node network to see whether or not the average subnet size increased. For each replication of the experiment, we grafted just one subnet from the 128-node network into an untrained pool of 64 nodes minus the number of nodes in the grafted subnet, thus entering a subnet of an average of 9.8 nodes, fixed in size, into a neural net that normally generates 7.1-node subnets. In each replication of a single subnet graft, we permuted over every possible placement of the grafted subnet into the 64-node net, principally to avoid any configuration that would artificially isolate any nodes and decrease the net size artifactually, examining only the best results from the permutations, in terms of task performance (i.e., percentage of characters correctly identified). After grafting, each 64-node net was trained, but with the provision that the grafted

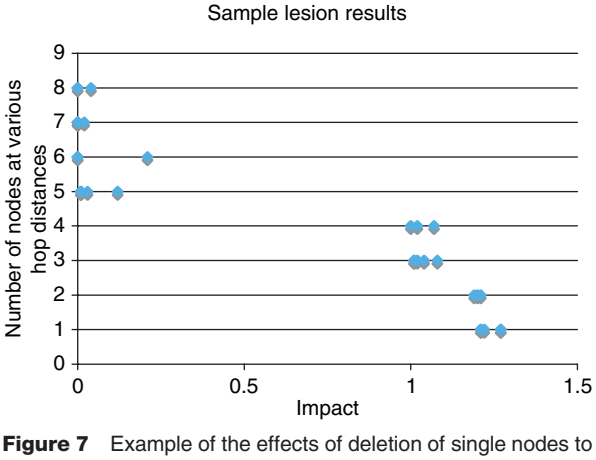

Figure 7 Example of the effects of deletion of single nodes to show connectivity structure within a neural net.

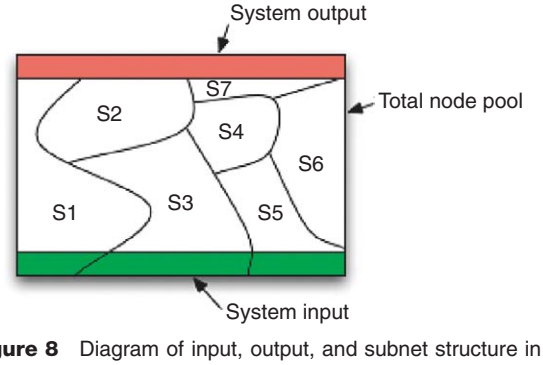

Figure 8 Diagram of input, output, and subnet structure in a trained neural net.

subnets were immutable. Grafted subnets contributed to the solution of the task, with the initially untrained net reconfiguring around the grafted component. For each neural net with a scaled-up graft, we found a smaller number of total subnets (and therefore larger average subnet size) than was the result in the original 64-node net experiment. There was an average decrease of 1.8 subnets to a mean of 7.2, with eight nodes per subnet including the grafted subnet and an average performance drop of 17% (failed character recognition or graph fragment completion). The decrease in number of subnets is significantly greater than the simple decrease expected by a subtraction of a subnet of 9.8 nodes; we trained nets on the same two tasks with 64 minus *N* nodes, where *N* is the size of each graft and is on average 9.8. Even when normalized to 64 minus *N* over 64, the control group produced a larger number (10) of smaller sized subnets that on average contained 5.4 nodes.

In summary, we found that when one subnet is scaled up in number of nodes, the other subnets in the network will scale up as well when trained, propagating the size of the new, larger unit throughout the net at the expense of subnet count as well as task performance. To maintain performance, we presume that other nodes would have to have been added in compensation, though we have not yet performed this experiment. Below, we will argue that the larger, grafted subnet is propagating its size through the net because of problems in input/output (IO) matching.

3.05.3.3.2 Possible causes of network scaling behavior Within any pool of nodes, such as the 64- and 128-node nets we used, there is always an interface to put input into the net, and register its output. However, subnets within that pool also have their own interfaces used to communicate across subnet boundaries. Subnet communication sends output from one subnet and feeds it to the input of another. As such, the number of subnet output neurons in the upstream subnet is tightly coupled with the number of subnet input neurons in the downstream subnet. Increasing the IO layer of any one subnet should cause subnets that are connected to the scaled subnet to widen their IO layers as well.

A quick look at the feedback nature of the nervous system suggests that IO matching will cascade (Figure 9). Potentially, in a strictly feedforward network, IO matching might only occur between the superscaled subnet and its immediate neighbors. However, any subnets that communicate not only with the scaled subnet, but also with each other would also have to scale their output layers to maintain compatibility with each other, causing a systemwide IO width increase cascade. Multiple types of compensation for systemwide scaling could possibly result, reconfiguring subnets in various ways. Even so, scaling any single component will inevitably tend toward a decrease in the number of resources dedicated to providing computational services as compared to the number of resources spent on wiring in the new, larger component.

At large scales, the increase in the number of subnets causes another kind of fragmentation of net structure when engaged in the same tasks. To maintain a relatively stable and low number of average hops, it is necessary for subnets to be directly connected to a number of other subnets that is proportional to the total number of nodes in the pool. This means as the pool grows larger, subnets will on average experience an increase in the number of other subnets to which they are directly connected. Ordinarily, downstream subnets converge on a single input layer, and upstream ones receive their input for multiple terminations of the same output layer. However, it may be the case that not all downstream subnets are sending information in the same encoding, or it may also be the case that some downstream subnets are transmitting information that could clobber other senders' output. In both cases, there is a need for a dedicated set of interfaces to the same subnet, such that input from different subnets may be coming in on different nodes.

As the number of nodes in a pool grows, so too does the likelihood that within any given set of

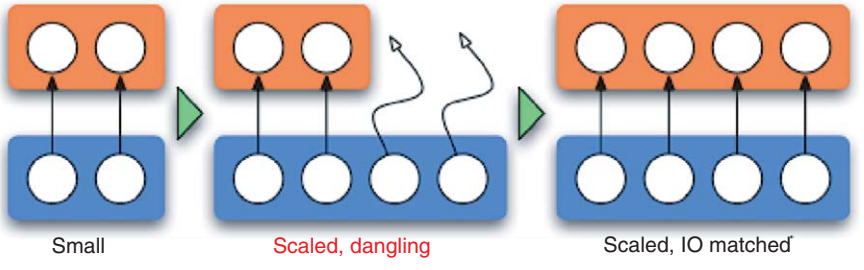

Figure 9 Diagram of the cascade of the magnitude of input/output (IO) units within a neural net.

subnets connected to the same subnet, some of the members of the set will require their own dedicated interface on the subnet to which they are connected. The result is a larger number of neurons in the interface layers and a smaller number of neurons in the hidden layers, increasing the number of nodes involved in IO, leaving fewer nodes available for actual computation. This phenomenon, like IO matching, makes subnets less efficient as the pool to which they belong scales up.

We suspect that simply inserting more neurons between the input and output neurons to improve processing power is not a viable way around the cascading scale effect, though we intend to investigate this kind of manipulation in future work. First, some subnets may already exist that cannot be significantly upgraded because they are at or close to the apex of the resource consumption to the computational power ratio. Secondly, certain important types of subnets, such as those that store memory, would require an increase in the IO layer to provide greater capacity, and more memory requires a larger interface to address that memory. Here too, the larger interface would trigger a cascading scale effect; any subnet interested in storing or retrieving information would need the ability to handle the memory's address space.

We believe that the decrease in both task performance and the number of modules as the result of grafting superscaled modules suggests why evolving brains scale in a coordinated manner. When a system is presented with a scaled-up component, it undergoes a systemwide reorganization that both changes underlying intermodule wiring strategies and the number and function of the modules themselves. The scaling of an area of the brain such as the somatosensory strip, S1, would have a profound impact on the rest of the brain. This cortical area participates not only in somatosensory analysis, but also is activated during word-finding tasks with a somatosensory component (Pulvermuller, 2001). The underlying mechanism that drives the correlation between behavioral complexity and relative but not absolute brain size may be the scaling properties of neural networks.

3.05.4 Overview: Distinguishing Developmental and Computational Structure from Constraint

3.05.4.1 What Are the Units of Brain Architecture in Development, Evolution, and Mature Function?

Developmental and evolutionary biologists and computational scientists attempting to understand the brain are all engaged in parallel quests to describe the fundamental units of brain structure and a syntax for their interaction. Each addresses a separate kind of change in the brain, over evolution, development, or while functioning. Much is to be gained by understanding how the requirements of each aspect of brain change reinforce and constrain each other. Much confusion is the result, however, if the structure in variation of these different types of brain change is arbitrarily assumed to be the same, or if terms are exported and imported between areas of study without particular attention to their referents. The unit of a cortical area is a good example of this. In the initial stages of studying mature cortex physiology, the cortical area was viewed as an extremely important level of analysis, in that each area was hypothesized to take its input and perform a particular transform of it before passing it up to the next, from area 17 to 18, to 19, and so on (Hubel and Wiesel, 1998). As is well known, neither the circuitry (DeYoe and Van Essen, 1988) nor the functional allocation proved to be very well described this way (for example, Schiller, 1993). Although debate persists for some particular cases (for example, Gauthier *et al.*, 1999; Haxby *et al.*, 2001; Grill-Spector *et al.*, 2004), most agree that perceptual or cognitive functions of even minor complexity are rarely associated uniquely with particular cortical areas and are usually highly distributed across the cortical surface (one example each from four independent functional domains: Andersen, 1995; Duncan and Owen, 2000; Haxby *et al.*, 2001; Pulvermuller, 2001). So, while the cortical area remains an extremely important aspect of cortical syntax, containing topographically ordered sensory and computed maps and highly constrained thalamocortical and downstream connectivity, comparison of present box diagrams of information flow for disparate functions would show much overlap and subdivision.

The researchers examining the developmental neurobiology of the cortex also began by concentrating on the cortical area, the holy grail of this enterprise to determine if particular cortical areas were genetically unique in the absence of patterning input. The essential hypothesis to be tested was whether the structure of mature cortical areas could be identified in the initial deployment of cells. Thus, researchers were very interested to determine if location in the cortical germinal zone was faithfully transmitted to the mature cortex, to determine if the nature of cortical areas could be fixed at the time of neurogenesis (Austin and Cepko, 1990; Rakic, 1990), or whether the properties of cortical areas could survive embryonic transplant

(O'Leary and Stanfield, 1989) or thalamic rewiring (Pallas, 2001). Though the picture is very far from complete, we now know that the primary sensory and motor areas may be identified by the graded pattern of gene expression that serves both to organize the polarity of cortical maps and direct early thalamocortical and intracortical connectivity (Ragsdale and Grove, 2001). Whether any comparable specification exists for areas other than the primary sensory and motor regions is not known, but it appears unlikely, and even within the most specified regions, much residual plasticity is retained (Kingsbury and Finlay, 2001). Developmental neurobiology has therefore produced evidence for unique specification of only a few of the multiple areas that are seen in the mature brain.

Comparative neurobiologists found evidence for a conserved set of primary regions across mammals (Krubitzer, 1995), but as this paper has argued, there is reasonable evidence that cortical areas proliferate predictably as a function of cortex size, and very little evidence to suggest that the relative size or number of cortical areas is related to niche-specific requirements or special adaptation (Nudo and Masterton, 1990; Kaskan *et al.*, 2005). We suggest that the comparative evidence is consistent with essentially three types of cortical areas, a set of primary sensory regions that are genetically specified and conserved across mammalian brains, a set arising from axonal sorting interactions in relatively small brains and similar to other features of local cortical topography such as ocular dominance columns and cytochrome oxidase blobs, and a final set arising from some unknown mechanism at very large scales.

The relationship of the concept of a cortical area in evolution, development, and mature function is subtle, important, and complex, but it is no longer the simple concept that the cortical area is the computational unit of the functioning cortex, the genetically specified fundamental unit of the developing cortex, and the selected-on unit of cortical evolution. Are there other candidates for fundamental units of brain development and evolution?

The developmental radial unit and its outcome in the mature brain (Rakic, 1990), the cortical column, is certainly a central aspect of the story, but our first explorations in the computational aspects of scaling suggest we might look for units at an intermediate level of analysis. Subnets emerged in neural nets trained on generic tasks, revealed by their relatively greater interconnectivity. These subnets have properties in computer simulations that demonstrate how cascading IO scaling is also likely to be a problem in real-world brain scaling. Subnets may be an essential unit of a neural wiring strategy, using modularity at a micro scale, where clusters of neurons implement logical units of computation, rather than the higher-level behaviors that have been the focus of most modular accounts of the brain. The demonstration of similar subnet structure in the brain and investigation of their scaling properties in brains of different sizes would be useful: possible candidates might be like the axonal architectures that link the cortical representations of extended visual contours or other spatial structures that can appear either early in development (Fitzpatrick, 2000) or later as a result of learning (Gilbert *et al.*, 1996).

A particular subnet need not be restricted to use within a single class of behavior; it can be used concurrently across different and functionally unrelated subnets. To grow larger, a subnet would recruit neurons from subnets with which it already shares neurons. Conversely, to contract, a module would relinquish neurons shared with neighboring micromodules increasing the number of neurons dedicated to a single module. Subnets might also be replicated across the brain, especially when it is cheaper to replicate than connect; the probability a subnet will replicate is inversely related to its size and directly related to the number of other subnets that depend on it.

3.05.4.2 Allometric Constraints May Show Us the Scaling Properties of Brain Networks

At this point, we may turn around to look at some of the most highly conserved aspects of mammalian brain scaling and raise them as potential points of interest for their network scaling properties. At the most basic level of proliferation, the change in number of motor neurons will be less than the proliferation of secondary sensory neurons as brains enlarge. Why?

Looking at the proliferation of the thalamus, it is quite notable that the primary sensory regions, the lateral geniculate, medial geniculate, and ventrobasal nuclei have their terminal birth dates earliest and proliferate at a greatly reduced rate compared to the rest of the thalamus and, of course, with respect to the cortex. For the pulvinar and frontally connecting regions of the thalamus, neurons are even conscripted in large brains from new regions to expand their size. Our initial work would suggest that the primary thalamic regions might be kept small, perhaps even to some detriment to acuity, to minimize the number of neurons wasted on IO functions and allow the proliferation of large numbers of

reusable subnets (Gilbert *et al*., 1996). Finally, it is clear that the size of large regions of the brain, cortex, olfactory bulb, hippocampus, and cerebellum may enlarge differentially, usually at a rate characteristic of whole radiations. How does the wiring between large regions stay exempt from the scaling and wiring constraints we have described thus far?

3.05.4.3 Where Do We Look for Species-Specific Adaptations in the Brain?

We have described several kinds of sources for species differences in the brain already, including the residual variation left after allometrically predictable variation is taken into account, the obvious possibility of modifications of neuronal architecture and function, and our two-hit model for placing new functions in areas destined to become disproportionately large in large brains by virtue of their developmental placement. We end with a plea that further investigation of the nature of brain scaling and neuroanatomical evolution abandon models of brain function that have not been used in sophisticated analysis of brain function for the last 25 years, and consider the network structure of the brain, and how computations might be deployed over networks of different scales. Hypothesis development in this next phase of understanding brain evolution will require that the hypotheses be explicit about the nature of networks and the nature of the computations embodied there.

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3.06 Mosaic Evolution of Brain Structure in Mammals

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Glossary

allometric	Changes in the quantitative value of a trait (e.g., brain size) with changes in some other measure of size (e.g., body mass). Positive allometry refers to a relationship in which the trait changes more rapidly than size (e.g., as in neocortex size relative to brain size); negative allometry refers to a relationship in which the trait changes more slowly than size (e.g., brain size relative to body size).
correlated evolution	The tendency of traits to change together with statistical predictability across independent evolutionary events.
grade differences	A significant difference between phylogenetic groups in the quantitative value of a trait after controlling for another variable (usually size).
haplorhine	The phylogenetic suborder of primates that includes apes, monkeys, and tarsiers.
insectivore	A taxonomically varied grouping of mammals including the order Insectivora (shrews, hedgehogs, and moles) and tenrecs.
orbital convergence	The degree to which the orbits face in the same direction.
partial correlation	The correlation between two variables with the effects of variation in other variables held constant.
strepsirhine	The phylogenetic suborder of primates that includes lorises and lemurs.
taxon (pl taxa)	A grouping of organisms according to their placement within a system of hierarchical classification, usually based on phylogenetic relationships.

3.06.1 Introduction

Organisms tend to evolve in a coordinated fashion. Hence, as overall size increases, so does the size of limbs, muscles, tendons, and internal organs, and this is necessary for the organism to function efficiently. A large proportion of the variance in the size of individual body parts is therefore in some sense explained by overall size. On the other hand, parts also vary independently of overall size: "The concept of mosaic evolution dictates that organs will evolve in different ways to meet varying selective pressures" (Gould, 1978, p. 66). For example, whereas the size of the testes correlates closely with the size of other organs and overall body size, testes of promiscuously mating species are large relative to body size as an adaptation for sperm competition (Harcourt *et al.*, 1996). As with the organs of the body, individual brain components may vary in adaptively specialized ways, independently of variation in whole brain size. Unlike bodily organs, however, brain components have extensive neural interconnections to support the integrated processing of information, potentially limiting the importance of mosaic evolution. Nevertheless, individual components are grouped within structurally and functionally differentiated neural systems specialized for handling particular cognitive operations, upon which natural selection might act at least partly independently of evolutionary change in other systems:

> . . . a relatively large brain may result from differential increase in the size of the cortical mass, subcortical structures, or the cerebellum. . .It is axiomatic that those areas of the brain that do increase in size are often related to specializations in certain sense organs, and correlate with the collection, storage and retrieval of specific kinds of data relevant to the niches the animals exploit (Eisenberg, 1981, p. 276).

Comparative tests of this hypothesis are of three broad types, each of which examines variation in the size of individual components after accounting for the effects of overall brain size or of the size of other brain components: tests for taxonomic differences in component size, tests for correlated evolution between separate components of the same functional system,

and tests for correlated evolution between brain components and behavioral ecology. Most analyses of these issues use the invaluable data on brain structure volumes produced by Stephan *et al.* (1981, 1991). Because many of the components measured by Stephan *et al.* are anatomically and functionally heterogeneous, and because functional systems are distributed across these components, they tend to show strong patterns of covariation (Finlay and Darlington, 1995). For example, the visuomotor system includes nuclei in the thalamus, pons, cerebellum, and neocortex, so natural selection on visuomotor abilities may have caused coordinated evolution of all four structures as well as the fiber tracts connecting them. This means that analyses must be capable of distinguishing between patterns of coordinated evolution resulting from selective size change in distributed neural systems and patterns of coordinated evolution resulting from developmental processes (see Cortical Evolution as the Expression of a Program for Disproportionate Growth and the Proliferation of Areas).

3.06.2 Taxonomic Differences in Relative Size of Brain Structures

A prominent feature of brain structure variation in mammals is the extent of variation in the size of the neocortex. In humans, the neocortex comprises approximately 75% of total brain volume, whereas in some shrews the equivalent figure is under 10% (Frahm *et al.*, 1982). However, the presence of a proportionately large neocortex in large-brained species is not in itself evidence for selection specifically on the neocortex, because the size of the neocortex scales with positive allometry relative to other brain parts (Frahm *et al.*, 1982; Finlay and Darlington, 1995). This allometric relationship appears to be due to the scaling of cortical connectivity and therefore of the white matter component of the neocortex (Frahm *et al.*, 1982; Ringo, 1991; Zhang and Szenowski, 2000; Barton and Harvey, 2000) (see Scaling the Brain and Its Connections). Nevertheless, statistically significant differences in neocortex volume between taxa are apparent even after taking allometric scaling into account. Compared with insectivores, primates have a relatively large neocortex, and within primates, haplorhines have a larger neocortex than do strepsirhines (Barton and Harvey, 2000). After controlling for the size of other brain structures, the neocortex is on average approximately five times larger in primates than in insectivores, with some comparisons yielding 10-fold differences (Barton and Harvey, 2000). The differences in neocortex size are mirrored by differences in the size of visual cortical regions relative to the rest of the neocortex (Barton, in preparation) and by differences in the volume of a subcortical visual structure, the lateral geniculate nucleus (Barton, 1999), suggesting that visual specialization may underlie neocortical evolution in primates (see below) (see The Evolution of Visual Cortex and Visual Systems).

The mosaic nature of change in brain proportions during primate evolution is clearly apparent when the sizes of major components are plotted against body mass (Figure 1; see also Barton, 2000). Some

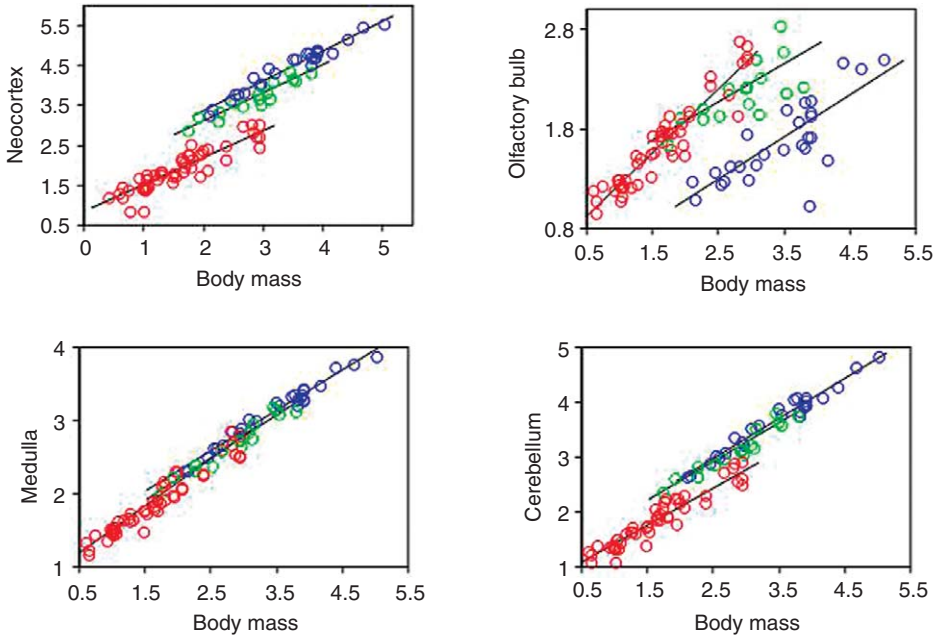

Figure 1 Taxonomic differences in the volume of four brain structures. Log-transformed structure volumes are plotted against log-transformed body masses. Red circles, insectivores; green circles, strepsirhine primates; blue circles, haplorhine primates.

components (e.g., the neocortex and cerebellum) show clear grade differences between taxa, whereas others (e.g., the medulla) do not. In addition, there are different grade effects for different structures: both neocortex and cerebellum size are greater in primates than in insectivores, but only neocortex size differentiates between strepsirhine and haplorhine primates. On the other hand, cerebellum size is greater in apes than in other catarrhines (Rilling and Insel, 1998) and the cerebellum is also enlarged in some cetaceans (Marino *et al.*, 2000). The main olfactory bulbs are larger in strepsirhine primates and insectivores than in haplorhine primates, a difference that is at least partly associated with adaptive radiation into nocturnal and diurnal niches (Barton, 2006). Finally, comparison of individual structures in species with similar brain sizes reveals the extent of system-specific size change. The superior colliculus is approximately 10 times larger in ground squirrels than in rats, despite the overall similarity in brain and body size (Kaas and Collins, 2001).

An important methodological point arising from the patterns of mosaic evolution illustrated in Figure 1 is that correlation coefficients and regression slopes computed across species without regard to phylogenetic effects are incorrect (Nunn and Barton, 2001). For example, the slopes computed across phylogenetic grades will give the impression that the size of the neocortex scales with a much steeper exponent than is the case for other structures, but this is largely an artifact of the evident phylogenetic effects (Barton and Harvey, 2000). This is important, because scaling exponents have been taken as being indicative of developmental mechanisms limiting mosaic brain evolution (Finlay and Darlington, 1995).

3.06.3 Correlated Evolution between Components of Functional Systems

If specific neural systems were targets of natural selection, the components of such systems, which may be distributed across major brain subdivisions, should have changed in size together, but independently of change in other systems. Tests for correlated evolution use phylogenetic comparative methods such as independent contrasts (Felsenstein, 1985; Harvey and Pagel, 1991). Correlated evolutionary change in functionally linked brain components, independent of change in other structures, is indicated by significant multiple regression coefficients or partial correlations between contrast values. For example, contrasts in visual cortex size correlate significantly with contrasts in lateral geniculate nucleus (LGN) size, holding constant the effects of variation in other brain components (Barton *et al.*, 1995), indicating that the visual system has evolved independently of overall brain size (see The Evolution of Visual Cortex and Visual Systems). In general, partial correlations between structures within neural systems seem to be ubiquitous: among a range of brain systems, anatomical and functional connections closely predicted significant positive partial correlations between pairs of structures in two mammalian orders (Barton and Harvey, 2000). In some cases, it has been possible to test the prediction of correlated evolution at the level of individual nuclei. In the cortico-cerebellar system, connected nuclei in the hind-, mid-, and forebrain have evolved together (Whiting and Barton, 2003). Of the four main vestibular nuclei and three cerebellar nuclei, the two that have direct connections (the lateral vestibular and middle cerebellar nuclei) are those that show correlated evolution after controlling for the size of other structures (Figure 2). Hence, anatomical connections

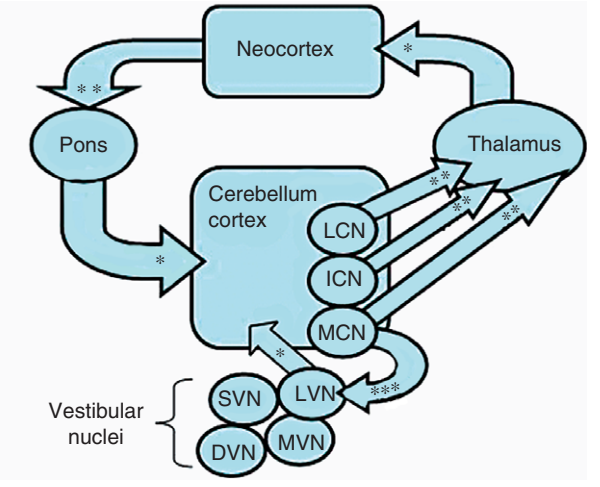

Figure 2 Correlated evolution among components of the cortico-cerebellar-vestibular system. The diagram summarizes analyses presented in Whiting and Barton (2003). Independent contrasts in the volume of each component were regressed on volume contrasts in connected components and in other brain structures. Asterisks represent significant partial regression coefficients between contrasts in the connected components, controlling for other brain structures. For example, the pons and neocortex exhibit significantly correlated evolutionary change, controlling for variation in the rest of the brain. The two nuclei in the cerebellum and vestibular complex that have direct anatomical connections (MCN and LVN) correlate even after accounting for variation in other cerebellar and vestibular nuclei, indicating that functional anatomy predicts correlated evolution at a detailed level. MCN, medial cerebellar nucleus; ICN, interposed cerebellar nucleus; LCN, lateral cerebellar nucleus; LVN, lateral vestibular nucleus; SVN, superior vestibular nucleus; DVN, descending vestibular nucleus; MVN, medial vestibular nucleus. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

predict detailed patterns of correlated evolution. The presence of connections between nuclei across major subdivisions of the brain explains why those subdivisions have tended to evolve in a coordinated fashion (Finlay and Darlington, 1995; Barton and Harvey, 2000).

The fact that brain nuclei co-vary in accord with their functional connections can be used to help resolve uncertainty among neuroscientists about aspects of brain organization. Comparative analysis may therefore contribute to systems neuroscience. One example concerns debate about the structural and functional integrity of the amygdala. On the basis of anatomical, pharmacological, and electrophysiological data, Swanson and Petrovich (1998) called into question the existence of the amygdala as a structurally and functionally coherent unit, arguing that the term amygdala refers to a collection of disparate nuclei that are really parts of different neural systems. Some other researchers, however, have drawn attention to the dense connections between nuclei within the amygdala (see Aggleton, 2000). The problem lies in deciding what constitutes sufficient evidence to conclude that the amygdala either does, or does not, exist as a structurally and functionally integrated entity, hence breaking the cycle of claim and counterclaim based on affinities among nuclei and differences between them, respectively. The crux of the matter is how such nuclei evolved. Like all complex biological traits that function conspicuously well, neural systems exist by virtue of the fact that they evolved by the process of natural selection (Young *et al*., 2000). Therefore, the question of whether particular components constitute parts of a unified neural system or structure is essentially an evolutionary question: did they evolve together, in a coordinated fashion, and in a way that cannot be attributed merely to their integration within a larger, more global system (e.g., the limbic system, or even the brain as a whole)? Phylogenetic analysis of comparative data in primates and insectivores clearly shows that the amygdala coheres as an evolutionary unit: after controlling for variation in a range of other brain structures, including other limbic structures, separate groups of nuclei in the amygdala show significantly correlated evolution (Barton *et al*., 2003), hence refuting the claim that they are parts of entirely different neural systems (see Evolution of the Amygdala in Vertebrates).

To what extent can the evolution of central neural structures be linked to specializations in certain sense organs (Eisenberg, 1981)? Analysis of data on the primate visual system supports the hypothesis that various features of the morphology of peripheral visual structures, including the degree of orbital convergence, size of the eyes, the density of retinal ganglion cells, and the size of the optic nerves, correlate with the relative size of the visual cortex (Barton, 2004; Barton, in preparation). Similarly, among insectivores, species differences in the organization and fine structure of cortical areas are associated with differences in peripheral sensory specializations (Catania, 2000).

3.06.4 Correlated Evolution between Individual Components and Behavioral Ecology

Comparative studies have begun to reveal the ecological correlates of mosaic brain evolution in mammals. In primates, visual system evolution and olfactory system evolution are both associated with ecology, though in different ways (Barton *et al*., 1995; Barton 1996, 1998, 2006, in preparation). Diurnality is associated with increased relative volume and cell number in parvocellular, but not magnocellular, layers of the LGN, and decreased olfactory and accessory olfactory bulb volume (Barton *et al*.,1995; Barton, 1998, 1999). The evolutionary dissociation between parvo- and magnocellular pathways accords with experimental data on functional segregation within the visual system and on behavioral attributes of different species. Parvocellular projections to the visual cortex mediate fine-grained, acute and color vision, abilities that are characteristic of diurnal primates (Allman and McGuinness, 1988; Allman, 1999). Parvocellular LGN size and visual cortex size also correlate positively with the degree of frugivory and with social group size, independently of the correlation with activity period (Barton 1998, 1999), suggesting that visual system evolution may have been affected by several aspects of lifestyles. The size of the accessory olfactory bulbs, which mediate pheromonal processing, are, as expected, more closely associated with social variables, such as group size (in platyrrhines) and spatial dispersion (in strepsirhines) than with ecological variables such as diet (Barton, 2006). Correlated evolution between individual brain structures and behavioral ecology has also been documented in other mammalian taxa. In carnivores, relative olfactory bulb size correlates positively with home range size and negatively with aquatic habits (Gittleman, 1991). In bats, wing area and habitat complexity correlate with the relative size of the auditory inferior colliculi (among echolocating species) and with relative hippocampus size (Safi and Dechmann, 2005).

3.06.5 Explaining Brain Size as a Function of Mosaic Evolution: Visual Specialization in Primates

The functional significance of brain size has been extensively debated (e.g., Jerison, 1973; Holloway, 1974; Striedter, 2005). In general, larger brains contain more neurons and synapses and are therefore in some sense computationally more complex than smaller brains. Support for the idea of mosaic brain evolution suggests that we can be more specific and describe at least the broad types of information processing that have been enhanced during brain expansion (or compromised during brain size reduction) in particular lineages. Primates again provide an example. In addition to being large-brained, primates are visually specialized. These visual characteristics include the following: a high degree of orbital convergence associated with stereoscopic vision; a concentration of ganglion cells in the central retina; a greatly expanded representation of the central field in visual cortex; a distinctive pattern of projections between eye and brain; a distinctly laminated LGN within which information from the same hemifield of each retina is brought into visuotopic register in separate layers, before converging on numerous single binocular neurons in the visual cortex; and relatively numerous and extensive visual cortical regions (Allman and McGuinness, 1988; Allmann, 1999). This suite of visual features was a fundamental component of the adaptive shift in the evolution of the first primates, but there is variability in most or all of these features within the order. This interspecific variability correlates with overall brain size. The degree of orbital convergence and the relative size of the LGN and primary visual cortex correlate with overall neocortex size and brain size relative to body size (Barton, 1998, 2004). Hence, the evolution of large neocortices and large brains relative to body size was associated with disproportionate expansion of the visual system. Thus far, this is the only documented case in which size variation in a specific neural system has been related to variation in brain size. Motor systems may also be implicated: the evolutionary expansion of the neocortex correlates with increases in cerebellum size, independently of overall brain size (Whiting and Barton, 2003). Given the role of the cerebellum in the sensory control of movements, these results may together indicate that brain size evolution in primates is associated with enhancements of fine visuomotor control. Broader understanding of mammalian brain size evolution will require investigation of mosaic size change across a variety of neural systems and phylogenetic lineages.

3.06.6 Conclusions

The components of any adaptive complex, such as the mammalian brain, by definition undergo coordinated evolution. At the same time, functional differentiation of neural systems within the mammalian brain potentially creates scope for a mosaic pattern of evolutionary change overlying the allometric covariation of individual components. Comparative analyses unequivocally show that specific systems and structures have evolved in part independently of change in overall brain size and that such system-specific change correlates with behavioral ecology. The fact that patterns of correlated evolution predict anatomical and functional relationships means that phylogenetic comparative analysis can be used to test hypotheses about brain organization. The increase in neocortex and overall brain size during primate evolution is associated with disproportionate expansion of the visual system and with other aspects of visual specialization, such as increased orbital convergence, as well as with cerebellar expansion. Hence, primate cognitive specialization may be based on the elaboration of visual and visuomotor systems.

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3.07 Developmental Studies on Rewiring the Brain: What They Tell Us about Brain Evolution

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Glossary

arealization	A process by which neuroectoderm that will give rise to the cerebral cortex is partitioned into anatomically and functionally distinct areas.
cerebral cortex	A telencephalic structure where learning, memory, reasoning, and sensory perception occur.
lateral geniculate nucleus (LGN)	A subdivision of the thalamus that normally processes and relays visual information.
medial geniculate nucleus (MGN)	A subdivision of the thalamus that normally processes and relays auditory information.
thalamus	A diencephalic structure that processes and relays information from the senses to the cortex.
transcription factor	A molecule that regulates the transcription of other genes.

3.07.1 Introduction

Understanding neural development can inform us about how a brain structure evolves. By examining the development of a cortical region, one can elucidate the role that molecular cues and afferent inputs play in determining the evolution of a cortical structure, its function, and associated behaviors. Cross-modal experiments provide insight through a gain-of-function approach, whereby inputs of one sensory system are redirected to a different sensory modality. This allows the role of intrinsic and extrinsic factors to be distinguished and their relative contribution to cortical structure, function, and behavior to be determined. Here we will discuss how molecular cues in early development may influence the evolution of a cortical structure, as well as how rewiring visual inputs to innervate the auditory pathway provides insight into the role of patterned electrical activity as a key extrinsic factor determining the ultimate organization and function of a structure (see A History of Ideas in Evolutionary Neuroscience, Relevance of Understanding Brain Evolution).

3.07.2 Early Development and the Evolution of Cortical Structure: Molecular Influences on Cortical Arealization

The mammalian cerebral cortex develops as a continuous sheet of cells that is divided into anatomically and functionally distinct areas during the course of development (see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates). This results in a pattern of cortical areas that is generally consistent among individuals within a species but varies between species, especially with respect to size and areal position (Krubitzer and Kahn, 2003). Influence over the pattern of these areas can potentially come from two sources: extrinsic sensory input, which reaches the cortex in an area-specific manner, and intrinsic molecular cues, which are encoded in the genome and expressed during development. Identifying the contributions of each of these is essential for understanding the mechanisms underlying the formation and diversification of the cerebral cortex. The first indication of arealization in the developing cortex is regional gene expression (Rubenstein *et al.*, 1999). Two studies have used mice in which thalamocortical axons fail to reach the cortex to examine the effect of blocked sensory input on early arealization

(see The Role of Transient, Exuberant Axonal Structures in the Evolution of Cerebral Cortex). Interestingly, in these mice (*Gbx2*$^{-/-}$ or *Mash1*$^{-/-}$), the initial expression of regionally expressed cortical genes appears normal (Miyashita-Lin *et al.*, 1999; Nakagawa *et al.*, 1999). This indicates that patterned gene expression occurs independent of extrinsic factors and argues that cortical pattern formation may be investigated using a logic similar to that used to study pattern formation in other embryonic tissues.

Identifying interactions among networks of transcriptional factors and signaling pathways has been critical in understanding the basic mechanisms of embryonic patterning (Wolpert, 2002). While a variety of transcription factors have been implicated in cortical arealization (O'Leary and Nakagawa, 2002; Sur and Rubenstein, 2005), one of the most heavily studied is Emx2. This molecule is expressed in a gradient across the caudal cortex, and a loss of Emx2 function results in an expansion of the rostral cortical markers at the expense of markers for the caudal cortex (Bishop *et al.*, 2000). Constitutive overexpression of Emx2 in mouse cortical precursors using *nestin cis*-regulatory elements is capable of altering both the size and position of primary sensory areas, and this change is complementary to that seen when Emx2 function is reduced (Hamasaki *et al.*, 2004). Intercellular signaling also appears to be involved in arealization, with the most well-studied pathway being the Fgf receptor (Fgfr) pathway. Fgf8, which is a secreted ligand in this pathway, is expressed in a high-rostral, low-caudal gradient across the mouse cortex during development. One study has shown that Fgf8 is capable of changing the location and patterning of mouse cortical areas when ectopically expressed early in embryogenesis (Fukuchi-Shimogori and Grove, 2001). Furthermore, mice homozygous for a hypomorphic Fgf8 allele show rostral shifts in the expression of arealization marker genes (Garel *et al.*, 2003), and projections that originate in the caudal cortex project ectopically into the rostral cortex (Huffman *et al.*, 2004). Fgf8 and Emx2 appear to interact with one another during cortical development (Fukuchi-Shimogori and Grove, 2003; Garel *et al.*, 2003; Hamasaki *et al.*, 2004), although the nature of this interaction awaits clarification.

Thinking about cortical arealization as a network of molecular interactions can help us understand how changes in cortical properties may have arisen in evolution. Subtle mutations at the genomic level may result in alterations in molecular interactions that can lead to profound phenotypic changes when amplified over the course of development. This, in turn, provides a mechanism for bringing about gross phenotypic change in evolution (reviewed in Pires-daSilva and Sommer, 2003). It is possible to imagine, for example, that a mutation causing a subtle change in expression level or binding affinity in a patterning molecule such as Fgf8 may result in a significant change in cortical arealization, which would then be subject to evolutionary selective pressure. This can also help us understand how the wiring of sensory input into the cortex may change during evolution. In addition to mediating early arealization, intrinsic factors also function in establishing thalamic input into the cortex (Lopez-Bendito and Molnar, 2003). For example, loss of function of the transcription factors Pax6 and Emx2 disrupts normal thalamocortical pathway development (Bishop *et al.*, 2000; Hevner *et al.* 2002; Jones *et al.*, 2002), and the Ephrin signaling pathway also appears to be involved in targeting thalamic projections into the cortex (Gao *et al.*, 1998; Vanderhaeghen *et al.*, 2000). Thus, molecules involved in establishing thalamocortical connections may act as substrates for evolutionary changes in cortical wiring.

3.07.3 Development of Structural Organization: Sensory Influences on Cortical Organization and Connectivity

Although early patterned gene expression in the cortex appears to occur independent of extrinsic factors such as the amount and pattern of electrical activity in input pathways, these can exert a substantial influence at both early and late stages of development and provide insight into the evolution of a cortical structure. For instance, while ocular dominance columns are present before eye opening (Rakic, 1976; Crowley and Katz, 2000; Crair *et al.*, 2001), the existence of retinal waves of spontaneous activity suggests that electrical activity generated within the developing brain may also contribute to the early establishment of central visual connections (Meister *et al.*, 1991; Wong *et al.* 1993).

Subsequently, visual experience plays a vital role in shaping the organization and connections of visual cortex. A balance of activity between the eyes appears to be essential for normal cortical development during the critical period, when visual experience has its maximal effect, with disruptions

to the amount or the pattern of electrical activity in only one eye having the greatest impact on cortical structure. For instance, monocular deprivation during the critical period induces robust changes in the anatomy and physiology of visual cortex (Wiesel and Hubel, 1965; Hubel *et al.*, 1977; LeVay *et al.*, 1980). In contrast, binocular deprivation during the critical period has little influence on ocular dominance columns, indicating that the balance of activity rather than the absolute level of activity is critical for the formation of intracortical connections during the critical period (Crowley and Katz, 1999). Even though these loss-of-function experiments suggest that input activity has a profound influence on the organization of the cortex, they cannot distinguish between the contributions made by the overall amount of activity and the specific pattern of activity in inducing these cortical changes.

Artificially induced strabismus, which alters the spatial correlation between the two eyes but not the overall level of activity, causes ocular dominance columns within primary visual cortex to become exclusively monocularly driven (Lowel and Singer, 1992). More generally, gain-of-function paradigms, such as the one used in rewiring experiments, allow separation of the relative influence of patterned visual activity from that of the amount of activity or of intrinsic factors in specifying the function and organization of a cortical area. In these experiments, visual input is redirected to the auditory pathway by inducing retinal ganglion cell axons to innervate the medial geniculate nucleus (MGN) through surgical removal of its normal inputs at birth (see Figure 1). This creates an alternative target for retinal axons and allows functional connections to form in the auditory thalamus, conveying visual information through existing thalamocortical connections to primary auditory cortex (A1). Such rerouting has been done in mice (Lyckman *et al.*, 2001), ferrets (Sur *et al.*, 1988; Roe *et al.*, 1990, 1992, 1993; Sharma *et al.*, 2000), and hamsters (Schneider, 1973; Kalil and Schneider, 1975; Frost, 1982; Frost and Metin, 1985).

Retinal axons do not enter the MGN during normal development or in adulthood, but do so when the MGN is deafferented during an early developmental window. Molecular characterization of the denervated MGN indicates that removal of normal inputs upregulates molecules that promote sprouting in other systems, presumably attracting axons of the optic tract to branch and innervate the nucleus (Ellsworth, 2004). Remarkably however, once retinal axons enter the MGN, they pattern themselves in a manner similar to that in their major thalamic target, the lateral geniculate nucleus (LGN; see Figure 2a). Indeed, experiments examining the patterning of

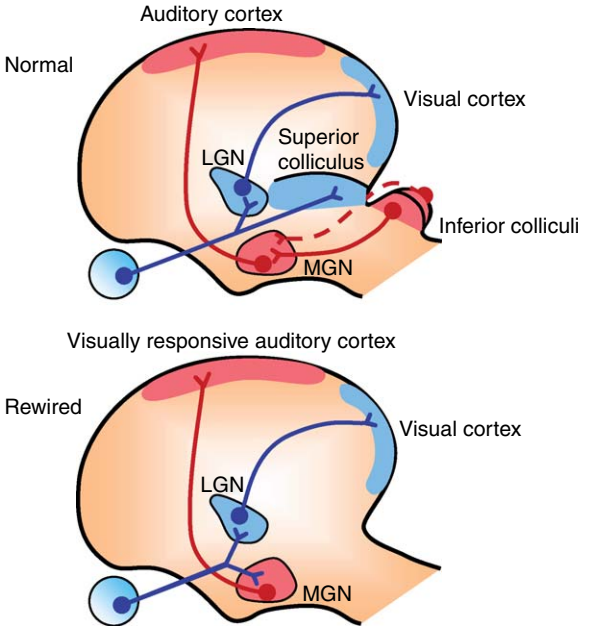

Figure 1 Schematics of the principal visual (blue) and auditory (red) pathways in normal (upper panel) and rewired animals (lower panel). LGN, lateral geniculate nucleus; MGN, medial geniculate nucleus. Modified from Sur, M., and Rubenstein, J. R. 2005. Patterning and plasticity of the cerebral cortex. *Science* 310, 805–810.

retinal innervation in the LGN and the rewired MGN suggest that the same intrinsic molecules facilitate pattern formation in both nuclei. In the normal mammalian visual pathway, retinal input is organized retinotopically in the LGN and is segregated into discrete eye-specific regions. A similar eye-specific segregation occurs in the rewired MGN (Ellsworth *et al.*, 2005), with input from the right and left eye showing little overlap (Figure 2b). There appears to be conservation of patterning molecules across sensory modalities, with molecules such as the ephrins and their respective Eph receptors expressed in multiple gradients throughout the developing thalamus, including the LGN, MGN, and ventrobasal (VB) nucleus. In addition, in ephrin knockout mice, this eye-specific patterning is disrupted similarly in the LGN and rewired MGN (Figure 2a; Ellsworth *et al.*, 2005). Thus, the ephrins appear to shape rewired retinal projections in the same way they influence normal LGN patterning. Therefore, ephrin expression in the MGN and throughout the auditory pathway may provide a scaffold as well as impose target-derived constraints on the extent to which connections along the auditory pathway are shaped by visual input.

Nevertheless, visual activity, which has a very different spatial and temporal pattern than auditory activity, leads to visual responses in

rewired A1 that resemble responses in primary visual cortex (V1) (Sur *et al.*, 1988; Roe *et al.*, 1990, 1992; Sharma *et al.*, 2000). Extracellular electrophysiology and optical imaging of intrinsic signals find that neurons in rewired A1 develop visual response features such as orientation and direction selectivity (Roe *et al.*, 1992; Sharma *et al.*, 2000) as well as an orderly retinotopic map (Roe *et al.*, 1990). That is, rewired A1 neurons are selective for different attributes of a visual stimulus such as a direction of stimulus motion, a particular line orientation, or a retinotopic location (size and receptive field location in visual space) of the stimulus. Each neuron has a slightly different preference for these features compared to its neighbors, such that a coherent stimulus feature map develops in rewired A1, similar to the one found in V1. Optical imaging experiments reveal that a systematic map of orientation information develops in rewired A1, which is similar to the orientation map found in V1 (Sharma *et al.*, 2000). This orientation map in rewired A1 contains iso-orientation domains, where the neurons all respond to the same preferred orientation, organized around pinwheel centers (Figure 3).

In addition to influencing the organization of visual response feature maps, the visual inputs directed to rewired A1 also shape its local and long-range

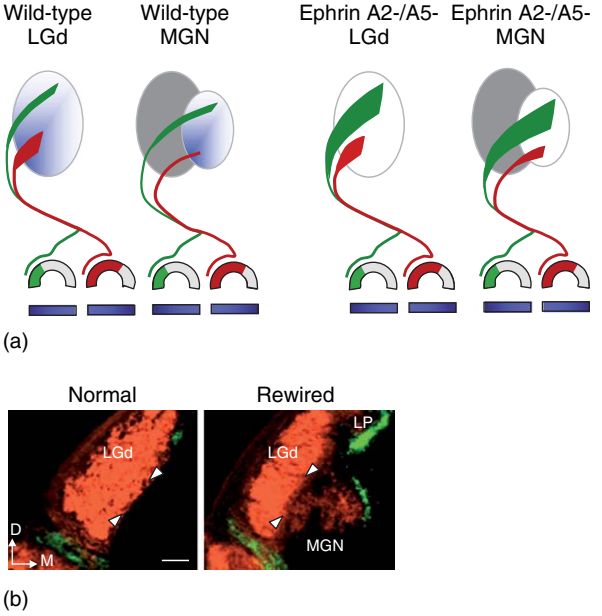

Figure 2 Target-mediated cues influence normal and novel retinal projections. a, Schematic representation of Eph–ephrin interactions in the retina and retinal targets in wild-type and ephrin knockout mice. Contralateral projections are labeled in red; ipsilateral projections are labeled in green. Ephrin expression is represented by the blue gradient within the target nuclei, while Eph receptor expression is depicted by the blue gradient under each retina. Left, in wild-type mice, ipsilateral axons arise from the temporal retina and express high levels of EphA receptor. As a result, these axons target regions of the dorsal lateral geniculate nucleus (LGd) with low ephrin-A expression. A similar ephrin gradient is also apparent in the MGN. As a result of these parallel ephrin gradients, eye-specific projections are similar in the LGN and rewired MGN: in rewired wild-type mice, ipsilateral retino-MGN projections target regions of the MGN with low ephrin expression. Right, in ephrin-A knockout mice, ipsilateral axons still show high EphA-receptor expression but target broader regions of the LGN and MGN. Ipsilateral axons spread ventrally in both the LGN and rewired MGN of ephrin knockout mice. b, Representative coronal sections in a normal (left) and a rewired (right) mouse. In the rewired mouse, the retinal axons overshoot the medial boundary of the LGd and project into the MGN. Enhanced retinal projections into the lateral posterior (LP) nucleus are also seen in rewired mice. Retinal axons are labeled with alexafluor conjugated CTB. Contralateral projections are labeled red and ipsilateral projections are labeled green. White arrowheads mark the LGd/MGN boundary. Scale bar: 0.1 mm. Modified from Ellsworth, C. A., Lyckman, A. W., Feldheim, D. A., Flanagan, J. G., and Sur, M. 2005. Ephrin-A2 and -A5 influence patterning of normal and novel retinal projections to the thalamus: Conserved mapping mechanisms in visual and auditory thalamic targets. *J. Comp. Neurol.* 488, 140–151.

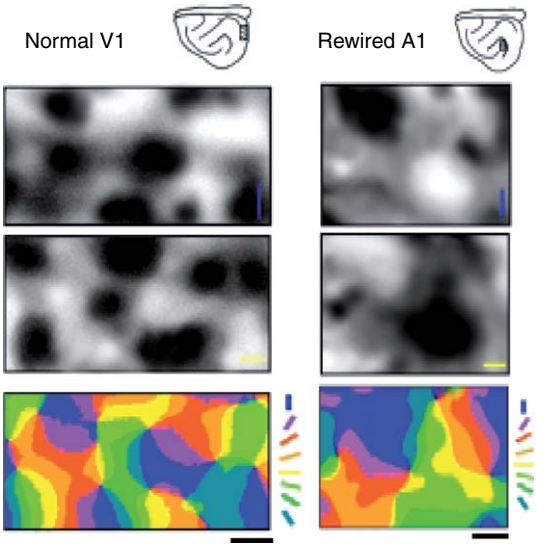

Figure 3 Orientation maps in normal visual cortex and rewired auditory cortex demonstrate the role of input activity in cortical development. Left, lateral view of normal primary visual cortex (V1) in the ferret brain. The upper two panels show activity maps in normal V1 using optical imaging of intrinsic signals in response to vertical and horizontal grating stimuli, respectively. Dark regions denote areas of high activity. The bottom map is a composite map of all orientations tested. The color key to the right of the panel shows the orientations represented. Right, lateral view of rewired primary auditory cortex (A1) in the ferret brain. The upper two panels represent the single orientation activity maps generated in rewired A1 under the same conditions as the left panels. The bottom map is a composite map of all orientations tested. The color key to the right of the panel shows the orientations represented. Scale bars: 0.5 mm. Modified from Sharma, J., Angelucci, A., and Sur, M. 2000. Induction of visual orientation modules in auditory cortex. *Nature* 404, 841–847.

connections such that they resemble connections in V1 (Sharma *et al.*, 2000). Rewired A1 neurons form connections between domains with the same orientation preference, just like V1 neurons. The patchy connections seen in V1, which are often elongated along the orientation axis of the injection site, are also observed in rewired A1 (Gao and Pallas, 1999; Sharma *et al.*, 2000). This differs significantly from the band-like connections that extend along the isofrequency axis in normal A1. At the same time, despite the similarities in the organization of visual information and connectivity of rewired A1 and V1, there are several notable differences. Rewired A1 orientation domains are larger and less orderly, as are horizontal connections relative to V1. In addition, the receptive fields in rewired A1 are larger (Roe *et al.* 1992) and spatial acuity of the rewired auditory pathway is lower than the normal visual pathway (von Melchner *et al.*, 2000). This may result from the large contribution of retinal W cell inputs to the rewired MGN (Roe *et al.* 1993). These differences may also reflect underlying structural constraints imposed by A1 that cannot be modified by experience (e.g., certain patterns of connections within and between the A1 cortical layers). Even though receptive fields and orientation domains in rewired A1 are larger than in visual cortex, these rewiring experiments provide powerful evidence that patterned visual activity plays an instructive role in the establishment of cortical connections by modifying the function and organization of a cortical area.

3.07.4 Specification of Cortical Areas: The Relationship between Inputs, Outputs, and Function

In addition to having a profound influence on cortical organization and physiology, rewired visual inputs can influence behavior. A study of unilaterally rewired ferrets suggests that patterned visual inputs influence behavior and drive the outputs of A1 (von Melchner *et al.*, 2000). Unilaterally rewired ferrets were trained to discriminate between light and sound (Figures 4a and 4b). After training, the ferrets were tested with a light presented in the rewired visual field. The normal and rewired ferrets primarily responded at the visual reward spout, an expected result given that the rewired hemisphere receives visual information through both the normal visual inputs to visual cortex and the rewired projection from the retina to the MGN to auditory cortex (Figures 4c and 4d). The normal visual projection from LGN/lateral posterior (LP) nucleus to the rewired hemisphere was then ablated, and after a period of recovery the ferrets were retested with a light presented to the rewired visual field. The rewired ferrets still responded primarily at the visual reward spout, indicating that the intact projection from the retina to the MGN to auditory cortex is capable of mediating the response to the visual stimulus (Figure 4c). The normal ferrets, however, responded at chance levels at the visual reward spout when retested after ablation of the LGN/LP and superior colliculus (SC) (Figure 4d). Finally the auditory cortex was ablated, and the rewired ferrets were again retested after a period of recovery. The rewired ferrets now responded at chance levels at the visual reward spout, indicating that the animals were no longer able to identify the visual stimulus, presumably because they were blind in the rewired visual field (Figure 4c). Thus, the rewired projection from the retina through the MGN to auditory cortex is able to mediate visual behavior and this visual input influences the behavioral function of the auditory cortex.

In addition, rewired visual projections in mice influence learned behavior mediated by subcortical pathways, such as conditioned fear (Newton *et al.*, 2004). In fear-conditioning experiments, a discrete auditory cue is paired with a mild foot shock, which induces rapid conditioned fear after as few as one tone-shock pairing (Fendt and Fanselow, 1999; LeDoux, 2000). In contrast, a discrete visual cue is less effective, requiring many more light-shock pairings to elicit a defensive response to the light alone (Heldt *et al.*, 2000). Dense direct connections from the MGN to the lateral nucleus of the amygdala (Figure 5a) are thought to be crucial for auditory cued conditioned fear responses (Rogan and LeDoux, 1995; Doron and LeDoux, 1999; see also LeDoux *et al.*, 1984; LeDoux, 2000; Namura *et al.*, 1997). In contrast to the direct auditory pathway from the MGN to the amygdala, visual inputs primarily reach the amygdala through indirect pathways (Doron and LeDoux, 1999; Shi and Davis, 2001). Visually cued conditioned fear is thought to be mediated by projections from the LGN to V1/V2 to visual association area TE2/perirhinal cortex (Pr) to the amygdala, or by projections from LP to V2/TE2/Pr to the amygdala (see Figure 5a; Shi and Davis, 2001).

A study of adult sham lesion and rewired mice suggests that the rewired visual input to the MGN mediates the acceleration of visually cued fear conditioning (Newton *et al.*, 2004). Under these circumstances, the rewired visual inputs to the MGN are able to elicit the output fear response normally associated with an auditory stimulus. In these experiments, adult sham lesion and rewired

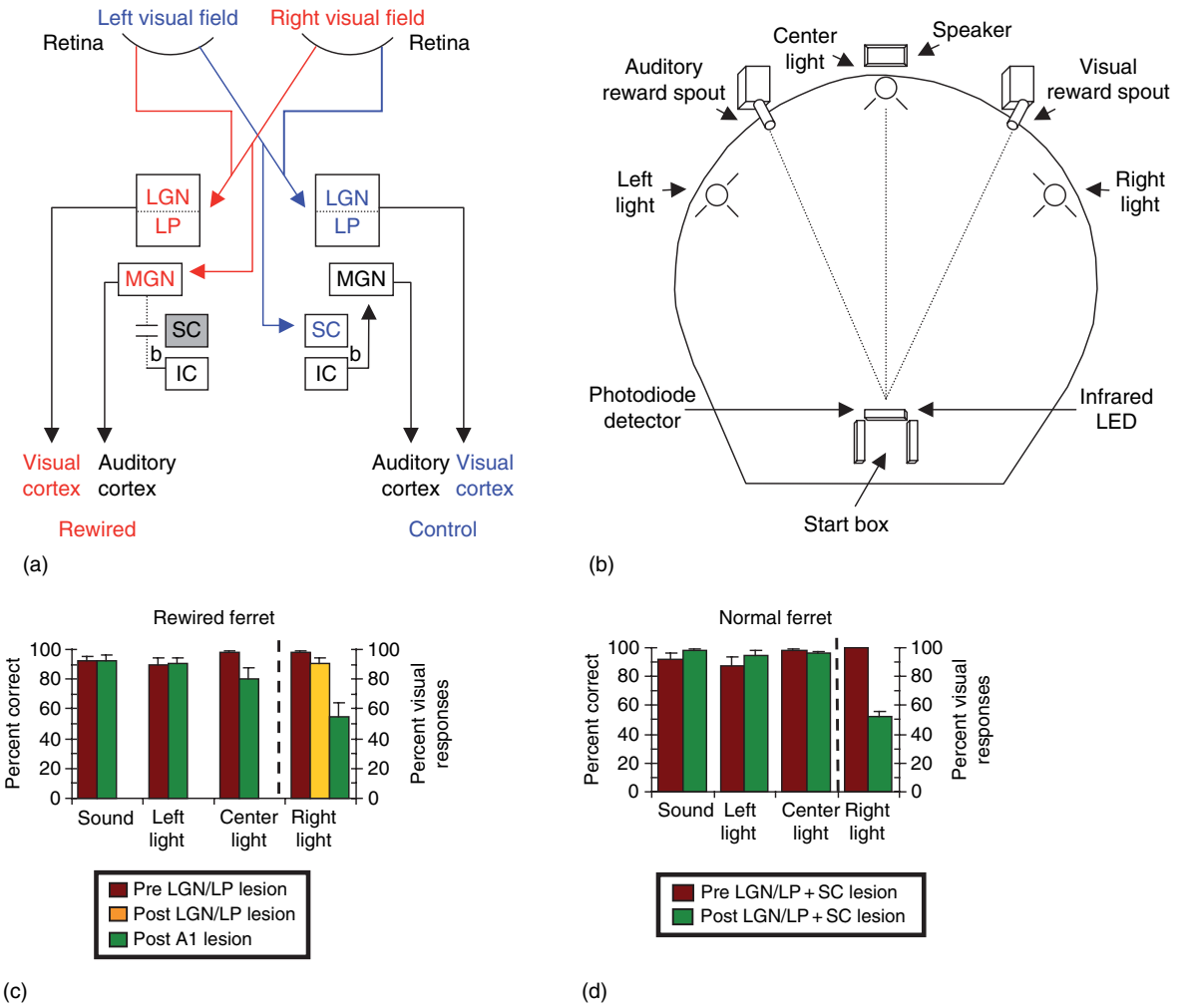

Figure 4 Retinal projections routed to the auditory pathway mediate visual behavior in rewired ferrets. a, Pathway from the retina to the visual thalamus, including the LGN and the lateral posterior (LP) nucleus, and to the superior colliculus (SC) in the control hemisphere (right); and to the LGN/LP and MGN in the rewired hemisphere (left). The SC and adjacent brachium (b) of the inferior colliculus (IC) were ablated neonatally in the left hemisphere. Visual projections in each hemisphere represent the contralateral visual field. b, Experimental apparatus and the design of the behavioral experiment. Dashed lines denote the borders of the left and right monocular fields and the direction of central gaze. Animals were rewarded at the right spout after a light in the left monocular field, and at the left spout after a sound from a central speaker. Subsequently, their responses to light in the right monocular field were tested. Animals initiated trials by standing in the start box with their muzzle between the infrared LED and a photodiode detector. c, Response of a rewired ferret to sound and light stimuli under three separate conditions: after training with sound and light stimuli, but before the ablation of the visual LGN/LP pathway (red bars); after the LGN/LP lesion (yellow bar; only the response to the right light is shown); and after the A1 lesion in the rewired hemisphere (green bars). Response bars (mean ± s.d.) depict the performance in the final 10–19 days in the pre-LGN/LP lesion condition, and in the first 10–18 days in the post-LGN/LP and post-A1 lesion conditions. d, Response of a normal ferret to sound and light stimuli under two separate conditions: after training with sound and light stimuli, but before the ablation of the LGN/LP and SC pathways (red bars) and after (green bars). Response bars depict performance in the final 9 days in the pre-LGN/LP + SC lesion condition, and in the first 12 days in the post-LGN/LP + SC condition. Modified from von Melchner, L., Pallas, S. L., and Sur, M. 2000. Visual behaviour mediated by retinal projections directed to the auditory pathway. *Nature* 404, 871–876.

mice underwent three sessions of fear conditioning with either a visual or an auditory cue (three cue-shock pairings per session) and behavioral testing after each session. The cued testing behavior of the different groups after one fear conditioning session are depicted in Figure 5b. Consistent with previous studies, after one session of fear conditioning, light-conditioned sham lesion mice did not freeze significantly more during the cue presentation compared to the habituation period (Figure 5b). Light-conditioned rewired mice, however, froze significantly more during the cue presentation after only one session of fear conditioning, as did tone-conditioned sham lesion and rewired mice.

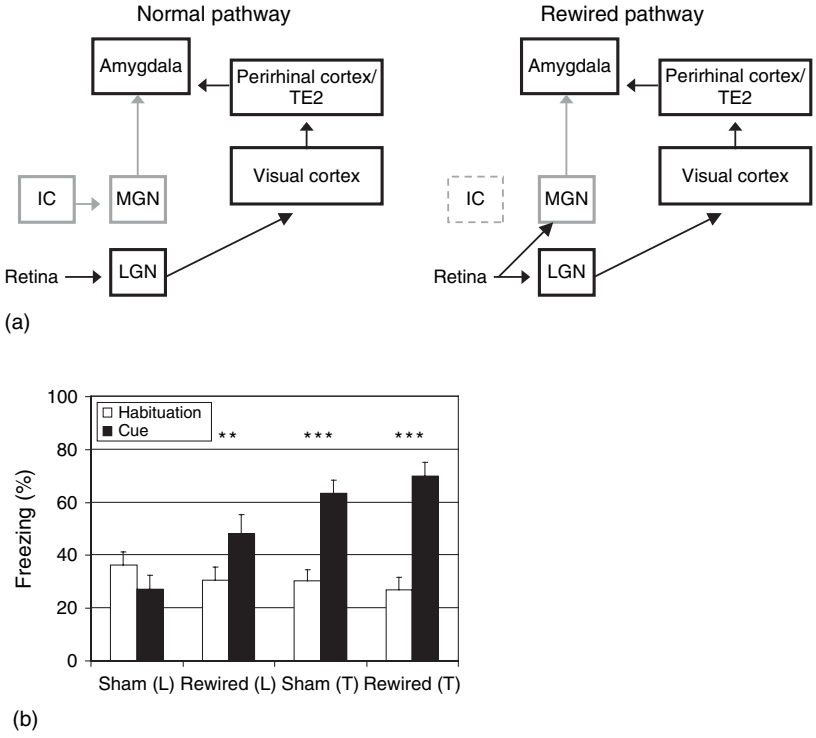

Figure 5 Retinal projections to the auditory pathway mediate visually cued learning in rewired mice. a, Simplified schematic of the principal visual (black) and auditory (gray) cued fear conditioning pathways in normal (left) and rewired mice (right). The IC (shown as a dotted box) was lesioned bilaterally in neonatal mice to induce retinal projections to the MGN. IC, inferior colliculus; LGN, lateral geniculate nucleus; MGN, medial geniculate nucleus. b, The mean freezing per group during the habituation (white bar) and cue presentation (black bar) periods of the cued testing session after one session of fear conditioning, with error bars denoting the standard error of the mean (significant paired *t*-tests, ** $p < 0.01$, *** $p < 0.001$). Modified from Newton, J. R., Ellsworth, C., Miyakawa, T., Tonegawa, S., and Sur, M. 2004. Acceleration of visually cued conditioned fear through the auditory pathway. *Nat. Neurosci.* 7, 968–973.

These findings indicate that the behavioral function of a target (in this case, the amygdala) is influenced by its inputs, and that it can activate the output response associated with the target. Thus, existing pathways can convey novel information to central structures, and this information is capable of mediating behavior.

3.07.5 Rewiring Experiments and Brain Evolution

Despite the limitations of rewiring experiments, this line of research demonstrates that the developing brain has an extraordinary capacity to reorganize itself and adapt to its inputs. Furthermore, the influence of molecular cues in early development on cortical arealization and the impact of rewired visual inputs on the organization and the connectivity of subsequent auditory structures provide insight into the evolution of a cortical area. In essence, these gain-of-function experiments provide information on certain principles by which novel connections might become functional and even adaptive, setting the stage for evolutionary change. During evolution, targets may express novel molecular cues as a result of mutations, and these, in combination with existing molecular cues, may form new structural patterns and functional connections. The electrical drive provided by these novel inputs can be utilized by target structures to form network connections that enable these structures or their downstream targets to process the novel information. Certain downstream pathways driven by the novel input may confer substantial adaptive advantage, as exemplified by rapid visually cued fear conditioning in rewired mice caused by novel retinal inputs to the MGN and subsequent utilization of the projection from the MGN to the lateral amygdala.

More generally, the studies discussed in this article suggest that there is a dynamic interplay between intrinsic and extrinsic factors throughout the evolution of a cortical region. Both factors play a critical role in determining the overall structure and function of a cortical area, but tend to exert their greatest influence at different times and in complementary ways during development.

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3.08 The Evolution of Neuron Classes in the Neocortex of Mammals

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Glossary

Afrotheria A clade of placental mammals that is thought to have originated in Africa at a time when it was isolated from other continents. This clade contains Paenungulata (elephants, hyraxes, and sirenians), Afrosoricida (tenrecs and golden moles), Tubulidentata (aardvarks), and Macroscelidea (elephant shrews). Molecular data indicate that the Afrotheria diverged from other placental mammals 110–100 Mya.

Anthropoidea A primate clade that includes New World monkeys, Old World monkeys, apes, and humans. Tarsiers are the sister group to the Anthropoidea.

Boreoeutheria A clade of placental mammals that encompasses Euarchontoglires and Laurasiatheria. This taxonomic division within the Boreoeutheria occurred 95–85 Mya in the Cretaceous.

Cetartiodactyla A clade of placental mammals that includes cetaceans (whales, dolphins, and porpoises) and artiodactyls (even-toed ungulates). This phylogenetic grouping is based on molecular evidence indicating that cetaceans evolved from within the artiodactyls and have their closest relationship with the hippopotamus and then ruminants (cattle and deer).

Euarchontoglires A supraordinal clade of boreoeutherian placental mammals that includes Rodentia, Lagomorpha, Dermoptera, Scandentia, and Primates.

Hominidae A primate clade that includes great apes (orangutans, gorillas, chimpanzees, and bonobos) and humans. The hylobatids (gibbons and siamangs) are the sister taxon to the Hominidae.

homoplasy A structure that is the result of convergent evolution, where organisms that are not closely related independently acquire similar characteristics. Homoplasy is contrasted with homology, which means that structures have a common origin derived from a shared ancestor.

Laurasiatheria A supraordinal clade of boreoeutherian placental mammals that includes Cetartiodactyla, Perissodactyla, Carnivora, Pholidota, and Eulipotyphla.

marsupials A clade of mammals in which the female has a pouch where the young is nurtured through early infancy. There are approximately 280 species of living marsupials, with the majority being native to Australia and the remainder living in South America (however, there is a single North American native marsupial species, the Virginia opossum).

monotremes A clade of mammals that lay eggs, rather than giving birth to live young like marsupials and placental mammals. The extant representatives of this group, the platypus and the echidnas, are indigenous to Australia, New Guinea, and Tasmania. Fossil evidence, however, suggests that monotremes were once more widespread.

placental mammals A clade of mammals in which the fetus is nourished during gestation by a placenta. This group encompasses the majority of living mammals.

Xenarthra A clade of placental mammals present today only in the Americas. This group includes sloths, anteaters, and armadillos. Molecular data indicate that the Xenarthra diverged from boreoeutherian mammals 100–95 Mya.

3.08.1 Introduction

A diverse array of neuron types populates the mammalian neocortex. Physiological activity within a cortical area, in turn, is largely determined by interactions among these different cell types and incoming afferents. Neurons in the cerebral cortex of mammals can be divided into two major classes on the basis of morphology and function, pyramidal excitatory cells, and inhibitory interneurons. Each class includes many subtypes that can be identified by their size, shape, dendritic and axonal morphology, and connectivity. These neuronal subtypes exhibit a variable distribution among cortical layers and regions, and some are differentially represented among species (see Hof *et al.*, 1999, 2000; Hof and Sherwood, 2005). Neurons can be further classified based on their expression of various proteins, such as neurofilament proteins (NFPs), calcium-binding proteins, and neuropeptides.

The morphology of a given neuron, particularly of its dendritic arborizations, reflects the size of its receptive field and the specificity of its synaptic contacts. Thus, the structure of the dendritic arbor as well as the distribution of axonal terminal ramifications confer a high level of subcellular specificity in the localization of particular synaptic contacts on a given neuron. The three-dimensional distribution of the dendritic tree is also a key factor with respect to the type of information transferred to the neuron. A neuron with a dendritic tree restricted to a particular cortical layer may be receptive to a very limited pool of afferents, whereas widely expanding dendritic branches typical of large pyramidal neurons will receive highly diversified inputs in the cortical layers through which the dendrites course. Considering the variability in cortical morphology, size, and cellular organization in mammals, it is important to investigate how specific characteristics of cortical microcircuitry differ among species, and how these cellular phenotypes could be used to assess taxonomic affinities and functional differences among species.

3.08.2 Neuronal Typology and Chemical Specialization

3.08.2.1 Pyramidal Neurons

Pyramidal neurons are the principal excitatory neuronal class in the cerebral cortex. They are highly polarized neurons, with a major orientation axis orthogonal to the pial surface of the cerebral cortex. Their cell body is roughly triangular on cross section, although a large variety of morphologic types exist with elongate, horizontal or vertical fusiform, or inverted perikaryal shapes. They typically have a large number of dendrites that emanate from the apex and from the base of the cell body. The span of their dendrites may cover several millimeters and their somata are found in all cortical layers except layer I, with predominance in layers II, III, and V. Small pyramidal neurons in layers II and III of the neocortex have a restricted dendritic tree and form vast arrays of axonal collaterals with neighboring cortical domains, whereas medium to large pyramidal cells in deep layer III and layer V have a much more extensive dendritic tree and furnish long corticocortical connections. Layer V also contains very large pyramidal neurons disposed in clusters or as isolated, somewhat regularly spaced elements. These neurons are known to project to subcortical centers such as the basal ganglia, brainstem, and spinal cord. Finally, layer VI pyramidal cells exhibit a greater morphologic variability than those in other layers, and are involved in certain corticocortical as well as corticothalamic projections. The dendrites of pyramidal cells show large numbers of dendritic spines that receive most of the excitatory synaptic inputs. As many as 40 000 spines can be encountered on a large pyramidal neuron.

The major excitatory output of the neocortex is furnished by pyramidal cells. Their axon extends in most cases from the base of the perikaryon and courses toward the subcortical white matter and gives off several collateral branches that are directed to cortical domains generally located within the vicinity of the cell of origin. While many of these branches ascend in a radial, vertical pattern of arborization, a separate set of projections also travels horizontally over long distances. One function of the vertically oriented component of the recurrent collaterals may be to interconnect layers III

and V, the two major output layers of the neocortex. Horizontal intrinsic connections are positioned to recruit and coordinate the activity of modules of similar functional properties while inhibiting other domains. Together these recurrent projections function to set up local excitatory patterns and coordinate the output of neuronal ensembles. NFP immunoreactivity, chiefly recognized by the expression of dephosphorylated epitopes of NFP medium and heavy molecular weight subunits, characterizes a subpopulation of pyramidal neurons in the neocortex of mammals that exhibits clear regional and species differences in their distribution and densities (Hof *et al.*, 1992, 1996; van der Gucht *et al.*, 2001, 2005; Kirkcaldie *et al.*, 2002; Boire *et al.*, 2005; Figure 1). The expression of NFP has been studied most comprehensively in primates, where it has been shown that this protein is enriched in a subset of large pyramidal neurons that have an extensive dendritic arborization, are distributed in well-defined laminar positions, form highly specific long corticocortical projections, and show regional specificity in their distribution patterns (Campbell and Morrison, 1989; Campbell *et al.*, 1991; Hof and Nimchinsky, 1992; Carmichael and Price, 1994; Hof and Morrison, 1995; Hof *et al.*, 1995b; Nimchinsky *et al.*, 1995, 1996, 1997; Preuss *et al.*, 1997, 1999; Sherwood *et al.*, 2003a; Vogt *et al.*, 2001, 2005). Although the precise function of NFP is not completely understood, its restricted distribution among certain subsets of corticocortical circuits in primates suggests that it confers unique neurochemical and morphologic properties subserving a range of highly specialized functions in neocortical connectivity, as well as selective vulnerability to neurodegenerative diseases unique to humans (Hof *et al.*, 1995a, 1995b; Nimchinsky *et al.*, 1996; Bussieère *et al.*, 2003; Hof and Morrison, 2004). NFP may be present, to some degree, in functionally homologous subsets of cortical output neurons in many species.

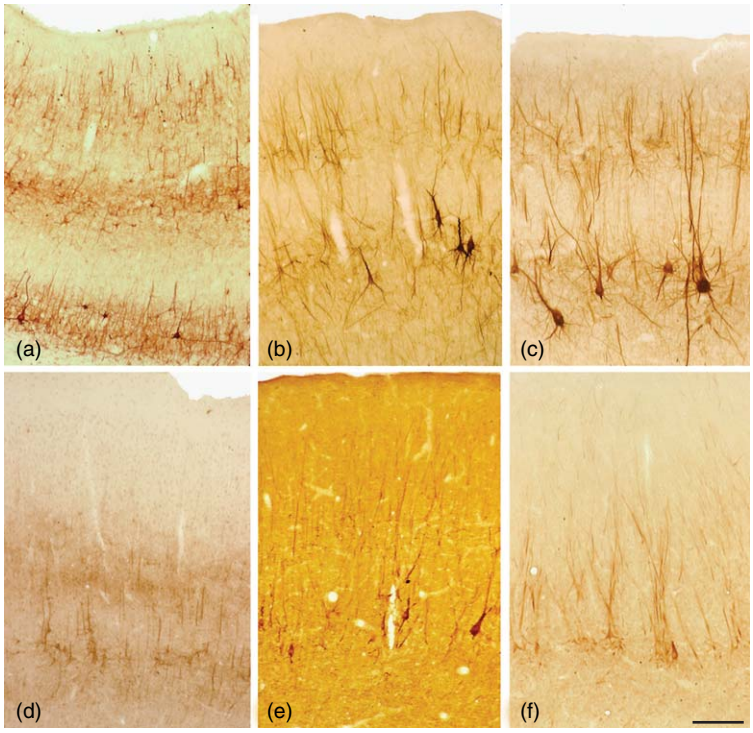

Figure 1 Examples of cytoarchitecture and cellular typology using an antibody against NFP. a, Organization of the primary visual cortex in a long-tailed macaque monkey (*Macaca fascicularis*). NFP-immunoreactive neurons are predominant in layers III, IVB, and V. b and c, Distribution of NFP-immunoreactive neurons in the primary visual cortex of two carnivores, the dog (b, *Canis familiaris*), and the California sea lion (c, *Zalophus californianus*). Note the prominent labeling of very large cells in layer V and smaller cells in layer III, whereas layer IV in the middle is devoid of labeled neurons. The large size and extensive dendrites of NFP-immunoreactive neurons is obvious. d, NFP-immunoreactive cells in the visual area of the camel (*Camelus dromedarius*). The labeled neurons are relatively small and predominate in the deep layers. e and f, NFP immunolabeling in the visual cortex of the pigmy sperm whale (e, *Kogia breviceps*), and of the beluga whale (f, *Delphinapterus leucas*). Note the prominent clusters of large immunoreactive neurons in layer IIIc/V only. Scale bar (on f): 300 μm. Reproduced from Hof, P. R. and Sherwood, C. C. 2005. Morphomolecular neuronal phenotypes in the neocortex reflect phylogenetic relationships among certain mammalian orders. *Anat. Rec. A* 287, 1153–1163, with permission from John Wiley & Sons, Inc.

3.08.2.2 Spiny Stellate Cells

Spiny stellate cells are the other class of cortical excitatory neurons and are found in highest numbers in neocortical layer IV. The spiny stellate cell is a small multipolar neuron with local dendritic and axonal arborizations. These neurons resemble pyramidal cells in that they are the only other cortical neurons with large numbers of dendritic spines, but differ from them in lacking most of an apical dendrite and having a restricted dendritic arbor that generally does not extend beyond the layer in which the cell body resides. The axons of spiny stellate neurons are primarily intrinsic and form links between layer IV, that receives a major input from the thalamus, and layers III, V, and VI. In some respects, the axonal arbor of spiny stellate cells mirrors the vertical plexuses of recurrent collaterals, albeit in a more restricted manner. Given its axonal distribution, spiny stellate neurons appear to function as a high-fidelity translator of thalamic inputs, maintaining strict topographic organization and setting up initial links of information transfer within a cortical area.

3.08.2.3 Inhibitory Interneurons: Basket, Chandelier, and Double Bouquet Cells

There is a large variety of interneuron types in the cerebral cortex. These neurons contain the neurotransmitter γ-aminobutyric acid (GABA), and exert strong local inhibitory influences on postsynaptic neurons. The dendritic and axonal arborizations of interneurons offer important clues as to their role in the regulation of pyramidal cell function. In addition, many morphologic classes of GABAergic interneurons can be further defined by a particular set of neurochemical characteristics. Three major subtypes of cortical interneurons are classically described, principally based on rodent and primate studies, namely basket, chandelier, and double bouquet cells. It must be noted, however, that interneurons exhibit a rich variety of size and morphologies, such as clutch cells, neurogliaform cells, Martinotti-type and Cajal–Retzius cells, bipolar cells and other stellate cells, and multipolar neurons, which all have diverse representations depending on the brain region as well as on the species studied.

Basket cells are characterized by axonal endings that form a basket of terminals surrounding a pyramidal cell soma and provide most of the inhibitory GABAergic synapses to the soma and proximal dendrites of pyramidal cells. One basket cell may contact numerous pyramidal cells, and in turn several basket cells can contribute to the pericellular basket of one pyramidal cell. The basket cells have a relatively large soma and multipolar morphology with dendrites extending in all directions for several hundred micrometers such that the vertically oriented dendrites cross several layers. Their axon arises vertically, quickly bifurcates and travels long distances, forming multiple pericellular arrays as it spreads horizontally. The basket cells predominate in layers III and V in the neocortex and are numerous amid hippocampal pyramidal neurons.

Chandelier cells generally have a variably bitufted or multipolar dendritic tree. The defining characteristic of this cell class is the very striking appearance of its axonal endings. In Golgi or immunohistochemical preparations, the axon terminals appear as vertically oriented cartridges, each consisting of a series of axonal swellings linked together by thin connecting pieces making them look like old-style chandeliers. These neurons synapse exclusively on the axon initial segment of pyramidal cells. Most of the chandelier cells are located in layer III and their primary target appears to be pyramidal cells in layer III, and to a lesser extent layer V. One pyramidal cell may receive inputs from multiple chandelier cells, and one chandelier cell may innervate more than one pyramidal cell. This cell exerts powerful inhibition on postsynaptic pyramidal cell firing.

Double bouquet cells are mostly prevalent in layers II and III, and are also present in layer V of the neocortex. These interneurons are characterized by a vertical bitufted dendritic tree and a tight bundle of vertically oriented varicose axon collaterals that traverse layers II through V. Many double bouquet cells synapse on spines of pyramidal cells and most of their remaining synapses are on fine dendritic shafts, in striking contrast to the basket and chandelier cells. Another subclass of double bouquet cell has similar synaptic targets but primarily in layer V, thus influencing the activity of different populations of pyramidal cells.

GABAergic interneurons can also be classified in nonoverlapping subtypes based on their content in the calcium-binding proteins parvalbumin (PV), calbindin (CB), and calretinin (CR), as well as several neuropeptides (Hendry *et al.*, 1989; Andressen *et al.*, 1993; Condé *et al.*, 1994; DeFelipe, 1997; Gonchar and Burkhalter, 1997; Glezer *et al.*, 1998; Morrison *et al.*, 1998; Hof *et al.*, 1999; Markram *et al.*, 2004). CB- and CR-expressing interneurons share many morphologic similarities, and are mainly bitufted, bipolar, and double bouquet neurons, as well as a few pyramidal neurons, with minimal overlap among these subpopulations in the rodent and primate neocortex (Rogers, 1992; DeFelipe, 1997; Morrison *et al.*, 1998). PV-immunoreactive neurons are mainly observed in layers II

to V, and are principally basket and chandelier cells (Blümcke *et al.*, 1990; Van Brederode *et al.*, 1990; Hof and Nimchinsky, 1992; Condé *et al.*, 1994; Nimchinsky *et al.*, 1997). PV has also been reported to occur in certain pyramidal neurons in primate somatosensory and motor cortex (Preuss and Kaas, 1996; Sherwood *et al.*, 2004).

3.08.3 Relationships of Neurochemical Phenotype and Phylogenetic Affinities

3.08.3.1 General Phylogenetic Patterns

Phylogenetic patterns in the distribution of NFP and the three calcium-binding proteins appear to be associated with interspecific variation in other aspects of the cytoarchitecture of mammalian neocortex. Monotremes (i.e., echidnas and the platypus) are the sister taxon to all therian mammals. In these species, neurons containing CB, CR, and PV are found throughout the cortex, although CR-immunoreactive neurons are most dense in the piriform cortex where they predominate as a polymorphic phenotype. In monotremes (Hof *et al.*, 1999), CB and PV comprise morphologic types that resemble those found in placental mammals, including large PV-immunoreactive multipolar neurons that are similar to basket cells. In echidnas, however, PV is present in a unique cell type characterized by a large pyramidal-like or multipolar morphology that is common in layers V and VI. Such PV-labeled neurons have not been observed in any other mammalian species. It is also worth noting that the Australian echidna (*Tachyglossus aculeatus*) presents NFP-expressing cells in layer V of its neocortex (Hassiotis *et al.*, 2004). Although there are no studies of NFP staining in the most closely related taxon, the platypus, NFP is enriched in layer V neurons in the marsupial tammar wallaby (*Macropus eugenii*) throughout its cortex, as well as occasionally in layer III neurons of select sensory and association regions (Ashwell *et al.*, 2005). In placental mammals, NFP-containing pyramidal neurons are found frequently in layers III, V, and VI. Thus, NFP-rich cells in layer V may be interpreted as a conservative trait among mammals, with variable patterns of NFP expression in other layers having arisen in different lineages in subsequent evolution (Figure 1).

All species of marsupials examined to date display comparable staining patterns in the neocortex for PV, CB, and CR (Hof *et al.*, 1999). The most prevalent calcium-binding protein in marsupials appears to be CR, which is present in numerous small bipolar neurons located in layers II and III, as well as small pyramidal-like neurons in layers V and VI in the lateral cortex. Compared to CR-immunoreactive neurons, CB is more sparsely present in bipolar and bitufted neurons in the supragranular layers throughout the cortex, and in some larger multipolar neurons in the deep layers. A major difference between marsupials and other mammalian taxa is the remarkable paucity of PV-immunoreactive neurons and fibers. PV is observed only in a few small interneurons, whereas it is much more prevalent in other small-bodied mammals as well as in primates and carnivores. Surprisingly, PV-containing neurons in layer II have a morphology resembling double bouquet cells that are usually labeled by CB in rodents and primates. This may represent a neuronal specialization in certain marsupials that is not found in placental mammals.

A current limitation to interpreting regional patterns of distributions of neuron classes is the paucity of data from the phylogenetic groups that diverged close to the base of the adaptive radiation of placental mammals, the Xenarthra (i.e., sloths, anteaters, and armadillos) and Afrotheria (i.e., tenrecs, golden moles, elephant shrews, aardvarks, manatees, hyraxes, and elephants). Substantially more is known regarding variation in cortical architecture of the other placental mammals, the Boreoeutheria. Among boreoeutherian mammals, species showing a high degree of morphologic differentiation of neocortical areas, a variable development of layer IV, and substantial variation in neuronal size and packing densities across the cortical plate are also generally characterized by a balanced representation of the three calcium-binding proteins and morphological diversity of NFP-immunoreactive pyramidal neurons across cortical regions (Hof *et al.*, 2000). In contrast, species characterized by greater cytoarchitectural monotony throughout the cortical mantle, a poorly defined or lack of layer IV in most regions, and the presence of very large pyramidal cells in all neocortical areas, display a predominance of CB- and CR-containing populations in comparison to PV-immunoreactive neurons, and rather uniform NFP-containing pyramidal cell morphology. The first type occurs in primates, rodents, carnivores, and to some extent megachiropterans, as well as in tree shrews and lagomorphs. Most of the taxa that are characterized by this cortical organization are members of the supraordinal group Euarchontoglires, with the exception of carnivores and bats. In contrast, the second type of cortical organizational pattern is present in cetaceans, artiodactyls, and perissodactyls, which are all

members of the Laurasiatheria. Thus, the distribution of these particular aspects of the cortical phenotype follows a major taxonomic division that occurred about 80–90 Mya at the base of the radiation of boreoeutherian mammals (Murphy *et al.*, 2001a, 2001b). While these general patterns appear to differentiate cortical organization in major boreoeutherian clades, the lack of data from xenarthrans and afrotherians makes it difficult to clearly establish which character states are conservative and which are derived for placental mammals. This is a particular challenge because cortical cell types in monotremes and marsupials often differ significantly from placental mammals and from each other. Our examination of calcium-binding protein immunoreactivity in the xenarthran giant anteater (*Myrmecophaga tridactyla*), however, shows similarities with marsupials and cetartiodactyls in that PV-immunoreactive neurons are very sparse, whereas CB- and CR-immunoreactive neurons are expressed in a morphologically diverse population of cells that includes a high frequency of pyramidal neurons (Hof and Sherwood, 2005; Figures 2 and 3).

3.08.3.2 The Distribution of Some Neuron Classes Closely Matches Phylogenetic Affinities

Reports of the cyto- and chemoarchitecture of odontocete cetaceans, particularly of visual and auditory regions, and analysis of neocortical neurons in a few large artiodactyls have revealed commonalities in cortical organization between these sister taxa (Morgane *et al.*, 1988, 1990; Glezer *et al.*, 1992, 1993, 1998; Hof *et al.*, 1992; 1999). In both groups, PV is present only in sparsely distributed large stellate neurons located in layer IIIc/V. A few small pyramidal neurons in layer III also exhibit PV immunoreactivity in dolphins. In cetaceans and artiodactyls, CB- and CR-immunoreactive cells are far more numerous than PV-immunoreactive cells, occurring in large fusiform, bipolar, or multipolar neurons in layers I, II, and superficial layer III. CB-containing neurons are much less numerous and less intensely stained than CR-immunoreactive neurons. The CR-containing neurons located in layer I have a morphology quite comparable to that of the bipolar/bitufted CB- or CR-expressing neurons typically seen in layer II of other mammals such as rats, carnivores, and primates (Ballesteros Yáñez *et al.*, 2005), whereas the CR-containing neurons in layers II and III are much larger and more variable in shape than in other species, with a predominance of multipolar and fusiform types. These neurons have long dendrites that extend into layers I and III. Very large CR-immunoreactive neurons are also encountered in layers V and VI, especially in the neocortex of large artiodactyls, such as the giraffe (*Giraffa camelopardalis*), llama (*Lama glama*), and camel (*Camelus dromedarius*), whereas they are less numerous in the pig (*Sus scrofa*), and in smaller ruminants. A few pyramidal-like neurons in layer III are also faintly CR-containing in dolphins, and the large pyramidal neurons in layer IIIc/V contain low levels of CB. The distribution and morphology of NFP-immunoreactive neurons are also comparable in cetaceans and artiodactyls, but differ considerably from those in primates, carnivores, and rodents. In cetartiodactyls, NFP is expressed in very large pyramidal neurons located in the deep portion of layer III and in upper layer V (Hof *et al.*, 1992). These neurons are present as clusters of three to six neurons, regularly spaced throughout the cortical mantle and intensely labeled with prominent apical dendrites extending well into layer I, with no major regional variability in their densities.

Another cell class that shows a restricted phylogenetic distribution is the spindle-shaped neuron, which is found exclusively in the cerebral cortex of hominids (i.e., great apes and humans). Spindle-shaped cells are characterized by a vertical, fusiform morphology, very large size, and high levels of NFP immunoreactivity (Nimchinsky *et al.*, 1995, 1999). They are prevalent in a restricted sector of the anterior cingulate cortex (areas 25, 24a, and 24b; Vogt *et al.*, 1995) and are also numerous in the anteroventral agranular insular cortex (see The Evolution of Neuron Types and Cortical Histology in Apes and Humans, Role of Spindle Cells in the Social Cognition of Apes and Humans). These neurons are found exclusively in hominids and have not been reported in any other mammalian species investigated thus far (including other primate species; Nimchinsky *et al.*, 1999; Hof *et al.*, 2000). The volume of spindle-shaped cells is strongly correlated with encephalization, which is not the case for the other neuron types (Nimchinsky *et al.*, 1999). These spindle-shaped cells are a particular type of projection neuron, as they send an axon in the subcortical white matter, although their exact domain(s) of projection cannot be ascertained in the species in which they are present. They may, however, provide well-defined projections, similar to Meynert cells or Betz cells (Sherwood *et al.*, 2003b). Furthermore, although CR-immunoreactive pyramidal neurons have been reported in the entorhinal cortex of several mammalian species, CR-containing small pyramidal neurons in layer Va of anterior cingulate cortex (area 24) appear to be present exclusively in hominids, possibly indicating a recent evolutionary character in

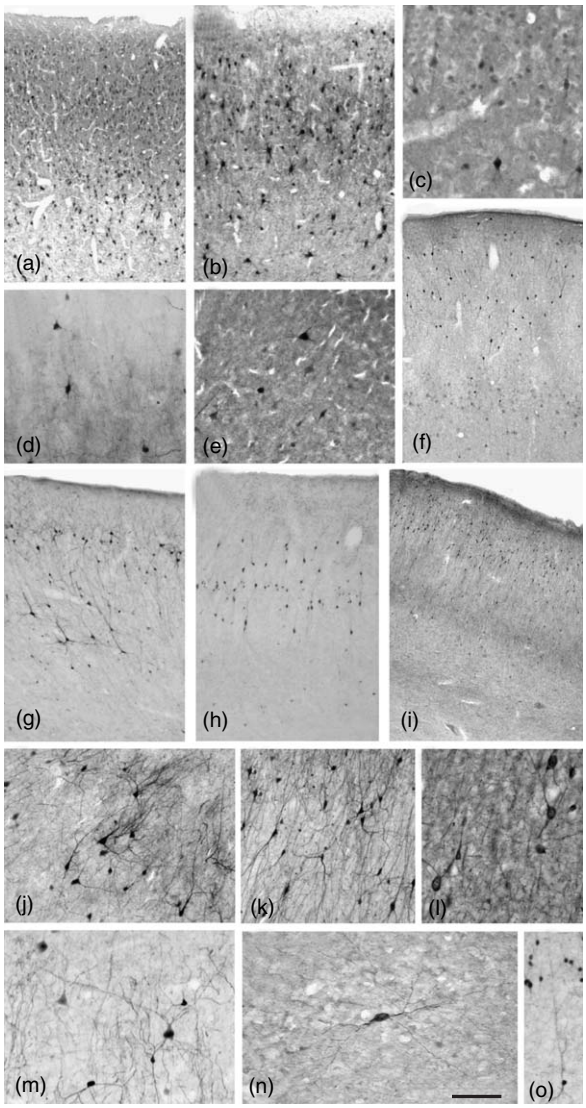

Figure 2 Immunoreactivity patterns of calcium-binding proteins in the neocortex of various mammals. a and b, CB (a) and PV (b) immunoreactivity in the primary somatosensory cortex of the echidna (*Tachyglossus aculeatus*). There is a large population of small neurons that express CB, whereas PV is contained in a heterogeneous collection of neurons, some very large and multipolar. This differs radically from other members of the Australian fauna (Hof *et al.*, 1999), such as the koala (*Phascolarctos cinereus*, c), which displays a rather sparse population of CB-containing cells. d and e, The giant anteater (*Myrmecophaga tridactyla*) is also characterized by a sparse population of relatively large CB-immunoreactive multipolar neurons (d) and PV-expressing neurons (e). These patterns are fully distinct from that in rodents (f, CR immunoreactivity in the somatosensory cortex of the chinchilla, *Chinchilla laniger*), carnivores (g, CR immunoreactivity in a dog motor cortex displaying bipolar neurons as well as pyramidal-like neurons), and a cetacean visual cortex (h, the bottlenose dolphin, *Tursiops truncatus*). Layers I and II are particularly enriched in the cetacean (layer II is located about one-third down from the top of the photomicrograph). Panels (i–l) show details of the chemoarchitecture of the frontal cortex of a Siberian tiger (*Panthera tigris*). CR-immunoreactive neurons exhibit a typical distribution predominating in the superficial layers (i, k), and large multipolar CB-immunoreactive (j) and PV-immunoreactive (l) neurons are encountered in deep layer III. Large multipolar CR-containing neurons are found in layer III of a dog (*Canis familiaris*) primary motor area (m). Note the large size of this neuron and compare it to the CR-immunoreactive giant neuron located in layer VI of a giraffe (n, *Giraffa camelopardalis*), and to a typical bipolar neuron in layer III of the mouse visual cortex (o, *Mus musculus*). Scale bar (on n): 300 μm (a, b); 400 μm (f–i); 50 μm (c–e, j–o). Reproduced from Hof, P. R. and Sherwood, C. C. 2005. Morphomolecular neuronal phenotypes in the neocortex reflect phylogenetic relationships among certain mammalian orders. *Anat. Rec. A* 287, 1153–1163, with permission from John Wiley & Sons, Inc.

this primate clade (Hof *et al.*, 2001). These observations of multiple novel cellular specializations suggest the occurrence of functional modifications along the hominid lineage during the last 15–20 My in cortical regions that play major roles in the regulation of autonomic function, cognition, self-awareness, emotionality, and vocalization (Figures 2 and 3).

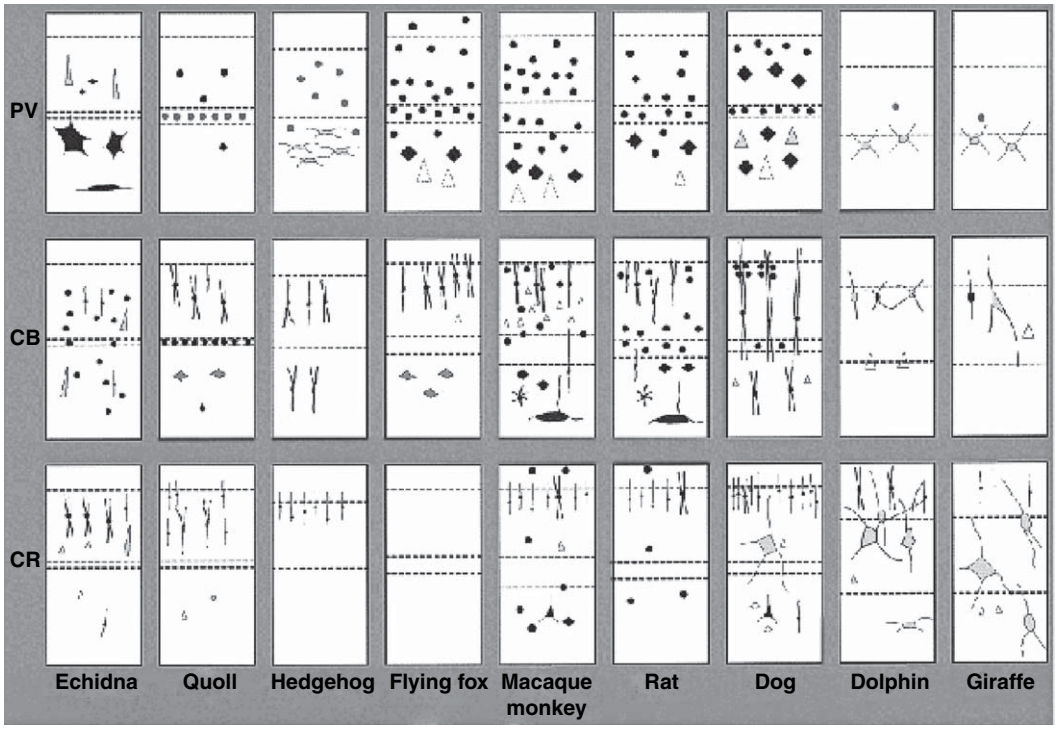

Figure 3 Schematic representation of the major calcium-binding protein-immunoreactive neuronal types in the mammalian neocortex. A few representative species of monotremes, marsupials, and placentals are shown. Many species have comparable neuronal types, especially with respect to small CR-immunoreactive bipolar neurons and CB-containing bitufted and double-bouquet cells. Primates, rodents, and carnivores, with the exception of CR, tend to show comparable patterns. The shading of the neurons indicates the relative intensity of the staining. Faintly labeled neurons are shown as empty symbols, moderately labeled neurons as gray symbols, and intensely labeled neurons as black symbols. Dotted triangles indicate the presence of PV-immunoreactive basket terminals on pyramidal neurons. Triangles represent pyramidal and pyramid-like neurons; black dots represent small multipolar or round neurons; diamonds represent large multipolar neurons and basket cells. Other symbols identify CB- and CR-immunoreactive bipolar, bitufted, and double bouquet cells, as well as neurons with atypical morphology. Note the presence in the monkey and the rat of CB-immunoreactive neurogliaform (represented as small star-shaped elements), and Martinotti cells (shown as large, ovoid horizontally oriented elements), in layers V and VI, that have not been reported in other species. On each panel, the dashed lines identify layers I and IV. Note the thick layer I and the absence of layer IV in cetaceans and ungulates. Layer IV is also not clearly defined in the hedgehog. Adapted from Hof, P. R., Glezer, I. I., Condé, F., *et al.* 1999. Cellular distribution of the calcium-binding proteins parvalbumin, calbindin, and calretinin in the neocortex of mammals: Phylogenetic and developmental patterns. *J. Chem. Neuroanat.* 16, 77–116, Elsevier.

3.08.3.3 Other Phylogenetic Distributions of Cortical Neuron Classes Indicate Convergent Evolution

Although many aspects of cortical architecture reflect phylogenetic affinities, it is likely that many cortical phenotypes present examples of homoplasy, where certain characters have evolved independently in nonrelated groups of mammals. In this regard, it is noteworthy that there are several traits shared by Carnivora and Euarchontoglires, such as the distribution and typology of calcium-binding protein-containing neurons (Glezer *et al.*, 1993, 1998; Hof *et al.*, 1999; Ballesteros Yáñez *et al.*, 2005). For example, in the dog neocortex, PV is present in a large population of morphologically diverse interneurons with a typology generally comparable to that observed in anthropoid primates. Some of these large multipolar neurons may be basket cells, due to the presence of PV-immunoreactive basket terminals around unstained pyramidal perikarya. Furthermore, CR is present in a very dense population of bipolar and double bouquet cells in layer II and the upper portion of layer III, as observed in primates and rodents. These aspects of chemoarchitecture in carnivores that are similar to primates, rodents, and other Euarchontoglires are not derived from a common ancestral state and hence have arisen due to convergent evolution. Other features of carnivore cortical organization resemble traits observed in their close relatives in the Laurasiatheria, the perissodactyls and cetartiodactyls, and so were probably inherited

from the last common ancestor of these taxa. For example, in canids, felids, and pinnipeds, gigantic intensely labeled CR-immunoreactive, multipolar neurons occur in layer III of agranular motor cortices. These neurons are morphologically comparable to the very large CR-containing neurons found in layers III, V, and VI throughout the neocortex of large artiodactyls and cetaceans (Hof *et al.*, 1996, 1999), which shows a poorly differentiated layer IV only in certain regions (Morgane *et al.*, 1990; Glezer *et al.*, 1993, 1998; Hof *et al.*, 1999). It is interesting that in spite of many similarities in cortical organization and connectivity between carnivores and primates, the dog neocortex displays several differences in neurochemical organization compared to anthropoids (Hof *et al.*, 1996, 1999). Canids have a high degree of cellular specialization in primary motor and sensory cortices, contrasting with fairly homogeneous patterns in association cortices. Large PV- and CB-containing pyramidal cells are present only in primary motor and visual regions, and high numbers of large PV-immunoreactive basket cells and multipolar CR-containing interneurons occur only in primary motor, somatosensory, and visual areas (Hof *et al.*, 1996).

Besides these observations in carnivores, other examples show that the distribution of cortical neuron classes exhibit a mosaic pattern of evolution, with some unique specializations, in particular lineages and other examples of homoplasy (for instance, a paucity of PV expression is observed in the neocortex of cetartiodactyls and marsupials). The predominance of CB and CR in the neocortex and subcortical systems of cetartiodactyls is similar to the calcium-binding protein distributions observed in microchiropterans and hedgehogs but differs from that in rodents and primates (Glezer *et al.*, 1993, 1998; Hof *et al.*, 1999). In addition, large multipolar PV-containing neurons are found in the deep layers of the neocortex of hedgehogs and dolphins, unlike in rodents, carnivores, and primates, where PV-expressing cells are more common in layers III and IV. It is also notable that megachiropterans are characterized by the absence of neocortical expression of CR, whereas their thalamic neurons do express it.

3.08.4 Functional Considerations

Calcium-binding protein-containing interneurons are known to influence the activity of pyramidal neurons in a manner specific to each cell class (Condé *et al.*, 1994; DeFelipe, 1997; Hof *et al.*, 1999; Ballesteros Yáñez *et al.*, 2005), and as such the role of calcium-binding protein in cortical integration is likely to be similar to a large degree among rodents, carnivores, and primates, suggesting that similar mechanisms exist across boreoeutherian mammals at least. However, differences are present at the level of particular neuronal subclasses, as recently revealed in a study of CB-expressing interneurons in primates compared to rodents, lagomorphs, carnivores, and artiodactyls (Ballesteros Yáñez *et al.*, 2005). These authors reported that, in the nonprimate species, axon bundles of CB-immunoreactive double bouquet cells are not observed except for some in the visual cortex of carnivores, indicating that although somata that resemble typical CB-expressing cell types from primates can be found in these species, their axonal projections are likely to differ from primates. Whether such differences in axonal organization can be extended to PV- and CR-immunoreactive neurons remains to be demonstrated.

The degree to which functional interpretations of biochemical neuron types can be applied to all mammalian orders is difficult to determine owing to large differences in morphological phenotypes and distributions for any given cell type across species. The relative rarity of PV-immunoreactive neurons in cetaceans and artiodactyls could be interpreted as an ancestral retention for the Laurasiatheria because it also occurs in other laurasiatherians such as echolocating bats and hedgehogs, which may show many plesiomorphic features (Glezer *et al.*, 1988). The neocortex of cetaceans and large artiodactyls appears to contain an inordinate number of cortical modules revealed by clusters of large NFP-containing pyramidal cells in layer IIIc/V (Glezer *et al.*, 1988; Morgane *et al.*, 1988; Hof *et al.*, 1992). Much cortical integration in cetaceans may take place in the cellular, thick layer I that contains 70% of the neocortical synapses in these species (Glezer and Morgane, 1990). Consistent with this observation, most CB- and CR-containing interneurons are located in layers I and II in cetaceans, and the few PV-immunoreactive cells lie in nearby layers IIIc/V and VI pyramidal cells. The PV-immunoreactive neurons may represent basket cells, and axons of CB- and CR-immunoreactive interneurons may be located in a position to interact with inputs to the neocortex and connect the apical dendrites of the deep layers of pyramidal neurons (Glezer *et al.*, 1988; Morgane *et al.*, 1988). It is possible that in cetartiodactyls calcium-binding protein-immunoreactive neurons play a comparable role in neocortical microcircuits as in primates and rodents. The similarities in neurochemical specialization of the cetartiodactyl

neocortex parallel the paleontological and molecular evidence, indicating that these species share a relatively recent common ancestor, and that much like primates, the evolution of the species with the largest brains (the delphinids) is a recent event (Marino *et al*., 2005).

Although there are major gaps in our knowledge of the evolutionary history of neocortical organization in mammals and of the chemical organization of the cerebral cortex in most species, collectively these observations indicate that brain organization and neurochemical cellular specialization reflect evolutionary relationships among many mammalian species.

Acknowledgments

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3.09 Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals

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Glossary

allometry	Variation of the size of any body part or organ relative to body size during ontogeny or phylogeny.
basal insectivora	The most primitive forms of extant mammals (Tenrecinae).
Cuvier's ratio	Relative brain size = brain weight/body weight.
isopoderal	The same body weight.
niche, ecological	The total of interactions of an organism in an ecosystem.
progression index	The ratio of the size of a brain or brain structure in a given species to the size of that structure in a hypothetical average isoponderal basal insectivore. It shows how many times a brain or brain structure is more or less developed in a given species than in a basal insectivore of the same body size.
size index	See 'progression index'.

3.09.1 Introduction: Brain Size and Evolution

The evolution of biological systems is characterized by an increase in functional efficiency, and an important indicator of functional efficiency is size (see The Evolution of Encephalization). Numerous neuroanatomical investigations have demonstrated that the size of the brain or a given brain structure is related to the size of the animal or species and the functional requirements of its habits (e.g., Stephan and Pirlot, 1970; Baron, 1974; Gittleman, 1991). The functional capacity of a brain or brain structure increases as its functional units increase. Large brains are more efficient and capable of more complex functions than small brains. Size is therefore an indicator of evolutionary progress. Since brain size shows variations, not solely related to body size, an important question with respect to brain evolution concerns the most appropriate statistical method for calculating biologically meaningful differences in the size of the brain and brain parts. This problem has been discussed in detail for Primates (Stephan *et al.*, 1988). The authors stated that a useful method (1) must be easily applicable to large numbers of specimens and species, (2) should provide similar values for related species sharing similar morphological and ecoethological features, and (3) should demonstrate that differences in values are compatible with known data on behavior and ecology. It is therefore reasonable to expect that brain size of *Homo sapiens* should have the highest value.

3.09.2 Methods of Comparing Brain Size

3.09.2.1 Reliable Measurements of Brain Size

For valid comparisons, solid data based on reliable measurements and appropriate scaling methods are required. The volume of a brain obtained after histological treatment (fixation, embedding, dehydration) is markedly less than the volume of the fresh brain. The degree of shrinkage differs in each brain and depends on the type of fixation (Bauchot, 1967; Stephan *et al.*, 1981). To obtain comparable data, the volume values must be transformed to fresh brain volumes using a correction factor (fresh-brain volume/fixed-brain volume). The volume of

fresh brain is obtained by dividing the weight of the fresh brain by the specific brain weight (1.036 g cm^{-3}; Stephan, 1960). The same correction factor is used for all brain structures, though the shrinkage may not be uniform. Such differences may be neglected because they are difficult to ascertain and are likely to be small.

3.09.2.2 Standard Brain Size Values

It is practically impossible and to some extent useless to get average values for each brain structure in each species. In comparative studies, it is more constructive to invest in as many species as possible than in many specimens from one species. To obtain brain part volumes representative of a given species, they must be transformed into standard volumes by a conversion factor (C_{sta}) based on the species' average brain size, which is easier to obtain than the average volume size of the various brain structures (C_{sta} = weight of standard fresh brain/weight of individual fresh brain). Employing standards produces good approximations of the unknown mean from a few or even just a single specimen.

3.09.2.3 Problems in Brain Size Scaling

To scale brains by their absolute size is not valid because available data show that, for example, brain weight (BrW) varies within the same genus. In the genus *Myotis* (mouse-eared bats), BrW ranges from 124 mg (*M. muricola*) to 485 mg (*M. myotis*).

Several attempts have been made to compare brains by their relative size (RBrS). Within extant mammals, BrWs vary from 62 mg, in the shrew *Suncus etruscus*, to about 7800 g in the sperm whale (*Physeter macrocephalus*). The ratio between the smallest and any given brain could be used as a relative measure to evaluate brain evolution. However, such ratios are biased. Closely related species of different BrW have different ratios. Furthermore, large mammals such as whales have higher ratios than humans. To compensate for the influence of body size, Cuvier (1845) expressed RBrS as the ratio of BrW/BoW (body weight). But these RBrS measurements, called Cuvier's ratios, are in general high in small species (4.23% in *Sylvisorex granti*, 3.33% in *Suncus etruscus*) and low in large species (0.019% in *P. macrocephalus*, 0.086% in the African elephant *Loxodonta africana*). Such differences exist even in closely related forms. In shrews of the genus *Sorex*, RBrS varies from 1.99% in *S. palustris*, with a BoW of 14.6 g, to 3.26% in *S. cinereus*, with a BoW of 5.16 g. The RBrS of humans (2.05%) is roughly identical to that of the mouse but smaller than that in several shrews. Therefore, RBrS expressed either by BrW ratio between the smallest and any given brain or by Cuvier's ratio does not meet the requirements and is inappropriate for scaling brain size. RBrS values determined by the two methods progress in opposite directions. When BoW is ignored ($BoW^0 = 1$), the values increase from small to large species. When BoW is fully taken into account (BoW^1), the values decrease with increasing body size. This means that in a given taxonomic group, brain size increases less rapidly than body size. The power of the BoW that gives similar RBrS in closely related species must therefore lie between 0 and 1 (see Principles of Brain Scaling).

3.09.2.4 Determination of Relative Brain Size

3.09.2.4.1 The allometric formula Although it seems reasonable to assume that a large body needs more neurons than a small body to control muscles, the relationship is not linear. In a plot of brain size relative to body size, the points do not scatter around a straight line but around a curved line representing a decreasing BrW/BoW ratio. In other words, increased brain size does not keep pace with increased body size. Moreover, not all isoponderal (same BoW) animals have the same brain size. Snell (1892) was the first to produce a very simple equation characterizing the relationship between brain size and body size: $h = k^s\ p$, with h representing brain weight; k, body weight; p and s, psychic and somatic factors, respectively. Snell's work can be considered the starting point of modern quantitative studies on brain evolution.

His power function was extended by Klatt (1919), Huxley (1924), and others to a wide range of biological phenomena that are characterized by differential growth. The term 'allometry' was introduced by Huxley and Teissier (1936), who also proposed more generalized symbols to be used in the allometric formula $y = bx^a$. The exponent of allometry a corresponds to s, which, according to Snell (1892), represents the relationship between BoW and that part of the BrW that may be attributed to somatic factors. Huxley (1924) stressed that the allometric exponent is not a ratio between two sizes but between two growth ratios. The coefficient b, corresponding to p, represents that part of brain size that exceeds the proportion of the brain dependent solely on body size and reflects the contribution to brain size from nonsomatic sources. Jerison (1973) also considered the brain to be the sum of two independent, somatic and nonsomatic, components.

3.09.2.4.2 Calculation of RBrS Based on Snell's power function or Huxley and Tessier's allometric formula, it is easy to calculate $RBrS = BrW/BoW^a$. The power function represents a curved line which in its logarithmic transformation plots a straight line: $\log BrW = \log RBrS + a \times \log BoW$. In this form, the allometric equation becomes a powerful tool for showing the differential size increase of the brain relative to body size. A major problem in calculating RBrS is determining the value of the exponent *a*. It is either estimated from an assumed model of the expected relationship or found empirically by fitting the equation to a set of data. As early as 1867, the Russian anthropologist Alexander Brandt hypothesized that brain weight should be proportional to the surface of the body, because an animal interacts with the environment through the surface of its body. Snell (1892) proposed a power of 2/3 for estimating the surface area of the body. Dubois (1897) was the first to calculate the exponent *a* by using two species with the same psychic level and found values near 0.56. However, the reliability of Dubois' method must be questioned. It assumes that the two species used for the calculation are at the same evolutionary level, and thus assumes that the problem is already solved. There are no grounds whatsoever to claim that two related species differing in size occupy the same evolutionary level (Bauchot and Platel, 1973). A major breakthrough in calculating *a* and RBrS came with the use of logarithmic transformation of the power function (Huxley, 1924). The data points scatter around a straight line in which RBrS is the y-intercept, and the exponent *a* is the slope of the line. The great advantage of the log form is to ease statistical handling (Figure 1).

Since the data points representing the selected species of a sample do not lie on a straight line, an important question concerns the production of a best-fit line, which minimizes deviation of the variables from the line. Three methods are generally used to produce this line. (1) The least-squares regression analysis (e.g., Huxley, 1932), which is the most commonly used, minimizes the sum of squared vertical residuals. (2) The principal major axis analysis minimizes the sum of squared residuals perpendicular to the line (Jolicoeur, 1965). (3) The reduced major axis analysis minimizes the sum of the areas of triangles bounded by the best-fit line and lines connecting it with the data points, parallel with the respective coordinate axes (e.g., Kermack and Haldane, 1950). The least-squares analysis has often been rejected as inappropriate for studying allometry because it assumes that one variable is measured with no errors. However, both BrW and BoW are subject to random fluctuations (Kuhry and Marcus, 1977). Steudel (1982) maintained that BoW should be considered the independent variable because allometry is essentially the variation of a structure relative to body size. Nevertheless, when the data points are highly correlated, the three procedures give similar results (Sprent, 1972).

3.09.2.4.3 Standards for brain size comparison RBrS is usually considered brain size relative to BoW. However, BoW is rejected by several authors because

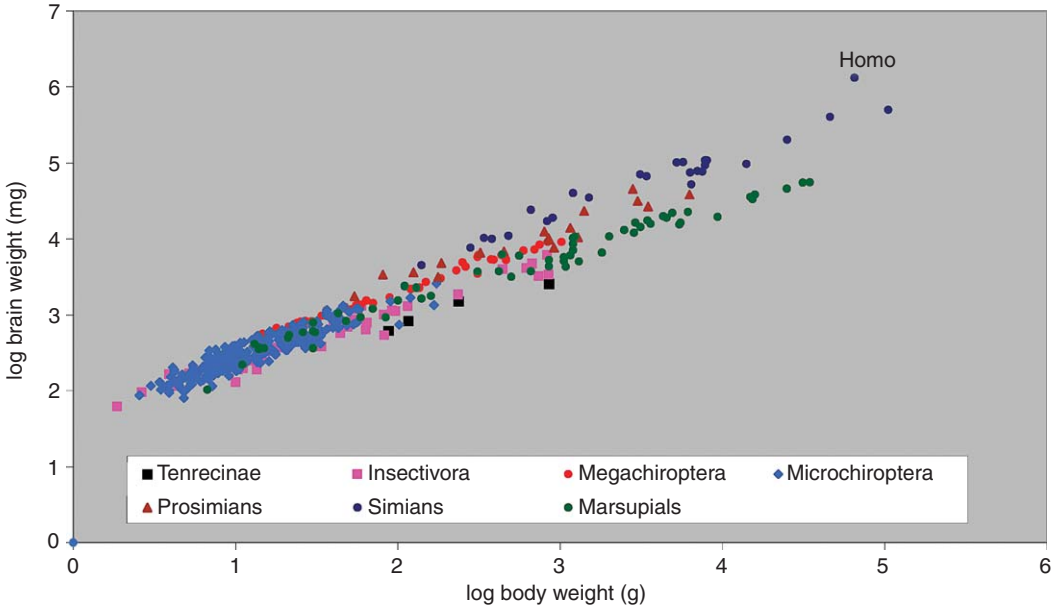

Figure 1 Brain weight (in milligrams) plotted against body weight (in grams) in a double logarithmic scale.

of strong individual variations due to actual food intake, gravidity in females, seasonal changes, age, and other factors. To avoid such problems, RBrS has sometimes been determined relative to body length. In some species, especially big mammals such as whales, body length is easier to measure than BoW. However, a one-dimensional measure is less accurate than BoW. Furthermore, length depends on shape. Long animals do not have larger brains than short animals of the same BoW (Stephan, 1954). A more frequently used standard of comparison is the size of a particular brain part, mainly the medulla oblongata, expressed as weight, volume, length, or cross-sectional area (e.g., Passingham, 1975) or the weight of the brainstem (e.g., Portmann, 1942). The use of the brainstem or medulla oblongata (OBL) as a substitute for body size is not recommended because it is not possible to determine their precise size macroscopically. Furthermore, they are not independent of brain size and are strongly influenced by higher brain centers and adaptations. Although the OBL is the most conservative of the major brain parts, the number of its pyramidal and extrapyramidal fibers is correlated with the isocortex (neocortex, NEO) and the striatum (STR). The sizes of the inferior olive and the ventral pons (VPo) increase with the cerebellum (CER) and NEO. VPo is often considered part of the brainstem and hence, the reference system. However, its percentages vary from 0.37% in *Echinops telfairi* (Insectivora) to 37.17% in *Homo* (Matano *et al.*, 1985a). The OBL of water-adapted animals is characterized by hypertrophy of the trigeminal nucleus (TR). In the semi-aquatic insectivore *Potamogale velox*, TR constitutes 21% of OBL, in contrast to 13% in the terrestrial hedgehog *Erinaceus europaeus*. Radinsky (1967) used the size of the foramen magnum as a standard for comparison when only skulls and no body size were available.

Despite intraspecific variations, BoW remains the most reliable standard for brain size comparisons. Since, in a given species, BoW is generally more variable than BrW, certain rules have to be applied in order to obtain the most reliable data. During ontogeny, the brain reaches adult size sooner than the body. The low RBrS found in animals living in captivity is due to their being overweight. Free-living chimpanzees were found to have BoWs uniformly around 46 kg (Stephan *et al.*, 1988), whereas values up to 80 kg are reported in the literature (Bauchot and Stephan, 1969). In general, the range of variation is rather small in wild animals. The brain can be considered an image of the body in reflecting not only differential muscle mass and sensorial areas but also the relative functional importance of various body parts. Furthermore, differences in body size also correspond to differences in the occupied niche.

3.09.2.4.4 Reference line slope Determining a common slope is crucial for interspecific comparisons of brain size. The slopes of the best-fit lines depend on the taxonomic level of the sample. It follows that the taxonomic level at which the allometric analysis is performed affects not only the slope, but also the residuals, which reflect size differences of the brain and its parts relative to the reference line. Authors who used samples belonging to several orders found slopes ranging from 0.66 (Bonin, 1937) to 0.91 (Stephan *et al.*, 1988). Many of them decided that the size increase of mammalian brains is best reflected by a slope of 0.75. Other authors have estimated the slope for lower taxonomic levels such as genera, families, or orders and found wide variation. Slopes of higher taxonomic groups are usually steeper than those of groups at a lower taxonomic level (Passingham, 1975). However, when using larger samples with a greater range of body size (>2), slopes become similar (Stephan *et al.*, 1988, 1991; Baron *et al.*, 1996). To avoid erroneous slopes, Rempe (1970) proposed a canonical method for determining the slope. This method gives each subfamily or family its appropriate weight when calculating a common slope. Taxonomic groups for which more data are available and/or those with a large BoW range have more influence on the slope than do those with poor data. For the total of 68 subfamilies or families of Insectivora, Scandentia, Chiroptera, Primates, and Marsupialia with more than one species examined, the common slope for brain size relative to BoW was found to be 0.66, which corresponds to the value postulated by Snell and others. The same method can be used to calculate the common slope for any brain part relative to BoW. The slopes of the five main components range from 0.58 (mesencephalon, MES) to 0.68 (telencephalon, TEL); the telencephalic components range from 0.57 (hippocampus, HIP) to 0.73 (NEO), and the brainstem sensory structures range from 0.50 (cochlear complex) to 0.62 (funicular complex). It follows that the size changes of different brain parts relative to body size take place at different rates (Table 1).

The slopes are important because of their effect on RBrS comparisons within species of different BoW. If a steep slope is used, RBrS systematically appears larger in species with small BoW than in species with large BoW within the same family. The RBrS values obtained from a steep regression line based on a mixed mammalian sample (mouse–elephant line) are often devoid of any biological

Table 1 Average progression indices of brain and brain structure volumes

	Slope	*y-intercept*	*MAM*	*MAR*	*INS*	*MIC*	*MEG*	*PRO*	*SIM*	*HOMO*
n			465	99	50	225	47	18	26	1
BrVol	0.66	1.506	237	194	154	185	289	409	800	3 010
OBL	0.61	0.776	144	146	132	142	137	153	176	187
MES	0.58	0.481	227	224	136	227	235	261	308	432
CER	0.68	0.552	306	222	167	309	320	459	633	2 057
DIE	0.65	0.350	281	256	169	216	374	523	706	1107
TEL	0.68	1.242	256	187	159	163	318	457	921	3 250
MOB	0.64	0.682	54	66	87	35	108	49	7	2
AOB	0.64	–1.903	276	642	138	218	0	414	61	0
PAL	0.63	0.799	62	74	103	44	97	63	38	55
AMY	0.60	0.232	154	122	114	166	164	170	210	401
SEP	0.61	–0.129	161	139	132	115	230	180	183	407
HIP	0.57	0.581	167	234	175	134	267	291	268	483
SCH	0.64	0.062	222	181	168	184	341	280	225	443
STR	0.67	0.089	302	223	207	240	424	542	830	1 393
NEO	0.73	0.346	896	512	292	509	1016	1902	4220	13 912

AMY, amygdala; AOB, accessory olfactory bulb; BrVol, brain volume; CER, cerebellum; DIE, diencephalon; HIP, hippocampus; INS, Insectivora; MAM, mammals; MAR, marsupials; MEG, Megachiroptera; MES, mesencephalon; MIC, Microchiroptera; MOB, main olfactory bulb; NEO, neocortex (isocortex); OBL, medulla oblongata; PAL, paleocortex; PRO, prosimians; SCH, schizocortex; SEP, septum telencephali; SIM, simians; STR, striatum; TEL, telencephalon.

meaning and may result in such oddities as a similar RBrS in *Homo* and small prosimians or higher values in small prosimians than in large simians. In contrast to the slope of the reference line, which determines the biological meaning of brain size and brain parts, the position of the reference line does not affect the RBrS ratios of different species.

3.09.2.4.5 The reference group A major problem in evolutionary biology is to decide which characters are primitive and which are advanced. Since it is impossible to obtain information on the quantitative composition of the brain in primitive fossil mammals, the Tenrecinae (Insectivora), probably the most primitive forms of extant mammals, were chosen as the reference group (basal Insectivora). Their brains are expected to be similar to those of the extinct forms considered forerunners of mammalian orders and thus provide the best possible approximation to the unknown brain structure of extinct primitive mammals. Stephan's (1967) progression or size indices (PIs or SIs) provide a simple and efficient tool for comparing the degree of evolution of brains and brain parts directly. The PI of a given brain structure in a given species is the ratio of the size (weight or volume) of that structure to the size of that structure in a hypothetical average isoponderal basal insectivore. A PI thus shows how many times a brain or brain structure is more or less developed in a given species than in a primitive insectivore of the same size. A critical evaluation of the use of PIs in statistical analyses was given by Jolicoeur *et al.* (1984).

3.09.2.5 Progression Indices of Brain Structures in Mammals

Major parts of the brain steadily increase in size from Tenrecinae (basal Insectivora) to Simians and *Homo* (Figure 2). The smallest increase in PIs was found for OBL, the largest for NEO. In humans, OBL is only 1.87 times larger than that of Tenrecinae and 1.42 times larger than the average index of Insectivora. The large brain components are heterogeneous structures, each containing cell populations relating to different functions. The OBL contains sensory nuclei receiving information about the environment as well as motor nuclei controlling precise muscle activities. The increased size of such large heterogeneous brain components does not likely reflect an increase in a specific function. Similar PIs may result from differential growth in functionally different brain centers (mosaic evolution, see Mosaic Evolution of Brain Structure in Mammals). For example, the PI of OBL (144) in the semi-aquatic *Neomys fodiens* (Insectivora) is similar to those of *Scotonycteris zenkeri* (146, Megachiroptera), *Molossus molossus* (145, Microchiroptera) and the marsupial *Aepyprymnus rufescens* (144). However, these animals differ considerably in the PIs of TR (194, 90, 97, 62) and those of the cochlear nuclei complex (CON) (53, 130, 209, 129) (Stephan *et al.*, 1991; Baron *et al.*, 1996) (Table 1).

The size increase of the MES from Insectivora to Primates is more pronounced than that of OBL. MES occupies the second place in Insectivora, Megachiroptera, and Primates. In Microchiroptera and marsupials, its enlargement is, however, greater

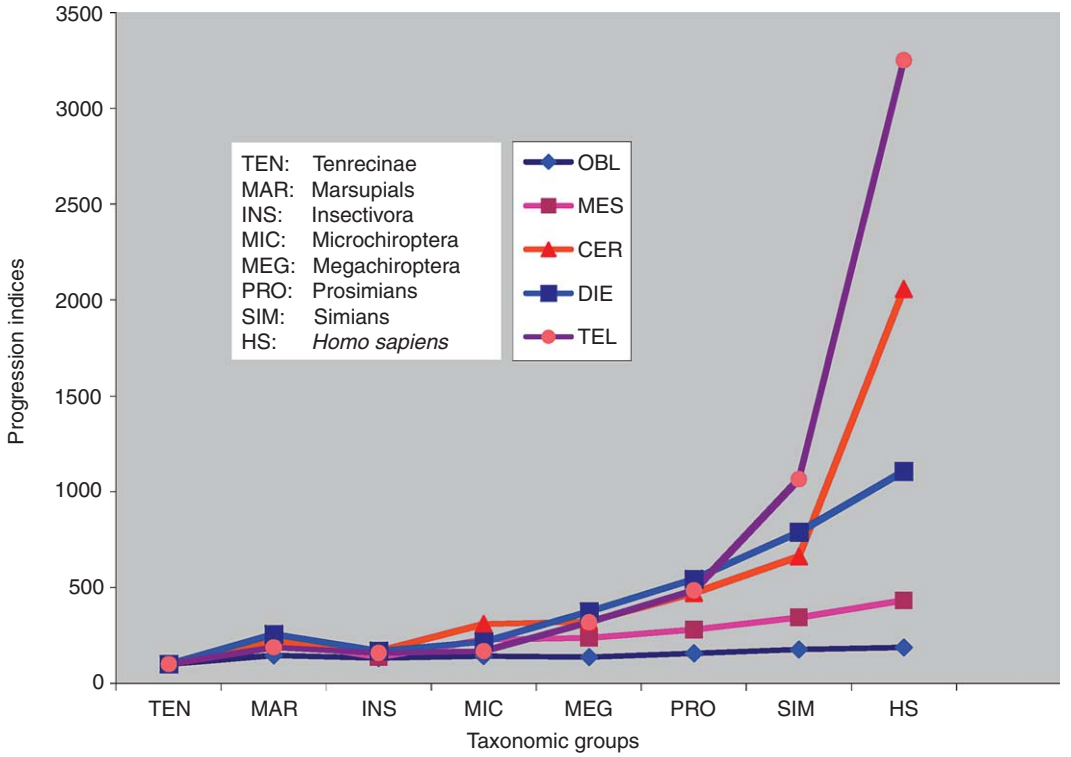

Figure 2 Progression indices of the five fundamental brain parts related to the average of the four species of Tenrecinae (PI = 100). Abbreviations for brain structures as in Table 1.

than that of TEL. The two components of MES, the tegmentum (MTG) and tectum (MTC), show divergent evolutionary trends in Chiroptera, with Megachiroptera having a larger MTG but smaller MTC than Microchiroptera. Of the tectal components, the visual colliculi superiores (SUCs) are better developed in Megachiroptera, whereas the auditory colliculi inferiores (INCs) are larger in Microchiroptera (Baron *et al.*, 1996). The two chiropteran suborders also differ in the quantitative organization of MTG. The auditory nuclei of the lateral lemniscus are more than three times larger in Microchiroptera than in Megachiroptera (Baron, 1974).

The size increase of the cerebellum (CER) is even greater than that of MES. In *Homo*, CER is 20.57 times larger than in Tenrecinae and 3.25 times larger than in nonhuman simians. In Microchiroptera, CER shows the greatest enlargement of the five fundamental parts. The size increase of the intracerebellar nuclei medial (MCN), interposed cerebellar nuclei (ICN), and lateral cerebellar nuclei (LCN) reflect the differential development of the three longitudinal zones of the CER (vermis-MCN, pars intermedius-ICN, hemisphere-LCN). The ICN is in all groups the smallest, while LCN is the largest or very close to the largest (Megachiroptera).The vermis-MCN component is concerned with postural tone, equilibrium, and locomotion of the entire body. The intermediate-ICN zone controls movements of the proximal limbs, and the hemisphere-LCN zone controls the distal parts. The evolutionary progress of CER in mammals is characterized by strong enlargement of the lateral zone. The medial zone is much less progressive and even smaller in apes and humans than in prosimians and monkeys. This evolutionary trend reflects the progression of complexity in motor patterns from simple movements to the very skilled movements of the hand and fingers (Matano *et al.*, 1985b). In bats, the high PIs of the LCN reflect the involvement of the anterior distal limbs in flight (Baron *et al.*, 1996).

The diencephalon (DIE) is clearly enlarged in the average Insectivora compared with the Tenrecinae. There is a steady increase from Insectivora to Primates and *Homo*. The average PI of the DIE of marsupials lies between those of Microchiroptera and Megachiroptera. In Insectivora, Megachiroptera, prosimians, and marsupials, it is the most progressive of the five encephalic main components. Studies of the ontogenetic development have revealed that DIE may be divided into four horizontal zones: the dorsal epithalamus (ETH), thalamus (THA), subthalamus (STH), and hypothalamus (HTH). Allometric

analysis in Insectivora and Primates reveals the existence of at least two major developmental gradients in the quantitative changes of the diencephalic zones during evolution. Though quite limited, the size increase of ETH and HTH is most accentuated in the earlier phyletic phases (from Insectivora to prosimians). THA and STH increase in a more continuous manner from Insectivora to higher Primates and *Homo*. The most progressive parts of the DIE have major direct connections with the NEO and the striatum (STR). ETH and HTH are functionally related to limbic structures (Baron, 1979).

The TEL is a highly heteromorphic brain part with purely cortical (NEO; schizocortex, SCH; HIP) and purely subcortical subdivisions (STR), as well as a mixture of cortical and subcortical elements (paleocortex, PAL; septum telencephali, SEP; amygdala, AMY). The olfactory bulbs are a special type of allocortex. Functionally, the TEL may be subdivided into olfactory components, limbic structures, motor centers, and higher brain centers. Only in simians and particularly in humans is TEL the most progressive of the five main components of the brain. In Microchiroptera and Marsupialia, its SI is second only to OBL.

Olfactory structures (main olfactory bulb, MOB; PAL) are clearly reduced in all taxonomic groups compared with Tenrecinae, except in Megachiroptera (Figure 3). PAL is a fairly heterogeneous brain part, with the components differing in their PIs. It comprises structures with PIs similar to those of MOB and structures characterized by a size increase. Of the olfactory cortices, the retrobulbar region (anterior olfactory nucleus) has similar PIs to MOB. In simians and especially in humans, the PIs of the prepiriform cortex and olfactory tubercle are clearly larger than those of MOB (Baron *et al.*, 1987). The deviations of PAL PIs from the trends in MOB may indicate changes in the functional role.

The limbic system (Figure 3) is composed of a group of moderately progressive structures comprising the HIP, SCH, SEP, and AMY. The progression of the entire limbic system is in Insectivora and Microchiroptera about 1.5, in marsupials 1.7, in nonhuman simians 2.2, in prosimians 2.3, in Megachiroptera 2.5, and in humans 4.4 times larger than in Tenrecinae. In nearly all taxonomic groups under consideration, HIP and SCH are more progressive than SEP and AMY. The only exception is in Microchiroptera, where HIP is clearly smaller than AMY.

The allometric size of AMY increases slightly in Insectivora (114) and marsupials (122) and moderately in Chiroptera (165) and prosimians (170). In nonhuman simians, it is more than twice (210) and in humans four times as large as in Tenrecinae. AMY can be divided into the lateral amygdala (LAM), or corticobasolateral group including magnocellular (MCB) and cortical nuclei, and the medial amygdala (MAM), combining several

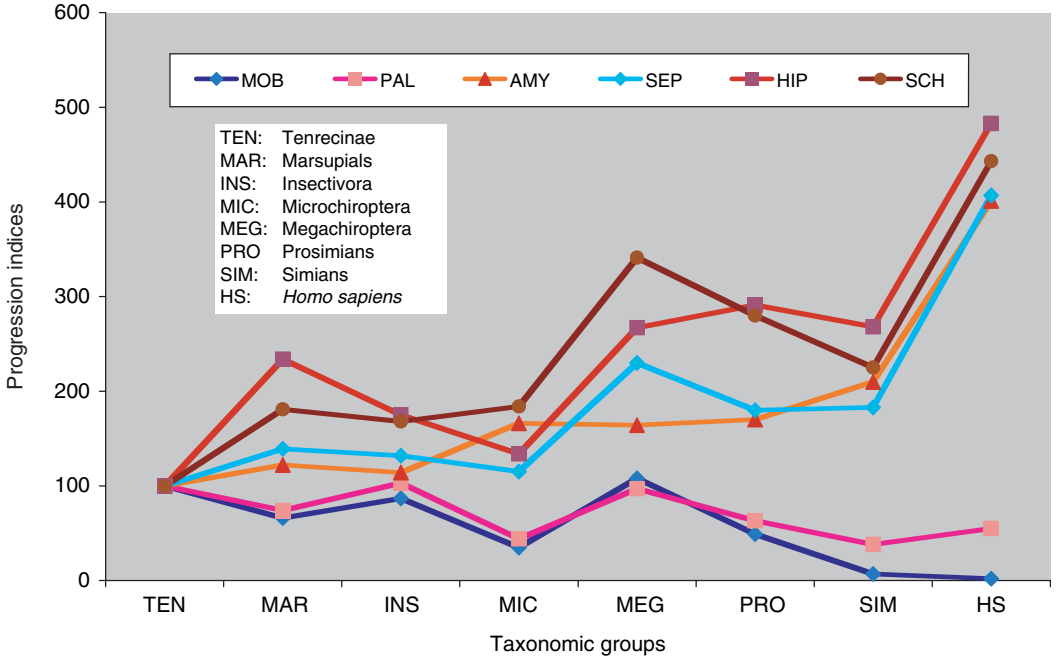

Figure 3 Progression indices of the olfactory and limbic brain structures related to the average of the four species of Tenrecinae (PI = 100). Abbreviations for brain structures as in Table 1.

distinct grisea. In all primate species, LAM has higher indices than MAM. LAM is best characterized by its connections with sensory systems in the NEO and PAL. Its progressive evolution suggests increasingly complex interactions with the environment. MAM is characterized mainly by its connections with autonomic centers in the brainstem (see Stephan *et al.*, 1988). The indices indicate that visceral functions are essentially the same in primitive and higher mammals.

In Microchiroptera, the PI of SEP (115) is lower than the average PI in Insectivora (132) and marsupials (139). In Megachiroptera, PI (230) is even larger than in Primates (182). In humans, SEP shows the same size increase (407) as AMY. Measurements of septal nuclei in some Insectivora and Primates (Andy and Stephan, 1966) indicate that all the major nuclei except for the nucleus septalis triangularis and bed nucleus of the anterior commissure contribute to the size increase of the septum during phylogeny.

The average PIs of SCH are highest in Megachiroptera (341) and prosimians (280). In humans, it is close to the PIs of the other limbic structures. The relative size within SCH of the two main components, entorhinal and presubicular cortices, do not change.

The average PI of HIP is lower in Microchiroptera than in Insectivora and lower in nonhuman simians than in prosimians. In *Homo*, HIP is the most progressive of the limbic structures. The data indicate that the progression of HIP is not continuous in the ascending primate scale. The marsupial average lies between those of Micro- and Megachiroptera. Not all of the HIP parts increase to the same extent. Investigations on HIP components from Insectivora through Primates show a marked enlargement of area CA 1 (see Stephan *et al.*, 1991).

STR (Figure 4) shows a steady increase in the eutherian taxonomic units under consideration. The index value in *Homo* is about 14 times as large as that of Tenrecinae. The most common function attributed to the STR is related to motor processes. However, there is increasing evidence of functions of the basal ganglia not easily characterized as motor. In fact, STR is not only connected with motor structures but also with the association cortex and limbic system.

Many studies support the conclusion of earlier investigations (e.g., Stephan and Andy, 1964) that the NEO is the most progressive and most discriminative component of the mammalian brain. The high progression indices may be considered the prime factor in mammalian evolution and indicative of the successful progress of mammals (see Cortical Evolution as the Expression of a Program for Disproportionate Growth and the Proliferation of Areas; Figure 4).

The differential growth of brain parts and nuclei as expressed by the progression indices clearly shows that adaptive radiation occurred at various taxonomic levels (e.g., Stephan *et al.*, 1988, 1991;

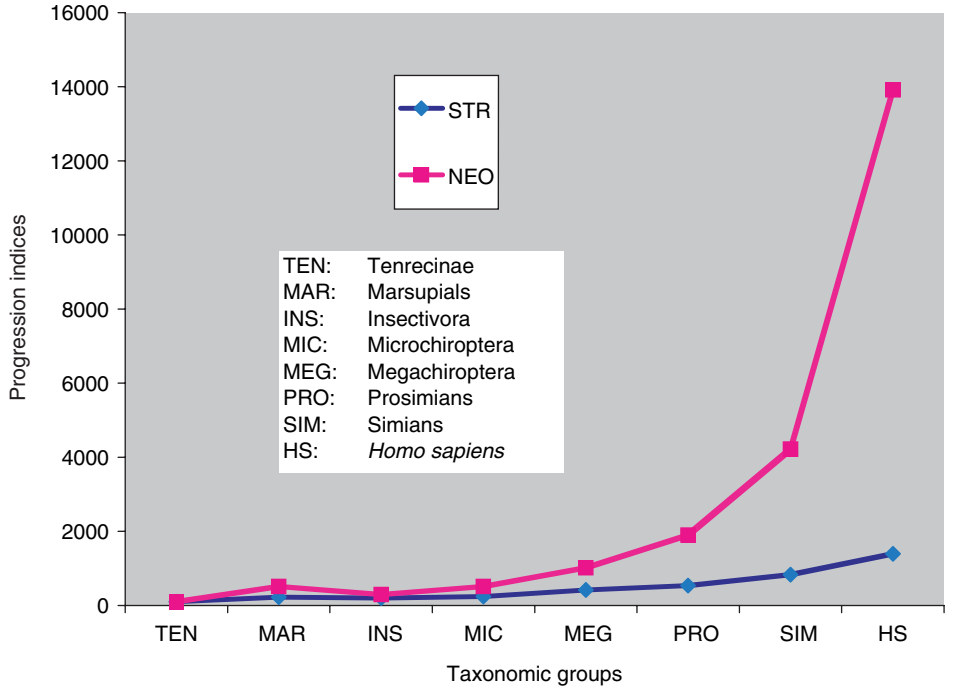

Figure 4 Progression indices of the STR and NEO related to the average of the four species of Tenrecinae (PI = 100).

Baron *et al.*, 1996). Thus, PIs measure differences between species by the amount of brain evolution that has occurred since their separation.

3.09.2.6 Brain Center Correlations

Brain components do not develop independently of each other. They are parts of a functionally integrated system, the output of which is coordinated behavioral patterns. One may, therefore, assume that most brain structures are more or less correlated in size, but that structures belonging to the same functional unit by virtue of direct fiber connections should show better correlation than those with no immediate functional relationship. However, to avoid misinterpretations, it should be stressed that strong correlation does not necessarily indicate functional interdependence. It is only one of the possible explanations. Correlations between the PIs of the major brain parts are almost exclusively positive and highly significant. The only negative correlations are with the olfactory structures MOB and PAL. However, none of the negative values even comes close to statistical significance, despite the large sample ($n = 466$). Moreover, the picture is largely the same for correlations at lower taxonomic levels except for Insectivora, with the only significant negative correlation being between olfactory structures and OBL, and for Microchiroptera and prosimians, with no negative correlations. The negative correlation in Insectivora is due to semi-aquatic species with their hypertrophied tactile trigeminal system and reduced olfactory system. The strongest correlations exist between NEO, STR, DIE, and CER, the highly integrative centers of sensory and motor modalities with multiple interconnections. Equally strong correlations in all taxonomic groups except simians are found for MOB and PAL, indicating direct projections from the MOB to PAL. The absence of correlation in simians is due to an increase of non-olfactory components during phylogeny. The correlations between limbic structures are highly variable and differ among taxonomic groups. Limbic structures are known to be interconnected by multiple pathways but differ in their precise function (Table 2).

3.09.2.7 Ecological Niche and Size Differences in Brains and Brain Parts

The brain reflects the complexity and specificity of a species' ecological niche. The characteristics of a niche that exert the strongest selective pressures on the survival of an organism and hence the evolution of its nervous system are (1) the trophic niche, including the type of food, its spatiotemporal distribution, and the feeding strategy, (2) the avoidance of predation, and (3) reproductive behavior. Other aspects of the ecological niche such as habitat structure and complexity, social organization and behavior, and other ecological descriptors may have a determining influence on the three groups of behavior and are widely interdependent. PI comparisons within each taxonomic group in which radiation of particular traits occurred independently clearly show the influence of similar selection pressures on brain evolution. To avoid similarity due to common selective pressures being confounded with similarity due to phylogenetic propinquity, authors use methods that correct for phylogeny, such as the independent contrasts technique (Felsenstein, 1985; Purvis, 1991; Pagel, 1992).

To understand the impact and nature of ecological pressures on brain evolution, structures with well-defined functions are more revealing than heterogeneous brain parts.

Numerous studies have shown that brain size and structure vary with ecoethological adaptations and in particular with dietary specialization (Pirlot and

Table 2 Correlations between the progression indices for 465 mammalian species

	MES	*CER*	*DIE*	*MOB*	*PAL*	*AMY*	*SEP*	*HIP*	*SCH*	*STR*	*NEO*
OBL	0.746	0.560	0.554	–0.029	0.025	0.482	0.386	0.446	0.408	0.492	0.440
MES		0.734	0.720	–0.173	–0.164	0.683	0.389	0.435	0.501	0.651	0.626
CER			0.817	–0.172	–0.145	0.674	0.344	0.587	0.515	0.865	0.880
DIE				–0.034	–0.017	0.530	0.549	0.707	0.661	0.924	0.871
MOB					0.934	–0.019	0.445	0.419	0.385	–0.058	–0.185
PAL						–0.017	0.479	0.403	0.347	–0.024	–0.133
AMY							0.180	0.399	0.335	0.593	0.527
SEP								0.598	0.709	0.393	0.378
HIP									0.803	0.697	0.537
SCH										0.565	0.421
STR											0.907

Abbreviations as in Table 1.

Stephan, 1970; Stephan and Pirlot, 1970; Baron, 1974; Eisenberg and Wilson, 1978; Clutton-Brock and Harvey, 1980; Baron *et al.*, 1996). It has been shown that quantitative brain composition varies not only with food type but also with the occupied foraging biotope. The impact of predation pressure on brain evolution has not received much attention. One reason may be that many defense mechanisms are related to social behavior and strongly depend on the habitat's characteristics.

The impact of sexual selection on brain evolution has been shown in several species. Sex-linked HIP size differences were found to be related to differences in home range size and in solving spatial problems related to navigation (Jacobs *et al.*, 1990). Size differences in hypothalamic structures implicated in both reproductive and social behavior are reported in dimorphic voles (Shapiro *et al.*, 1991)

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3.10 Organization of a Miniature Neocortex – What Shrew Brains Suggest about Mammalian Evolution

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Glossary

chemoarchitecture	The appearance of the fine structure of a brain area as revealed by various histological tissue stains.
encephalization	The relative size of the brain compared to the expected size of the brain for a given body weight.
eutherian	A placental mammal. Marsupial and placental mammals form the two major groups of extant mammals.
lissencephalic	Refers to a neocortex that is smooth, lacking gyri and sulci.
neocortex	The six-layered sheet of neural tissue that forms the outer layer of the mammalian forebrain.

3.10.1 Introduction

A well-documented trend in mammalian evolution has been an increase in absolute and relative brain size (Jerison, 1973). This is evident from fossil skulls of early stem mammals and brain endocasts that have been identified for some early eutherians. An outstanding review of the current understanding of early mammalian evolution and brain–body size relationships can be found in a recent text by Kielan-Jaworowska *et al.* (2004). From the available evidence, it seems clear that early mammals had small brains with little neocortex. As a result, small-brained mammals, particularly members of the order Insectivora, have been of long-standing interest for investigations of mammalian brain evolution (Ebner, 1969; Lende, 1969; Kaas *et al.*, 1970; Valverde and Facal-Valverde, 1986; Glezer *et al.*, 1988; Michaloudi *et al.*, 1988; Stephan *et al.*, 1991; Regidor and Divac, 1992). These species (Insectivora) are thought to resemble ancestral mammals in a number of respects. For example, fossil evidence suggests that ancestral mammals were shrew-like in size, habits, and external form.

Despite this crucial fossil evidence regarding the morphology of ancestral species and the overall size and proportions of their brains (Kielan-Jaworowska, 1983, 1984, 1986; Jerison, 1990; Kielan-Jaworowska *et al.*, 2004), the internal organization of ancestral brains cannot be determined from fossils. Instead, our best attempts to reconstruct this organization must come from analysis of the brains of extant species. The general approach is to examine the diversity of brain organization across modern lineages of mammals, and to determine those characters that are shared by most species and therefore likely inherited from a common ancestor, and those characters that are unique to specific lineages of mammals and therefore most likely independently and more recently evolved. Kaas (1982, 1987, 1989) provides several reviews of this approach and the conclusions that can be drawn from considering comparative studies of modern mammalian brains.

However, in addition to these comparative studies, it also seems reasonable to consider how brains are organized in modern species that resemble ancestral species in many respects. The aim of this article is to outline relatively recent results from investigations of shrew neocortex, because these species represent some of the smallest mammals on the planet and they resemble ancestral mammals in a number of respects. This is of course the major reason for the long-standing historical interest in the brains of small mammals that exhibit primitive characteristics.

At the same time, we must keep in mind that living species are not ancestors, and the mosaic nature of evolution may (as usually does) result in many primitive features combined with more derived traits that do not represent an ancestral condition. Nevertheless, small-brained mammals such as shrews can give us some insights into possible ancestral brain organization. By learning how the smallest mammal brains are organized, we can at

for sensory areas that 'may' have existed in early mammals with diminutive areas of neocortex.

It is possible that there are limitations on the size and form of small sensory areas due to functional and developmental constraints. If a minimal number of neurons is necessary for processing information in sensory areas, we might predict that cortical areas would occupy a greater proportion of cortex in very small-brained mammals. Alternatively, if we attribute much of the size increase of cortex in larger mammals to simply dealing with information from a larger body, then we might predict that sensory areas in very small mammals would be proportionally reduced in correspondence to their equally small bodies. Areas could overlap extensively in a tiny brain, as has been reported for S1 and M1 in the North American opossum (Lende, 1963; but see also Beck *et al*., 1996) or they might retain the sharp borders characteristic of virtually all primary and secondary areas of larger-brained mammals. If some characteristics of cortex organization are typical for very small brains, we might postulate similar conditions for ancestral species with little neocortex. Thus in addition to providing important comparative information regarding the organization of cortex in insectivores and mammals in general, studies of shrews with very small brains may help guide theories of cortical evolution by indicating which hypotheses about small-brained ancestral mammals are most tenable.

3.10.2 Brain Size in Ancestral Mammals

It is commonly stated in the literature that ancestral mammals had small brains with little neocortex. However, this is best appreciated by viewing some of the fossil evidence to scale. Figure 1 provides schematics of fossilized skulls redrawn in outline form from Kielan-Jaworowska *et al*. (2004). Early stem mammals are shown in Figure 1a. No attempt has been made to illustrate forebrain size for the early stem mammals as endocast data were not available. However, it is clear that the absolute size of the head was relatively small in these species, and that within the cranium, the forebrain occupied a relatively small proportion of the available space (see Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals).

Considerably more information is available from early eutherian mammal fossils (Figure 1b). These specimens were also small animals with relatively small heads; however, there is an apparently much larger space for the forebrain (see The Evolution of Encephalization). Although endocranial data are available for only four specimens from the Mesozoic (Kielan-Jaworowska *et al*., 2004), the relative sizes of

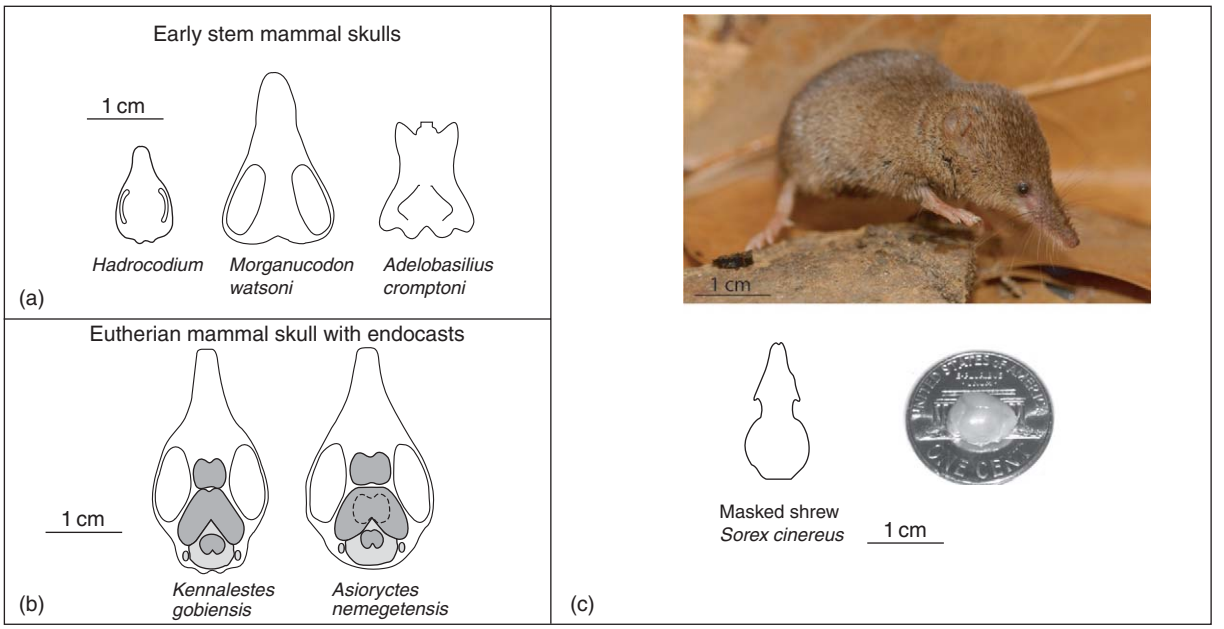

Figure 1 The skulls of early mammals compared to the skull and brain of a masked shrew. a, Reconstructions of skulls of some early stem mammals shown at true size. b, Schematics of early eutherian mammal skulls with endocast data showing brain sizes and subdivisions. The dotted line in *Asioryctes* indicates the possible location of the rhinal sulcus, suggesting these species may have had very little neocortex. c, A masked shrew with an outline of a masked shrew skull (bottom left) and a masked shrew brain on a penny (bottom right). Note that all illustrations are life size and at the same scale across the panels. Schematics for (a) and (b) are redrawn from illustrations in Kielan-Jaworowska, Z., Cifelli, R. L., and Lou, Z. X. 2004. Mammals from the Age of Dinosaurs. Origins, Evolution, and Structure. Columbia University Press.

the cerebral hemispheres, olfactory bulbs, and cerebellum are apparent. The presumptive neocortex was lissencephalic, and the two hemispheres were widely separated posteriorly such that the superior colliculi were exposed. One of the most interesting clues to brain organization in these species comes from the hint of the rhinal sulcus in *Asioryctes nemegetensis*. Evidence for this landmark is not conclusive; however, it does suggest, along with the exposed colliculi, that these early mammals had relatively little neocortex (see The Origin of Neocortex: Lessons from Comparative Embryology). In addition to this direct fossil evidence, it has been well established that neocortex occupies a proportionally larger part of the brain in large brains compared to small brains, in general, across different mammal species (e.g., Finlay and Darlington, 1995; Finlay *et al*., 2001; see The Evolution of Neuron Classes in the Neocortex of Mammals, Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications). This in turn means that neocortex occupies a proportionately smaller part of small brains in general.

From these considerations it seems clear that neocortex was relatively small in ancestral mammals, and that a major trend in evolution has been an increase in absolute brain size and in the size of the neocortex (see What Fossils Tell Us about the Evolution of the Neocortex). This makes the brains of shrews interesting to consider from a number of perspectives. Figure 1c shows a masked shrew, weighing only 3 g, an outline of a masked shrew skull, and a masked shrew brain on a penny – all at the same scale (and at the same scale as the skulls in Figures 1a and 1b). It is hard not to conclude, from the swollen appearance of the shrew's cranium compared to the appearance of the available fossils, that an increase in relative brain size (i.e., encephalization) has played an important role in mammalian evolution. Of course our interest in shrew brain organization stems from the comparatively small size of the brain compared to other extant mammals (Stephan *et al*., 1991).

3.10.3 Organization of Neocortex in the Smallest Mammals

Despite the long-standing historical interest in shrews for comparative brain research, it is only recently that microelectrode recordings were made from their neocortex to determine how these brains are organized. By examining five different species of shrews using a combination of electrophysiological recordings and analysis of flattened brain sections, a fairly complete picture of their cortical organization was obtained (Catania *et al*., 1999). Some of the most compelling data come from the brain of the diminutive masked shrew. This species approaches the lower size limits for mammals (approximately 3 g) based on physiological considerations (Schmidt-Nielson, 1984).

An important and convenient finding in this species was a series of chemoarchitectural subdivisions that delineated cortical areas. Although cortical recordings were not made in this particularly small shrew species, the subdivisions visible in the flattened cortical preparation correspond to similar areas from which recordings were made in other shrews, allowing the different subdivisions to be identified (Catania *et al*., 1999). Such visible subdivisions are not apparent in all mammal brains, but when they do occur, they provide a detailed picture of brain organization.

Figure 2a shows the flattened cortex from a masked shrew that has been processed for the metabolic enzyme cytochrome oxidase. The various locations of

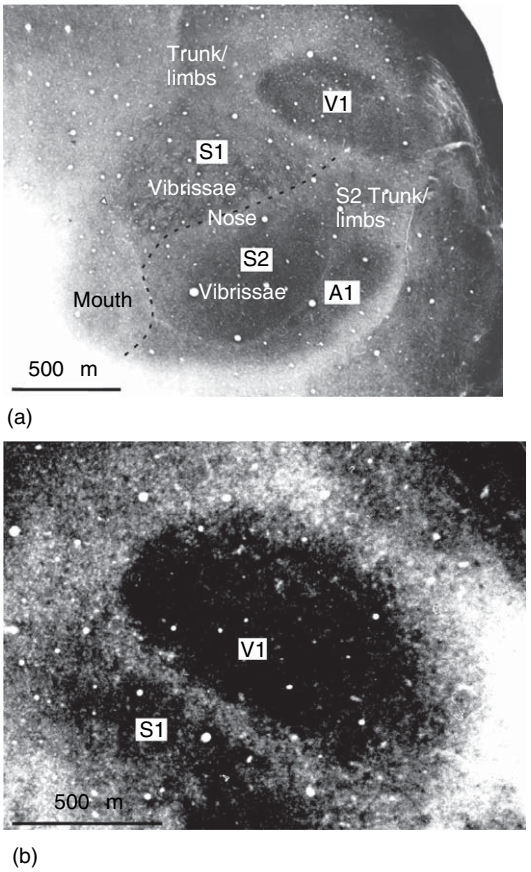

Figure 2 A section of cortex from *Sorex cinereus* (the masked shrew) demonstrating the direct adjacency of cortical areas in a single section processed for cytochrome oxidase. a, Each cortical area has a distinctive appearance, while subareas (such as mouth, vibrissae, and trunk) within S1 and S2 are also distinct. V1, primary visual cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; A1, primary auditory cortex. b, A close-up view of the border between S1 and the V1 in a masked shrew (opposite hemisphere from plate a). The small space suggests there are no intervening cortical areas.

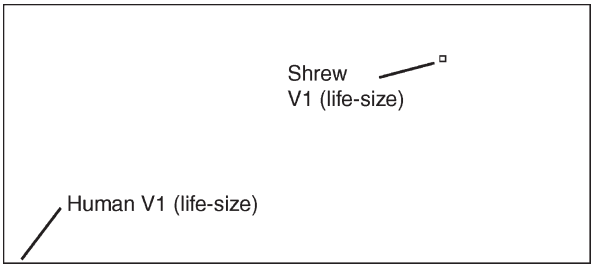

Figure 3 The relative size of primary visual cortex (V1) in a masked shrew (0.75 mm^2) compared to V1 in a human (approximately 2500 mm^2).

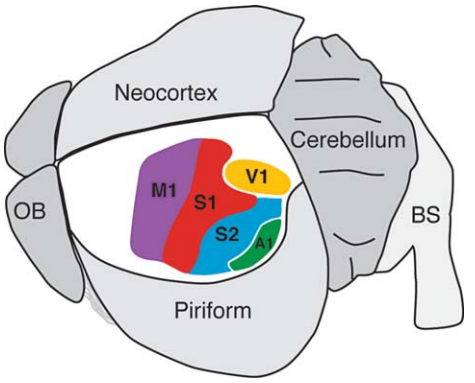

Figure 4 Summary of cortical organization in shrews. The sensory areas are located in caudal, lateral cortex. Two representations of the contralateral body surface correspond to S1, the primary somatosensory area, and S2, the second somatosensory area. Auditory cortex was found at the extreme caudolateral end of neocortex, partially surrounded by S2. Note the close adjacency of sensory areas. This condition seems to exclude the possibility of intervening higher level or association areas typical of other larger-brained mammals. OB, olfactory bulb; BS, brain stem; V1, primary visual cortex; A1, auditory cortex; M1, motor cortex. Adapted from Catania, K. C., Lyon, D. C., Mock, O. B., and Kaas, J. H. 1999. Cortical organization in shrews: Evidence from five species. *J. Comp. Neurol.* 410, 55–72.

different body-part representations are indicated. Evidence was found for a number of different cortical areas, including primary somatosensory cortex (S1), secondary somatosensory cortex (S2), primary auditory cortex (A1), and primary visual cortex (V1). In addition, a study by Nudo and Masterton (1990), examining the location of corticospinal projecting neurons, indicated the probable location of motor cortex (M1) in shrews (see Catania *et al.*, 1999, for details).

There are several obvious conclusions from these data. First, shrews have only a few cortical subdivisions. However, these areas are not overlapping, are delineated by chemoarchitectural features, and have sharp borders. The recording data from a number of other shrew species additionally suggest that these cortical areas are well organized internally (i.e., have topographic representations of sensory surfaces). Second, there is little or no room for intervening cortical areas between those visible in the cytochrome oxidase preparation. This is emphasized in an enlarged view of the S1–V1 border in the masked shrew (Figure 2b). For example, it is difficult to envision a secondary visual cortex (V2), with its characteristic topographic representation of the visual hemifield, compressed into the small space between V1 and S1. Finally, the absolute size of these areas provides a striking illustration of the changes that have occurred for some cortical areas in the course of mammalian evolution. Ancestral mammals probably had a V1 that was similar in size to V1 in shrews. Figure 3 is a schematic showing the actual area of masked shrew V1 (0.75 mm^2) compared to V1 in humans (about 2500 mm^2).

Figure 4 shows a summary of cortical organization in the masked shrew. This schematic illustrates the major finding, that the number of cortical sensory and presumed motor areas in shrews is extremely limited. Most mammals have at least some, and often extensive, cortical territory intervening between S1, V1, and A1. This does not appear to be the case in shrews, which have a relatively large S2 occupying much of this intervening territory. As a result, shrews appear to have fewer cortical areas than any other mammal that has been well studied.

3.10.4 Implications for Mammalian Brain Evolution

Comparative data from a wide range of mammals would suggest that ancestral mammalian cortex contained a number of cortical subdivisions, including at least S1, S2, a parietal ventral (PV) somatosensory area, V1, V2, probably at least three auditory areas including A1, in addition to a handful of motor areas. This conclusion stems from comparative studies in which these cortical areas were identified in virtually every studied mammal (for reviews, see Kaas, 1989, 1987; Krubitzer, 1995; Krubitzer *et al.*, 1993, 1995; Krubitzer and Kaas, 2005). The general idea is that characters, in this case cortical areas, found in a wide range of mammals are most likely homologous and therefore retained from a common ancestor. In contrast, those cortical areas that are found in only a few species (e.g., Broca's area) are likely to be more recent innovations unique to one or only a few lineages.

At the same time, however, it is important to consider, in addition to this comparative data, some of the constraints that may be imposed on brain organization based on the known morphology of ancestral mammals. Fossil evidence suggests that early mammals were relatively small and had small brains with little neocortex. Shrews are not

ancestral mammals, but they do have a tiny neocortex similar in size to that of the earliest mammals that gave rise to modern lineages. The results of studies investigating their cortical organization suggest that they have fewer cortical areas than would be predicted from comparative studies of other mammals, and indeed that they are unique in having fewer cortical subdivisions than any other mammal species that has been examined in detail. If this is a constraint imposed on small mammal brains generally, then it is possible that the earliest mammals also had very few cortical areas, perhaps fewer than would be predicted from comparative studies of larger mammal brains.

Acknowledgments

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3.11 The Effects of Domestication on Brain Size

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3.11.1 What is Domestication? What is a Domesticated Animal?

The domestication of animals can be defined as a special time-dependent and dynamic process whereby humans once started isolating some individuals of certain wildlife species and later began breeding these animals to provide a product or service useful to humans. Therefore, selection by nature is replaced by human-controlled 'artificial' breeding and selection of specific features for this animal group.

As is commonly recognized, the domestication process was a special revolutionary event during the cultural history of the human species, since no advanced civilization ever existed without domesticated animals and plants. This is still valid today when contrasting native human societies (e.g., bushmen, aborigines, and Indian tribes) who are still living as hunters and gatherers with the rest of modern human societies. The dynamics of domestication and its special consequences make for very interesting comparisons with their wild-type cohorts. Prior to domestication, nearly all members of a human society were involved in searching for their own food, whereas with progressive domestication in modern societies, only a relatively small group is involved in food 'production' and trade to supply the majority of the population with food, thereby allowing these individuals to become non-food-producing specialists (e.g., scientists, artists, politicians, businessmen, and soldiers). Additionally, with the enormous mass increase of the human population on earth, there is a corresponding increase in the number of many domesticated mammals. These increases led to further expansion of agricultural land with the consequence of suppression, destruction, and extinction of wild species and natural wildlife communities. Thus, domestication has wide-reaching consequences for the animal being domesticated, human evolution, and the niches that these nondomesticated animals occupy.

Domestication of animals began with the Paleolithic people. During the initial phase of domestication, they transformed from a nomadic shifting group to a more sedentary lifestyle with ensured resources for food, clothes, and other needs. According to archeological evidence, the first domestications of certain species happened at different times in the past, but most probably evolved independently in three major regions of the world: southwestern Asia and the Mediterranean region, South America from the Andes to Mexico, and East and Southeast Asia. Different domesticated animals and plants are characteristic for these different centers, although today there is some overlap in the animals domesticated. The first evidence of a domesticated mammal, the dog, is dated back to approximately 14 000 or 12 000 years ago. Domestication of the dog was followed by that of sheep and goats, approximately 11 000 BP, and then other animals later on. These data give a rough impression of the duration of domestication, although the actual start of this process most probably occurred even earlier. It must be remembered that bony remains of domesticated animals can be recognized only through differences from the original wild ancestor and these differences may not have emerged during the initial phase of domestication. Extremely long durations of domestication such as several thousand years are, however, valid for a few species. Several others were clearly domesticated much later and even recently some animals are still in the initial phase of domestication. This is particular true for some rodents, such as rats, mice, and gerbils, which were domesticated only a few decades ago. Nevertheless, these mammals may equalize their duration differences (in terms of number of years domesticated) with larger-sized 'older' domestications through apparently greater reproduction rates and generation shifts per year. Thus, when it comes to comparisons of this parameter, comparing years of domestication alone is problematic.

Of course, different domesticated mammals were selectively bred to serve very diverse human demands; e.g., for production of meat, fat, milk, and fur, as well as for work, research, and for use as pets. Differences in such usage occur not only from one species to another but within species as well. Consequently, selective breeding goals are numerous and constantly changing both within and across species.

As a group, domesticated mammals are not a taxonomic unit in a general zoological sense, but despite their numerous differences, they can be generally defined as a special group of animals thriving under human care. Once isolated from their natural niche, they were influenced and bred by humans for a greater number of generations. In contrast to their ancestors still living in the wild, domesticated animals as a group share a common 'ecological niche', the domestication, although this artificial niche has some degree of variability. In this sense, wild species kept in zoological gardens or circuses are not domesticated animals nor are other species or individuals under human care that are not purposely bred over many generations for special needs. Likewise, tame wild mammals are not domesticated. Taming and habituation may be of certain importance when selecting wild individuals for further breeding, but this is generally a modificatory effect and not a special phenomenon of domestication. Some domesticated animals may be rather untamed and aggressive and habituation is at least possible with wild as well as domesticated animals.

The number of wild ancestors of species that have been domesticated, often called the stem species, is rather small. Thus, only a few mammalian species of the five orders Rodentia, Lagomorpha, Carnivora, Artiodactyla, and Perissodactyla can be listed as having led to domesticated forms. These are summarized in Table 1. As a result of domestication, we recognize an enormous and arbitrary changeability and diversification of the organism. Domesticated forms are at first very much different than the

Table 1 Ancestral wild mammalian species and their domesticated descendents

Wild species	*Domesticated relatives*
Rodentia	
Mus musculus (House mouse)	Laboratory mice
Rattus norvegicus (Norway rat)	Laboratory rats
Ondatra zibethicus (Muskrat)	Ranch muskrats
Meriones unguiculatus (Mongolian gerbil)	Laboratory gerbils
Mesocricetus auratus (Syrian hamster)	Golden hamsters
Cavia aperea (Cavy)	Guinea pigs
Chinchilla laniger (Chinchilla)	Ranch chinchillas
Myocastor coypus (Coypu)	Ranch coypus
Lagomorpha	
Oryctolagus cuniculus (European wild rabbit)	Rabbits
Carnivora	
Mustela putorius (Polecat)	Ferrets
Mustela vison (American mink)	Ranch mink
Canis lupus (Wolf)	Dogs
Vulpes vulpes (Red fox)	Ranched silver foxes
Alopex lagopus (Arctic fox)	Ranched blue foxes
Felis silvestris (European wild cat)	Cats
Procyon lotor (Raccoon)	Ranch raccoon
Perissodactyla	
Equus przewalskii (Przewalski horse)	Horses
Equus africanus (African wild ass)	Asses
Artiodactyla	
Sus scrofa (European boar)	Pigs
Llama guanacoe (Guanaco)	Llamas and alpacas
Camelus ferus (Wild camel)	Dromedaries and Bactrian camels
Rangifer tarandus (Wild reindeer)	Domestic reindeers
Bubalis arnee (Arni, Indian wild buffalo)	Water buffalos
Bos primigenius (Aurochs)	Taurine cattle
Bos mutus (Wild yak)	Domestic yak
Bos javanicus (Banteng)	Bali cattle
Bos gaurus (Gaur)	Gayal or mithan
Capra aegagrus (Bezoar goat)	Goats
Ovis ammon (Mouflon)	Sheep

ancestral wild type in outer appearance, anatomy, physiology, ethology, and other biological parameters. In addition, domesticated mammals that derived from one wild ancestor are also impressively different, which becomes clear through differences between certain breeds, races, or strains. Very often extremely small 'dwarfs' or larger-sized 'giant' breeds were 'created' through domestication. Whereas the wild ancestors appear rather uniform and only slightly different from subspecies to subspecies, the domesticated derivatives are extremely different. This is especially important for a zoological interpretation and understanding when contrasting the results of domestication as a consequence of artificial human interference with evolutionary phenomena that occurred under natural conditions.

3.11.2 Domestication versus Evolution

Domestication and evolution are, in principle, characterized through the changeability of the animal, although the way in which changes occur for each process is different. However, both of these processes generally can be assumed as having started from an individual intraspecific variability of a wild species and a common gene pool. Only a certain part of the wild population, most probably individuals that had already gradually preadapted to future domestication, were isolated and followed the special genetic drift under artificial selection. Depending on the domestication time, this selection resulted in a special radiation of different breeds. It is, however, important to point out that no new species ever occurred due to domestication. All of the many well-defined different races of dogs, cats, and breeds or strains of other domestications are still the same species as their ancestors. Domesticated mammals still can, and in several cases they frequently do, interbreed with their wild relatives of common ancestry and produce fertile offspring. Changes from a wild ancestor to domesticated derivatives consequently constitute an intraspecific (within species) phenomenon. However, the variability of domesticated forms deriving from one ancestral wild type alone is especially great and impressive. This demonstrates, in principle, the general potency for changeability of the organism no matter what selective forces are in power, be it natural or 'human-designed'. Although changes due to domestication are not recognized within the variability of a wild species, these changes must at least derive from the wild type gene pool. In fact, this had already been recognized by Darwin (1868) in pre-Mendelian times, when he designated domestication as the greatest experiment man ever made with animals, that is, it is the greatest experiment with respect to the time it takes to domesticate, the number of individuals involved, and the results obtained. Diverse aspects of the domestication phenomenon are treated in the books of Clutton-Brock (1987), Mason (1984), Herre and Röhrs (1990), and Zeuner (1963), to name only a few.

Evolution, on the other hand, also is assumed to have started from an intraspecific variability of a species, but certainly with otherwise preadapted forms in a different genetic drift. This occurred during long geological times and by phylogenetic radiation to the origin of new species, reproductively isolated, and also different from the ancestor from which they radiated and other newly emerged sibling species. These changes are connected with special adaptations to certain lifestyles. In an interspecific (between species) comparison, differences in biological parameters for recent wild types are evident.

Given this scenario, and the ideas regarding domestication and evolution that have emerged, it is of interest to investigate and interpret the effects of domestication on the size and structure of the central nervous system.

3.11.3 Domestication and Total Brain Size

Rough estimates of the domestication effect on the brain size can be obtained through comparisons of brain cavity sizes of domesticated derivatives with those of their wild ancestors. Such investigations had already been performed at earlier times (e.g., Darwin, 1868; Klatt, 1912, 1921) using more or less bulky, but mostly anonymous samples of museum materials. As is usually common in skull comparisons, these studies used divariate analyses evaluating brain cavity sizes with reference to the greatest skull lengths. This was performed because brain size is dependent on body size and the greatest skull length of wild species normally serves as an indicator of body size. Such analysis generally resulted in smaller brain cases for the domesticated forms than for their wild ancestors with identical skull length.

However, comparisons of this kind are problematic for two reasons, especially when it comes to quantitative estimates of a domestication effect. First, in most cases, domestication has additionally influenced skull dimensions, as well as the total skull proportioning. Thus, in a very arbitrary way, skull conformity, including the skull length, also changed

due to domestication in a special mosaic mode (see Mosaic Evolution of Brain Structure in Mammals). Consequently, this reference measure does not reflect the body size of wild and domesticated forms to the same degree and therefore is not a reliable reference. This fact becomes especially clear through comparative investigations performed on identical materials concerning the three relationships of brain cavity size (third root)/skull length versus brain weight/body weight, and skull length/body weight (third root) for wild species and their domesticated relatives (Sorbe and Kruska, 1975; Kruska and Sidorovich, 2003). At comparable body weights, domesticated mammals normally have smaller and shorter skulls. A second problem is that brain cavity sizes are not always consistent with the actual brain sizes. Differences between these two parameters occur. Normally these are greater in larger skulls than in smaller ones. This is a problem even within mammalian species, such as in dwarf versus giant races of a domesticated species (Röhrs and Ebinger, 1983). Consequently, comparative analyses on skulls may be of help in characterizing trends, but not for specific details of brain organization.

More accurate quantitative results can be obtained if fresh brain weights are obtained from a large sample of healthy adult individuals whose body weights are known. Although such data are seldom available, there have been some samples of brains from several individuals of wild ancestry and related domesticated forms including different races, breeds, or strains. This material allows allometric calculations of the brain size to body size relationship for both the wild progenitor and the domesticated derivatives. In this respect, it is especially noteworthy to recognize that in this case, intraspecific analyses are dealt with. As commonly known, in a double log plot, the relationship of brain to body size can be characterized by the linear function with the formula

$$\log y = a \log x + \log b,$$

where y is the brain weight, x is the body weight, a is the slope of the allometric line, and b is the intercept with the y-axis in $x = 0$.

In such double log plots, the individual data points from differently sized individuals are arranged in a distribution ellipsis and allometric lines generally can be calculated in different manners. However, the main axis, at best, expresses the position of such distribution ellipses and consequently is better suited to comparisons.

Such investigations, respectively contrasting wild with domesticated forms, were performed in the past for several species (see Kruska, 1988, 2005) and they have produced similar results for diverse species in several ways.

At first, slope values of intraspecific allometrical calculations are rather similar for diverse species. They rank between $a = 0.20$ and $a = 0.30$, normally $a = 0.25$. Only occasionally do they reach $a = 0.40$, especially in species with a very prominent sexual dimorphism (see Evolution of the Sexually Dimorphic Brain). Consequently, these intraspecific lines clearly are less steep than interspecific lines when diverse species with a similar encephalization are concerned. These slope values are between $a = 0.56$ and $a = 0.63$. Second, the data from the wild ancestors are associated with one allometric line and those from the domesticated derivatives are associated with a significantly different allometric line. Both of these lines show identical slopes and thus run parallel to one another. This undoubtedly means that domestication has not affected the general dependency of the brain on body size or vice versa, but domestication has changed the brain size. Third, the domesticated individuals very often show a greater variability of brain size at any given body weight. Their distribution ellipses of data clearly are wider. This indicates that the selection pressure on brain size in the domestication process is less strong than natural selection. Fourth, on average, domesticated mammals have smaller brains than their wild ancestral relatives of similar body size. No brain size increase for the domesticated forms has ever been documented and most likely will never be obtained in the future. Moreover, to learn how strongly domestication has affected brain size on average, differences can be calculated from the $\log b$ values of the two parallel lines. This results in species-specific percentage decrease values for the domesticated compared with the wild ancestral type (=100%). As an example, Figure 1 shows the situation for wild European boars (*Sus scrofa*) versus domesticated pigs (*S. scrofa* f. dom.). Independent of body size, brain size decreased by 33.6% in pigs due to domestication. This result is also depicted in Figure 2.

Comparable investigations for some other species resulted in species-specific average decrease values due to domestication for the total brain size independent of body size. These data are listed in Table 2, which demonstrates that the dimensions of the decrease due to domestication vary considerably and range from 0% in laboratory mice to 34% in pigs. However, as is also evident, the brains of the less encephalized species of Rodentia and Lagomorpha have decreased relatively little and range from 0% in laboratory mice to 15% in laboratory gerbils. Moreover, with the exceptions of laboratory mice

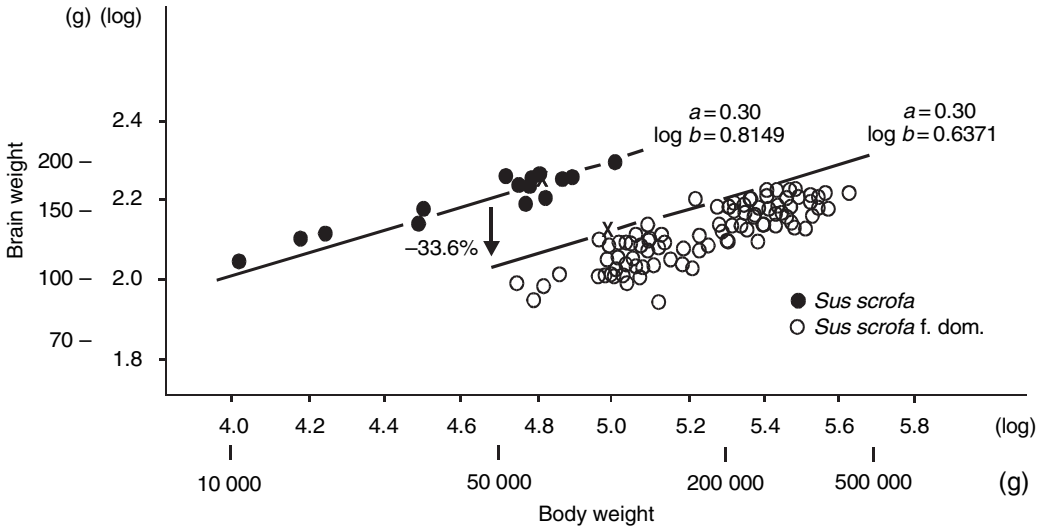


Figure 1 Intraspecific relation of brain to body size in European boars (*S. scrofa*) and different pig races (*S. scrofa* f. dom.) with average allometric lines. The body weights of boars are net body weights (total body weight minus viscera); those of pigs are actually total body weights. The allometric line for pigs therefore is displaced to the left by 45% subtraction of total body weight (10% for viscera, 35% for fat) to make this parameter comparable for both groups. Reproduced from Kruska, D. 1970. Vergleichend cytoarchitektonische Untersuchungen an Gehirnen von Wild- und Hausschweinen. *Z. Anat. Entwickl. Gesch.* 138, 291–324, with permission.

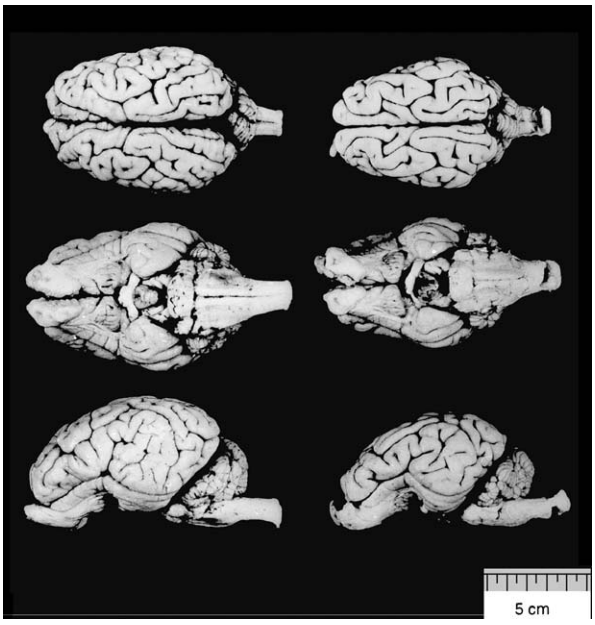


Figure 2 The brains of a European boar (left) and a pig (right) in dorsal, ventral, and lateral views. The boar and the pig were of comparable net body weight; European boar, 53.0 kg; pig, 52.8 kg. Reproduced from Kruska, D. 1970. Vergleichend cytoarchitektonische Untersuchungen an Gehirnen von Wild- und Hausschweinen. *Z. Anat. Entwickl. Gesch.* 138, 291–324, with permission.

Table 2 Body size-independent average values for total brain size decrease (DV) from the wild ancestral (=100%) to the domesticated types as resulting from intraspecific allometrical calculations

	DV (%)
Rodentia	
Mus musculus	0
Rattus norvegicus	
(Wistar albino)	−8
(DA pigmented)	−12
Meriones unguiculatus	−15
Cavia aperea	−13
Lagomorpha	
Oryctolagus cuniculus	−13
Carnivora	
Mustela putorius	−29
Mustela vison	−20
Canis lupus	−29
Felis silvestris	−28
Perissodactyla	
Equus przewalskii	*c.* −16
Equus africanus	*c.* −16
Artiodactyla	
Sus scrofa	−34
Llama guanacoe	−18
Ovis ammon	−24

For authorships of studies, see Kruska (2005).

and albino laboratory rats, the values from 12% in pigmented laboratory rats to 15% in laboratory gerbils are rather similar for four very diverse species (*Rattus norvegicus*, *Meriones unguiculatus*, *Cavia aperea*, and *Oryctolagus cuniculus*). In contrast to this, the decrease values for the more strongly encephalized Carnivora, Perissodactyla, and Artiodactyla species clearly are greater. They range from 16% in

horses and donkeys to 34% in pigs. Here again, four species (*Mustela putorius*, *Canis lupus*, *Felis silvestris*, and *S. scrofa*), which are ethologically very different, show rather similar and very large decrease values in brain size of approximately 30%.

These data indicate that there is a relationship among evolution, encephalization, and domestication. Species that have larger brains through natural selection and evolution appear to lose more brain tissue during the domestication process than do those species with naturally smaller brains. In addition, the rather similar decrease values of very different domesticated species led to the following conclusion: it seems that the convergent domestication effect is comparable to other convergent effects in phylogeny based on similar ecological niche adaptations that were reached by diverse species in different radiations and on different evolutionary plateaus (e.g., aquatic, arboricole, underground lifestyles). During domestication – of course – this is the case on an intraspecific level concerning brain size with exclusively regressive trends. Whereas changes in overall brain size due to domestication are of interest, the next important step in understanding the domestication process is to characterize the effect of domestication on individual and diverse parts of the brain.

3.11.4 Domestication and Brain Subdivisions

The question now is: are all the diverse subdivisions of the brain reduced to the same extent due to domestication as is the whole brain? This was addressed by comparative quantitative analyses in diverse species through equidistant serial sectioning of several individual wild type versus domesticated brains (Kruska and Stephan, 1973). This method allows calculations of fresh brain tissue volumes of diverse brain parts and adjacent allometrical comparisons of average data. Decreased values due to domestication are available for some species for the five fundamental brain parts including the telencephalon, diencephalon, mesencephalon, cerebellum, and medulla oblongata. Measurements of these structures resulted in a large range of different decrease values as is comparatively scaled in Figure 3. As can be seen, little conformity is evident between the diverse species concerning the arrangements of these brain parts from greatest to least decrease. However, with the exceptions of gerbils and mink (two species that were relatively recently domesticated), all the other species consistently show the telencephalon as having decreased to the greatest extent of all the brain parts. This is especially impressive since the investigated wild-type forms are differently encephalized. Very

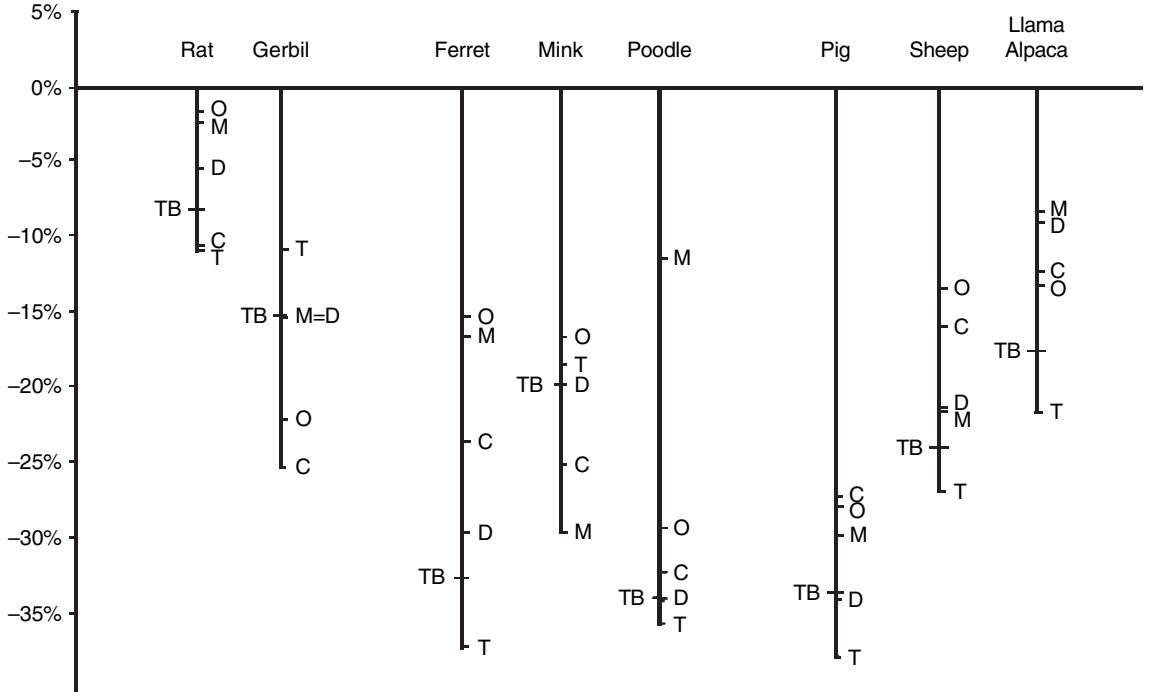


Figure 3 Scaling of size decrease values of total brain and the fundamental brain parts of several species from the wild to the domesticated forms (for authorships of studies, see Kruska, 1988, 2005). TB, total brain; T, telencephalon; D, diencephalon; M, mesencephalon; C, cerebellum; O, medulla oblongata.

roughly, such differences in encephalization on two general plateaus result from comparisons of relative values; e.g., in species of Rodentia, the telencephalon accounts for only 54% (wild gerbil) to 56% (Norway rat) of total brain size, whereas the corresponding values for species of Carnivora and Artiodactyla clearly are greater (polecat/American mink 69%; wolf 82%; European boar/mouflon/guanaco 72%).

In addition to these similar effects of domestication in several different species, it appears that within the telencephalon, the total neocortex (white and gray matter) has decreased to the greatest extent compared to other subdivisions of the telencephalon and even the total brain. This is indicated in Figure 4. Thus, those brain parts in which higher level functions are generated, such as the neocortex, are most affected by domestication. The implication is that under natural conditions, the neocortex, compared to structures that perform more life-sustaining functions, such as the nuclei of the brainstem, is expendable. Although similar decreases in the size of the neocortex are evident in an interspecific comparison,

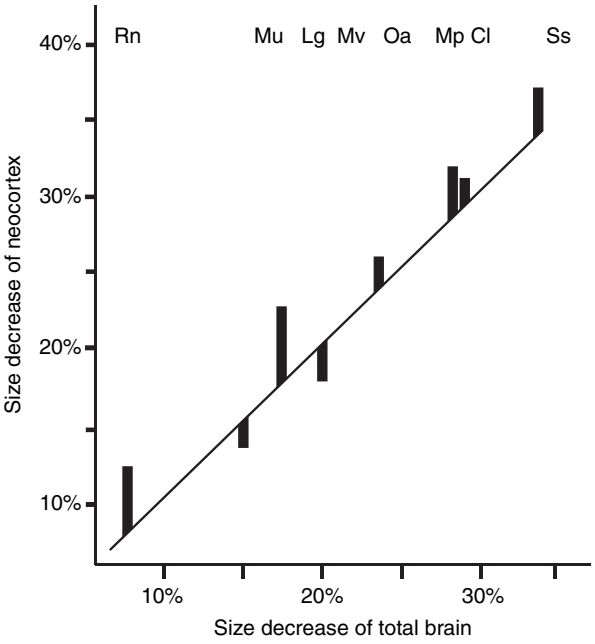


Figure 4 Relation between size decrease values of neocortex and total brain due to domestication in several species. The line serves as an orientation assuming that neocortex has decreased to same extent as total brain. Thus, bars above the line indicate a greater decrease intensity for the neocortex; those below the line indicate a lesser decrease intensity for the neocortex. Rn, *R. norvegicus*; Mu, *M. unguiculatus*; Lg, *L. guanacoe*; Mv, *Mustela vison*; Oa, *Ovis ammon*; Mp, *Mustela putorius*; Cl, *Canis lupus*; Ss, *Sus scrofa*. Reproduced from Kruska, D. C. T. 2005. On the evolutionary significance of encephalization in some eutherian mammals: Effects of adaptive radiation, domestication, and feralization. *Brain Behav. Evol.* 65, 73–108, with permission.

decrease values vary for different brain subdivisions intraspecifically as well. This is particularly true for several nuclear masses that serve certain functional systems, e.g., sensory systems for olfaction, vision, and hearing; motor structures of pyramidal and extrapyramidal quality; and limbic structures. Such species-specific decrease values are listed in Table 3. Consequently, all these diverse interior structures have also decreased from the ancestral wild form to the derived domesticated form. These data show a very variable mosaic picture regarding the decrease intensity not only between species, but also within species from one functional system to another and within the systems as well. Furthermore, concerning the latter, there are some exceptions; e.g., the olfactory bulb as a primary center of olfaction is more strongly reduced in contrast to secondary olfactory areas of the allocortex in gerbils, mink, pigs, and sheep, but this is not the case in microsmatic llamas. Similar results for decrease values were obtained for visual structures, such as the lateral geniculate nucleus versus the visual cortex. Both of these structures are visuotopically organized and one would expect rather similar decreases in both these nuclear masses. However, this is the case only for pigs, whereas in rats and mink the lateral geniculate nucleus is more strongly decreased than the striate cortex and in sheep this pattern is reversed. Again, within the auditory system of pigs, the cochlear nucleus and the medial geniculate body are decreased to a lesser extent than are the other structures, especially the auditory cortex. Similar inconsistencies occur for diverse motor structures.

Also of interest is the effect of the domestication process on size changes of limbic structures. In the mammalian telencephalon, these are mainly represented by the complexly organized hippocampus, the septum, the schizocortex (entorhinal region, pre- and parasubiculum), and some medial nuclei of the amygdala. These brain structures are interconnected with the anterior nuclei of the thalamus, with the habenular complex, and most prominently with the hypothalamus; together these form the limbic system. This system is in general assumed to represent the 'visceral brain' (MacLean, 1992). The hippocampus is the main center of this system and, although influenced by other brain regions (including the neocortex), it mostly acts endogenously. Thus, this nuclear formation plays an important functional role in several behavioral complexes, such as emotionally guided behavior and individual self-protection, but additionally in learning and memory. The hippocampus itself shows, of course, its own evolutionary changes with size increase and progressive structural differentiation in diverse mammalian radiations but

Table 3 Percentage decrease values of brain structures from the wild ancestral type to domesticated forms that serve in different functional systems

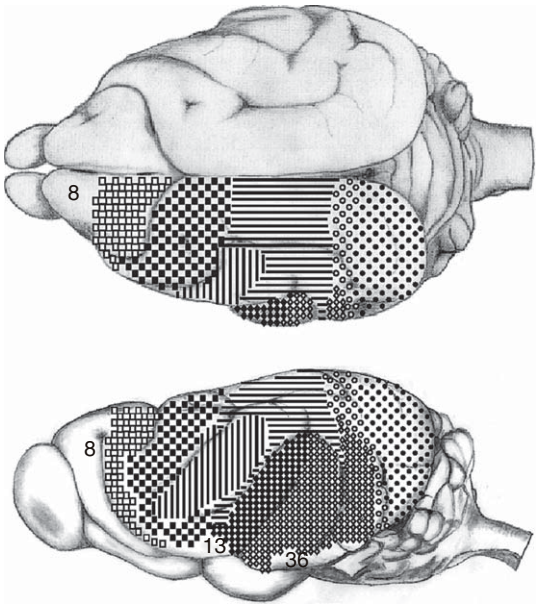
	Rat	*Gerbil*	*Mink*	*Poodle*	*Pig*	*Sheep*	*Llama*
Olfactory structures	−6	−9	−25	−33	−31	−22	−4
Visual structures	−4		−(27)		−41	−26	
Lateral geniculate body	−16		−(22)		−39	−25	
Superior colliculi	−3		−(26)		−32	−12	
Striate area (gray matter)	−12		−15		−41	−30	
Auditory structures					−30		
Cochlear nucleus					−15		
Superior olive					−28		
Lateral lemniscus					−33		
Inferior colliculi					−28		
Medial geniculate body					−20		
Auditory cortex (gray matter)			−16		−32		
'Motor' structures							
Cerebellum	−10	−25	−25	−32	−27	−16	−12
Corpus striatum	−11	−8	−16	−27	−29	−21	−9
Area gigantopyramidales (4 a.Br.)			−26			−30	
Area frontalis agranularis (6 a.Br.)			−25				
Limbic structures	−10	−4	−17	−34	−41	−35	−6
Hippocampus	−12	−1	−17	−42	−44	−41	−3

For authorships of studies, see Kruska (2005); values in parentheses are preliminary data that have not yet been published.

basically certain emotional reactions, especially aggression and other affective functions, general attention, as well as motivating and activating functions certainly seem to be guided, controlled, and regulated by this limbic center.

All of the various telencephalic structures of the limbic system are also smaller in domesticated mammals than in their respective ancestral wild-type forms. This can be seen in Table 3 for the sum of these structures and the entire hippocampus as well. Here again, some inconsistencies are evident when decrease values are compared within species, and such inconsistencies occur more prominently in a between-species comparison. Nevertheless, most notably the hippocampus decrease values are extremely high for poodle, pig, and sheep. In these highly domesticated mammals, this main limbic center is decreased in size by over 40%. This is a most remarkable degree of mass decrease, which exceeds even the decrease value for total brain and neocortex size. The fact that this brain part is characterized by very densely packed neurons suggests that the size changes induced by domestication might have a very special and immense functional effect on the hippocampus. Such functional and behavioral changes may enable humans to keep and handle large mammals without danger. Humans most probably have sought these changes unconsciously or consciously through breeding, especially during the initial phase of domestication. Some domesticated species, such as llamas, do not show such clear decreases in the size of the hippocampus.

Another, rather similar phenomenon of the mosaic of size changes due to domestication can be documented for the entire neocortex and is concerned with the subdivisions of the structure termed cortical fields or cortical areas (see Cortical Evolution as the Expression of a Program for Disproportionate Growth and the Proliferation of Areas). Unfortunately, there is only scattered information available for some of these in different species (Kruska, 2005) except for the mink (*Mustela vison*). In this species, the total neocortex was cytoarchitectonically investigated following the criteria of Brodmann (1909) and several different histologically defined areas were recognized and delineated by Danckers (2004). Danckers very accurately investigated both hemispheres of six (three male, three female) wild and six (three male, three female) ranch mink (Dark Standard) brains and calculated fresh volumes of gray matter for areas of neocortex using equidistant serial sections. She found no significant differences in gray matter volume of the diverse areas (at least the larger ones) in relative composition (total neocortex gray matter = 100%) between individuals and sexes within the wild type and within the domesticated form, respectively. On average, however, differences occurred between the wild and the ranch mink groups. On the basis of an allometrically calculated size decrease for total neocortex gray matter due to domestication at an amount of 21.5%, she arrived at rather different decrease values for different neocortical areas. These decreased values are indicated for some regions of different functional importance on the dorsal and lateral hemispheres in Figure 5. The



Cortical areas	Decrease values (%)
● Primary visual cortex (area 17)	15
○ Secondary visual cortex (areas 18, 19)	8
◆ Primary auditory cortex (area 50)	16
◇ Secondary auditory cortex (areas 20–22)	16
||| Primary somatosensory cortex (area 1)	28
≡ Secondary somatosensory cortex (areas 5, 7, 52)	22
■ Primary motor cortex (area 4)	26
□ Secondary motor cortex (area 6)	25

Figure 5 Surface extents of diverse cytoarchitectonical areas on the dorsal and lateral views of the cerebral hemisphere of a female wild mink and average decrease values of these due to domestication. Reproduced from Danckers, J. 2004. Cytoarchitektonische Arealisierungen des Neocortex beim Mink (*Mustela vison*) und vergleichend-quantitative Untersuchungen zwischen der Wild- und Haustierform. Diss. thesis, University of Kiel, with permission.

neocortical areas involved in motor functions of the pyramidal system and those for somatosensory functions are clearly more strongly decreased in size than are those serving visual and auditory functions. Most probably, such size changes from a wild to a domesticated type are somewhat different in other species in quality and quantity, since this seems to be dependent on the evolutionary and species-specific plateau of the wild ancestor's brain composition but additionally on the selection aims of domestic breeding. Nevertheless, the mosaic mode of these changes due to domestication is a general phenomenon (see Mosaic Evolution of Brain Structure in Mammals).

3.11.5 Domestication and the Sense Organs

Sense organs were compared in wild ancestral and domesticated derivatives although the data are limited. Nevertheless, decreases in the number of receptor cells in the olfactory epithelium of the nose and in the retina of eyes from boar to pig were comparable in quantity with those of the associated sensory brain nuclei. Decreases in certain structures of the ear, e.g., volume of tympanic cavity, size of auditory ossicles, bony cochlea, spiral length of cochlear duct, basilar membrane, and total number of cochlear hair cells in the organ of Corti, were also found in comparisons of wild and laboratory rats. Most often, eye weights were allometrically compared within several species with a general decrease due to domestication (Herre and Röhrs, 1990; Kruska, 2005). Very regrettably, topographic organization and counts of ganglion cells in the retina were studied in detail only in wolves and dogs (Peichl, 1992) and wild versus ranch minks (Steffen, 2000). A pronounced visual streak of high ganglion cell density from the nasal to the temporal side was characteristic only for the wolf retina, whereas in dogs this was not recognized or at least clearly less pronounced. Also, the total number of ganglion cells was decreased in dogs. Unfortunately, the investigated dogs were small breeds, approximately half the size of wolves, and body size differences were not taken into account.

The investigations on mink were based on intraspecific allometries and therefore are of special value for a quantitative approach. From a sufficient database of wild and ranch mink, it appears that ranch mink eyes are 17% smaller in weight and the surface area of the retina in 39 ranch mink versus 11 wild mink was on average 22% smaller for animals with the same body size. The relationship of eye to retina size remains the same in both types. Within the retinas of 10 wild mink, there was a scanty visual streak from the nasal to the temporal side. Although this streak was not very well pronounced, a comparative topography was never recognized in any of the 46 ranch mink retinas investigated (Figure 6). This is a convergent phenomenon since similar observations were made in canids. The topography of ganglion cells and their densities were highly diverse in the ranch mink; their total number was decreased on average by 15%. Receptor cells were also compared in quality, number, distribution, and relationship, with the general result of less clear organization and a decrease in number in the domesticated form. These changes found in the retina are quantitatively in good agreement with the results for the primary visual area of the hemispheres of this species.

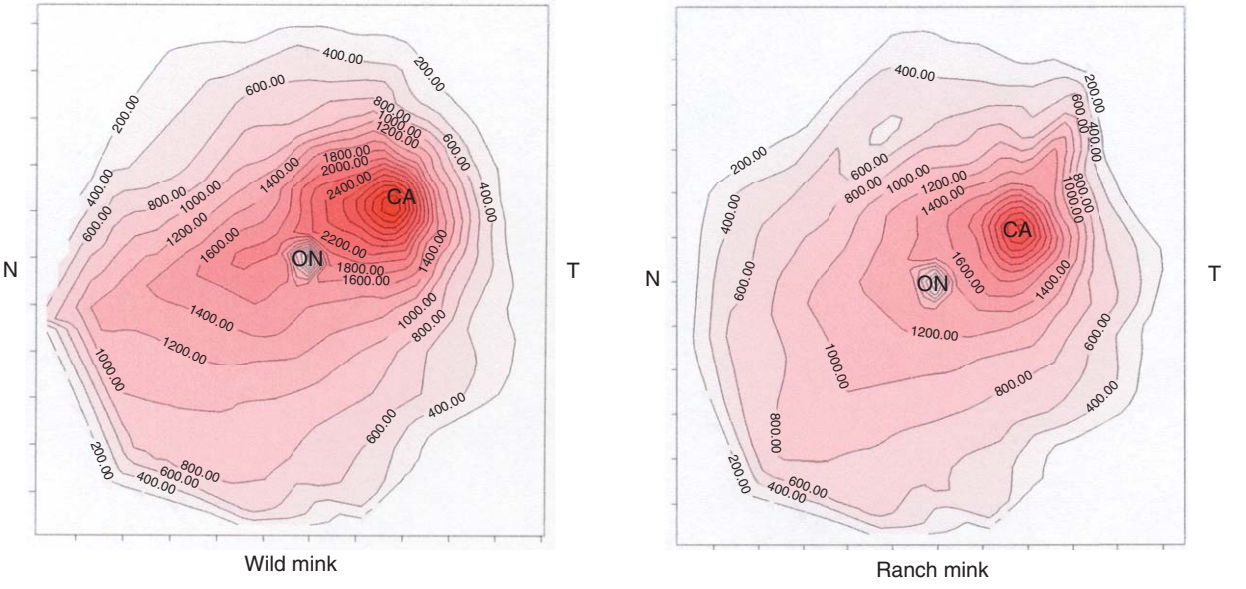


Figure 6 Topography of ganglion cells in the retinas of wild and ranch mink eyes expressed by isodensity maps. A 'visual streak' of high ganglion cell density clearly is more pronounced in the wild mink as is the larger number and greater density of ganglion cells in the central area (CA). N, nasal side; T, temporal side; ON, optic nerve. Reproduced from Steffen, K. 2000. Vergleichender quantitativer Nachweis sowie topographische Analyse von Ganglienzellen und Zapfen in der Retina von Wildmink (*Mustela vison energumenos*) und Farmmink (*Mustela vison* f. dom.). Diss. thesis, University of Kiel, with permission.

3.11.6 Conclusions

Brain size and structural composition are mainly genetically determined. The intraspecific differences of both these parameters from a wild ancestor to a domesticated derivative therefore must be evaluated as consequences of selective breeding through human interference during the domestication process. In this view, domesticated mammals in general provide proof for the intraspecific variability and the evolutionary malleability of the central nervous system. However, the actual size and structure of the entire brain itself cannot be the subject of selective breeding. Rather, it is likely that particular behaviors in different breeds were selected for, and the brain changes are an indirect result of this selection. Additionally, it can be argued that domestication in any case started with only some individuals in which the normal variability of the species gradually was preadapted to the special conditions of domestication. However, the quantitative comparative analyses revealed that domestication of very diverse mammalian species always is connected with a decrease in its functional performance that is evidence at least by behavioral changes. In any case, different degrees of total brain decrease and that of certain brain structures from species to species demonstrate that the phylogenetic and evolutionary plateau of cerebralization and specialization of the wild species and its species-specific peculiarities are rather complicated and likely apply to selective breeding for domestication. Nevertheless, the dimensions of intraspecific differences in brain size and brain composition between the wild ancestor and its domesticated derivatives are unique and are not observed in individuals that are caught in the wild and then tamed. Furthermore, keeping wild species in zoological gardens for several generations does not lead to brain size decreases. Likewise, feralization of domesticated mammals into the wild over many generations does not restore brain size to that of the wild species again, which means that "once domesticated, always domesticated" (Kruska, 2005).

Consequently, brain size changes due to domestication can be understood as special adaptations at the species level that are directed by human interference to the special ecological niche of domestication even though this might be very diverse and broad.

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3.12 How to Build a Bigger Brain: Cellular Scaling Rules for Rodent Brains

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Glossary

allometry Modification in the proportion of the various parts of an organism or structure, like the brain, as it increases in overall size.

anisotropic Any structure whose components – neuronal cells, in the case of the brain – are distributed unevenly in different directions. The brain, with its white matter tracts and gray matter regions organized in laminae and nuclei, is a highly anisotropic structure.

glial density Number of glial cells per mg or ml of tissue, measured as the number of glial somata or nuclei found per unit volume or mass.

hypermetric growth Growth of a structure that surpasses the rate of growth of another. Cerebral cortex is a typical example of hypermetric growth at the expense of other brain regions.

isometric growth Growth of a structure at the same rate of growth of another structure.

neuronal cells When determined by the isotropic fractionator, 'neuronal' refers to cells whose nuclei express neuronal nuclear antigen (NeuN), detected immunocytochemically.

neuronal density Number of neuronal cells per mg or ml of tissue, measured as the number of neuronal somata or nuclei found per unit volume or mass.

neuronal size Has often been used in the literature as shorthand for 'size of neuronal somata'. We define it here as the total size of a neuronal cell, including the whole of its dendritic and axonal arborizations and the immediately surrounding extracellular space.

neuropil The ensemble of axonal and dendritic arborizations in a brain region.

non-neuronal cells All the cells in the brain that do not express a neuronal phenotype. This includes glial and endothelial cells. When determined by the isotropic fractionator, 'non-neuronal' refers, by exclusion, to cells whose nuclei do not express NeuN, detected immunocytochemically.

power function Any mathematical equation of the form $Y = aX^b$, meaning that Y varies as a power function of X, that is, according to the exponent b of X. When b is larger than 1, Y grows faster than X. If b is a positive value below 1, Y grows slower than X. Negative values of b mean that Y actually decreases as X grows. If b is unity, the function becomes linear, as Y varies directly as X multiplied by a.

stereology The study of the three-dimensional properties of brain structures, or of any other tissue, through the measurement of microscopic sections of the whole. Usually, stereology involves the measurement of parameters such as cell or synaptic density within a tissue section of known volume, and extrapolation to the volume of the entire tissue.

3.12.1 Introduction

Brain size varies by a factor of 100 000 across mammalian species (Count, 1947). Several variables probably contribute to determine adult brain size within and across species: number of neurons, number of glial cells, cell body size, dendritic and axonal arborization volume, vasculature, and extracellular space. Although the cellular composition of the brain is one of the major determinants of its computational capacities (Williams and Herrup, 1988), little is known about how it varies with brain size. What are the cellular scaling rules that determine brain allometry? How do number of neuronal and non-neuronal cells contribute to structure size? What are their relative contributions across species of different brain sizes? Do different brain structures gain neurons and overall mass at the same rate?

3.12.2 Where Volumetry and Stereology Have Gotten Us

3.12.2.1 Bigger Animals Have Bigger Brains, and Bigger Brains Have More and More Cortex

Comparative studies of mammalian brain anatomy have been largely limited to analysis of volumetric data on large brain divisions of different species published by a small number of labs (Stephan *et al.*, 1981; Frahm *et al.*, 1982), often based on measurements of only one brain of each species. These have established that brain size is related to body size by a power law of exponent inferior to 1.0 (Martin, 1981; Fox and Wilczynski, 1986), such that brain size increases with body size, but at a slower pace (Figure 1a). The cerebral cortex increases hypermetrically in volume in relation to the remaining brain structures (Figure 1b), such that, within each order, the relative size of the cerebral cortex increases with brain size (Figure 1c): larger brains are more and more dominated by cortex (Frahm *et al.*, 1982).

In comparison, larger brains have isometrically larger cerebella, which accompany almost linearly the size of the cerebral cortex (Figure 1d), and retain a stable relative size with increasing brain size: larger brains have cerebella of the same relative volume (Figure 1e).

3.12.2.2 Bigger Brains Have Decreasing Neuronal Density

From visual inspection only, Nissl (1898) observed that neurons are distributed more sparsely in larger brains. Stereological measurements soon confirmed his observation, showing that neuronal density declines in the cerebral cortex as a power function of increasing brain volume with a small, negative exponent of −0.32 (Tower and Elliot, 1952). Further stereological studies showed that the numerical relationship between brain size and neuronal density is valid from the smallest mammalian species, such as insectivores (Stolzenburg *et al.*, 1989) to species with brains much larger than human, such as dolphin, elephant, and whale (Tower, 1954; Garey and Leuba, 1986; Haug, 1987; Figure 2a). Nonstereological measurements have found the same relationship in rodents (Herculano-Houzel *et al.*, 2006a). The direct, negative relation between cortical neuronal density and brain size is an interesting indication that intelligence does not bear a simple relationship to neuron density and thus to the degree of axodendritic complexity in the cerebral cortex (Tower and Elliot, 1952).

In principle, two factors might account for the decreased neuronal density in larger cerebral cortices: increased neuronal size (including the neuropil), and increased relative number of the interspersed glial cells. Both seem to apply: Ghouse Shariff showed in 1953 that smaller neuronal densities are associated with larger neuronal somata in the primate cerebral cortex, a finding that was later replicated in insectivores (Stolzenburg *et al.*, 1989); and it was soon confirmed that larger cortices do have larger and larger relative number of glial cells to each neuron (see The Evolution of the Cerebellum in Anthropoid Primates).

3.12.2.3 Bigger Brains Have Much More Glia

Friede (1954) defined the glial index of the cerebral cortex: the ratio between number of glial and neuronal cells (g/n ratio), which he believed to vary

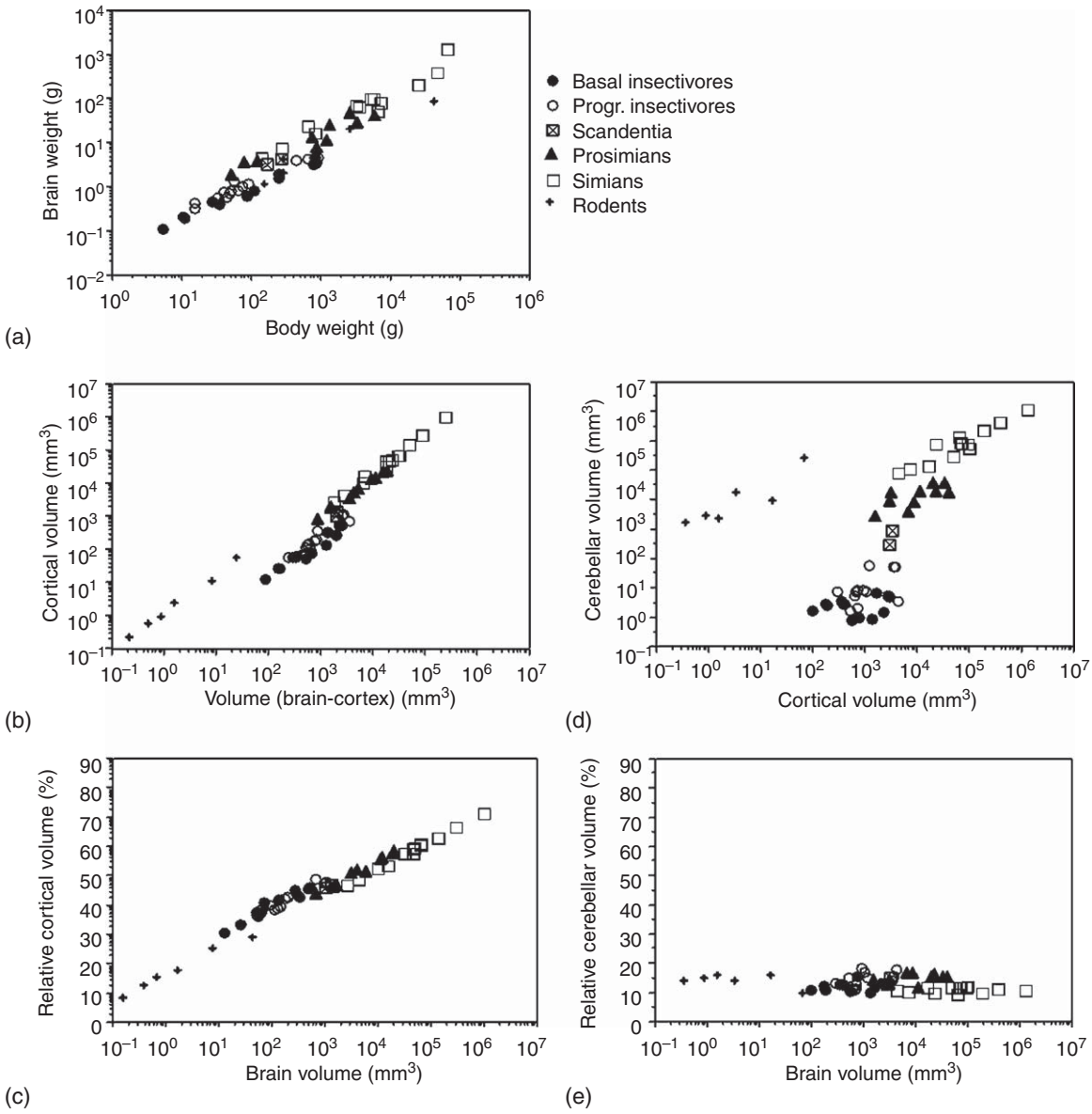


Figure 1 Brain scaling across mammalian species. a, Brain size scales hypometrically with body weight. Power law exponents relating the two parameters vary between 0.60 and 0.80. b, Absolute cortical volume (or mass, for rodents) scales hypermetrically compared with the remaining, noncortical brain structures. Power law exponents relating the two parameters vary between 1.10 and 1.20. c, Relative cortical volume (or mass, for rodents) increases with increasing brain size within each group. d, Absolute cerebellar volume (or mass, for rodents) scales hypometrically compared with cerebral cortex, with power law exponents of 0.8–1.0. e, Relative cerebellar volume (or mass, for rodents) remains invariant among all groups, regardless of increasing brain size. Data from Stephan, H., Frahm, H., and Baron, G. 1981. New and revised data on volumes of brain structures in insectivores and primates. *Folia Primatol.* 35, 1–29; Frahm, H. D., Stephan, H., and Stephan, M. 1982. Comparison of brain structure volumes in insectivora and primates. I: Neocortex. *J. Hirnforsch.* 23, 375–389; and Herculano-Houzel, S., Mota, B., and Lent, R. 2006a. Cellular scaling rules for rodent brains. *Proc. Natl. Acad. Sci. USA* (in press).

according to the phylogenetic position of the species, such that an increasing proportion of glia would indicate a more highly developed cortex. It was soon demonstrated, however, that the g/n ratio in the cerebral cortex increases with brain size rather than with an ill-defined phylogenetic scale that placed the human species on top (Figure 2b; see The Evolution of Neuron Types and Cortical Histology in Apes and Humans). Fin whales have a cortical g/n ratio of 4.54, compared to 1.78 in humans (Hawkins and Olszewski, 1957). Stolzenburg *et al.* (1989) later proposed that the g/n ratio actually increases with increasing thickness of the cortical wall rather than with total brain or cortical weight.

In any case, it has been widely believed that the relative glial expansion has trophic and metabolic

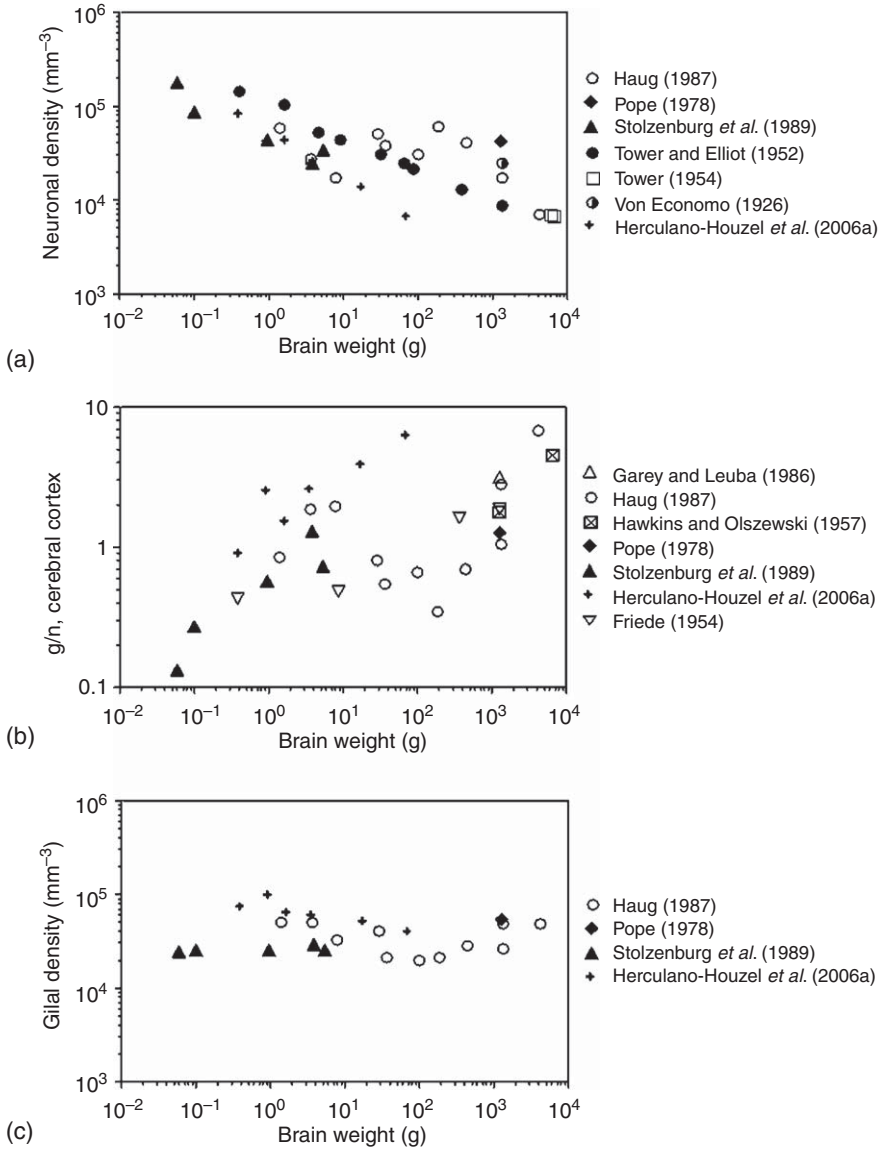


Figure 2 a, Scaling of neuronal density; b, g/n ratio; and c, glial density in the cerebral cortex of different species. All graphs were drawn from data reported in the references listed.

meaning, in accordance with the traditional view that these cells have a supportive function for neurons (Friede, 1954; Reichenbach, 1989). However, the numeric expansion of glial cells relative to neurons seems to contradict the observation that the neuronal need for metabolic support remains similar across species (Nedergaard *et al.*, 2003). Hypothetically, this discrepancy might be settled if, as the brain increases in size, a larger number of glial cells were a compensation for their small size relative to increasingly larger neurons. However, data on the relative scaling of neuronal and glial cells in the brain are lacking in the literature.

Interestingly, the increased g/n ratio is not accompanied by any major variation in glial density, which has been reported either to vary widely but independently of brain size (Haug, 1987), to remain stable (Stolzenburg *et al.*, 1989), or to show a marginally significant decrease (Herculano-Houzel *et al.*, 2006a) across mammalian species of increasing brain size. Inspection of data pooled from these sources shows that, compared to neuronal density and given the relatively high variation in experimental data obtained by different authors, glial density can be considered to remain relatively invariant across species of different brain size (Figure 2c).

3.12.3 The Need for Cell Counts, and the Trouble with Estimating Total Number of Cells with Stereology

Glia are widely said to be the most numerous cell type in the brain (Doetsch, 2003; Nishiyama *et al*., 2005), and to be 10–50× more numerous than neurons in humans (Kandel *et al*., 2000). Evidence for this assertion, however, is scant. Stereology, the only available tool for addressing cell numbers until recently, relies on the determination of cell densities in small sectors of tissue. Total cell numbers can in principle be estimated by multiplying cell density and volume of given brain regions, which is how mouse neocortex was estimated to have about 10 million neurons (Schüz and Palm, 1989), for instance.

There are, however, several problems with this approach to determining total number of cells in large brain regions or even the whole brain. First, brain tissue is highly anisotropic: neuronal density varies widely across cortical areas and subcortical nuclei, which would have to be sampled separately. This problem is solved in part by techniques with ingenious sampling strategies such as the optical fractionator, which allowed a recent estimate of the total number of neurons in the human cerebral cortex at about 20 billion (Pakkenberg and Gundersen, 1997). However, precise determination of neuronal density in the samples is still a key issue. Neuronal densities in human cerebral cortex have been estimated to be as low as 8750 mm^{-3} (Tower and Elliot, 1952), and as high as 48100 mm^{-3} (Shariff, 1953), with intermediate estimates of 24500 mm^{-3} (Von Economo, 1926), 25000 mm^{-3} (Haug, 1987) and 41300 mm^{-3} (Pope, 1978). Even if cortical volume were assumed to remain constant across samples, estimates of total number of neurons in cortex based on these densities would vary about 5×.

Precision of measurements of cortical volume, however, is also an issue: even with the highest estimate of neuronal density found in the literature, Shariff's value of 115.2 cm^3 for one cortical hemisphere would yield a total of 11 billion neurons in both hemispheres, a value that is still too low compared to Pakkenberg and Gundersen's (1997) more recent estimate of about twice that.

Even if measures of neuronal density and volume could be considered precise, stereological determination of total number of cells in the whole brain would require its parcellation into a prohibitive number of structures of defined volume and homogeneous density. This incompatibility explains why there are data in the literature on densities and total cell numbers in well-defined brain nuclei, but no attempts to use stereology to determine total number of neurons or non-neuronal cells in the brain of any mammalian species.

3.12.3.1 Why Stereological Estimates of Total Cell Number Yield Invalid Relationships

A further limitation of stereological determinations of total cell numbers is that these will, by definition, depend on the volume of the structure of interest, and therefore variations in number of cells estimated in this way cannot be compared to variations in structure volume across species. Stereological methods, thus, should not be applied to the examination of cellular brain scaling rules across species. Haug (1987) employed neuronal densities to calculate the total number of neurons in the cerebral cortex of various mammals, and reported it to vary as a power function of cortical volume with an exponent of 0.95. However, this is an invalid power law relationship that seems only to reflect variations in volume of the cerebral cortex, and does not reveal any real relationship with the number of cells. This is so because, since neuronal density varies as a very small power of cortical volume, the latter always varies much more than its density. Total number of neurons estimated as the product of density and volume will thus necessarily reflect mostly the variation of structure volume and be very little affected by variations in neuronal density, yielding invalid power law relationships of exponent close to 1, as Haug found. This is in strong contrast to the much larger exponent of 1.760 obtained when total number of cortical neurons are determined independently of cortical volume (Herculano-Houzel *et al*., 2006b; see below).

Similarly, Stevens (2001) has used the product of structure volume and neuronal density to examine how variations in total number of neurons correlate between the lateral geniculate nucleus (LGN) and primary visual area (V1) of several primate species (Stevens, 2001). The estimated exponent of 3/2, however, mirrors the 3/2 exponent relating LGN and V1 volumes across the species, since thalamic and V1 neuronal densities vary little compared to their volumes. It is most probable, therefore, that this exponent does not reveal a true relationship between number of neurons in the two structures. The questionable nature of the relationships reported by Haug (1987) and Stevens (2001) can be confirmed by shuffling neuronal density values across the species being compared, which, as expected, does not affect the exponents obtained, as they reflect mostly the variations in structure

volume. Volume-independent estimates of total number of neurons are required in order to determine how they really relate to brain volume.

3.12.3.2 The Problem with the Idea That Volume Indicates Computational Power and the Need of Total Cell Numbers

Many authors are interested in allometric rules of brain scaling from a functional point of view, as brain size, or encephalization, has long been accepted as an indicator of computational and cognitive capabilities and even intelligence (Jerison, 1985; Reader and Laland, 2002; Sol *et al*., 2005). In the absence of data on total number of neurons, different authors have focused their studies on the analysis of published volumetric data, derived mostly from Heinz Stephan's group (Stephan *et al*., 1981; Frahm *et al*., 1982). Thus, a number of studies have compared the absolute size of brain regions (Finlay and Darlington, 1995), their proportional size relative to one another (Barton and Harvey, 2000; de Winter and Oxnard, 2001), and relative to the whole brain (Clark *et al*., 2001). Based on the same data, these authors have proposed, respectively, that different brain regions evolve concertedly, in mosaic, or even as scalable versions of a same set of proportions, called cerebrotype, within a given taxon but not among them.

Strikingly, conclusions drawn from the same data can be conflicting. For instance, while the neocortical fraction of the brain increases from 14% in basal insectivores to 80% in humans (Frahm *et al*., 1982), the cerebellar fraction of brain volume varies little across species of various mammalian orders, a discrepancy taken to argue against the hypothesis that the cerebellum works in service of the neocortex (Clark *et al*., 2001). However, cerebellar and cerebral cortices increase concertedly both in surface area (Sultan, 2002) and in volume (Barton, 2002). Given that these parameters are adopted by most authors to indicate computational capacity, this evidence has been taken to suggest a functional dependence of one structure upon the other. A conciliatory view holds that cerebellum and neocortex evolved together, but with the cerebellum evolving more slowly than neocortex (Barton, 2002).

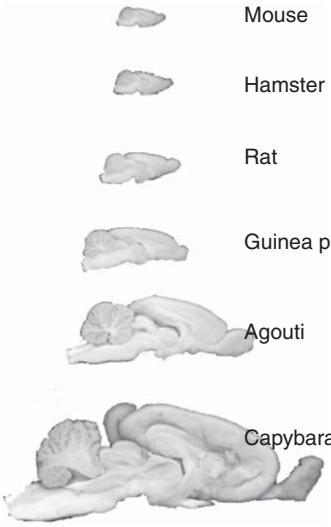
These conflicting interpretations demonstrate that cortical volume and surface, although informative measurements and widely used in the literature, particularly in relation to intelligence, cognitive abilities, and versatility, are only indirect indicators of computational capacity. As both cerebral (Douglas and Martin, 2004) and cerebellar (Leiner *et al*., 1991) cortices have modular structures, their computational capacity can be related more directly to the number of modules in each structure, and thus to the number of neurons in each structure, independently of total cortical surface or volume, characteristics that are affected by other variables such as non-neuronal volume. Thus, one way of clarifying the issue of how cerebral and cerebellar cortices are structurally, functionally, and evolutionarily related might be through the comparative analysis of the number of neurons in these structures.

3.12.3.3 A Nonstereological Way Out: The Isotropic Fractionator

The isotropic fractionator is a novel method developed recently in our lab which allows the nonstereological determination of the absolute number of neuronal and non-neuronal cells in different brain regions (Herculano-Houzel and Lent, 2005). It consists in transforming highly anisotropic brain structures into homogeneous, isotropic suspensions of fixed cell nuclei which can then be counted and identified immunocytochemically as neuronal or non-neuronal. The method can be applied either to the brain as a whole or to its dissected parts, such as cerebral cortex or cerebellum, and their respective number of cells can next be added up in order to obtain a whole-brain estimate. Estimates of total cell, neuronal and non-neuronal numbers in any brain structure can be obtained in 24 h, and vary by less than 10% among animals. Since the estimates obtained are independent of brain volume, they can be used in comparative studies of brain volume variation among species and in studies of phylogenesis, development, adult neurogenesis, and pathology. We have used the isotropic fractionator to compare the cellular composition of cerebral cortex, cerebellum, and remaining areas of the adult brain of six species of the order *Rodentia*, from mouse to the giant Amazonian capybara (Herculano-Houzel *et al*., 2006a), and are currently expanding this analysis to primate species (Herculano-Houzel *et al*., 2006b).

3.12.4 How to Build a Bigger Brain

Across the six rodent species examined, body mass varies over 1000-fold, from about 40 g in mouse to over 40 kg in capybara, while brain mass varies by less than 200×, accompanied by a smaller increase of 45× in total number of cells, an even smaller 23× increase in total number of neurons but a relatively large 86× increase in total number of non-neuronal cells (Figure 3). All data mentioned henceforth



Species	Body mass	Brain mass	#Cells	#Neurons	#Non-neuronal
Mouse	40 g	0.416 g	109 M	71 M	38 M
Hamster	168 g	1.020 g	166 M	90 M	76 M
Rat	315 g	1.802 g	331 M	200 M	131 M
Guinea pig	311 g	3.759 g	478 M	240 M	238 M
Agouti	2843 g	18.365 g	1941 M	857 M	1084 M
Capybara	47500 g	76.036 g	4870 M	1600 M	3270 M
Variation	1188	183	45	23	86

Figure 3 Total number of neuronal and non-neuronal cells in the brain of six rodent species. Average body mass, brain mass (in grams), total number of cells, neurons and non-neuronal cells (in millions) in the brain of six rodent species, shown in the same scale. Relative variations in each parameter from mouse to capybara are listed in the bottom.

regarding the cellular composition of rodent brains were reported in Herculano-Houzel *et al.* (2006a).

3.12.4.1 Bigger Brains Have a Constant Relative Number of Neurons in Cortex

As reported previously for other mammalian orders, relative size of the cerebral cortex increases significantly with brain size among rodent species (Frahm *et al.*, 1982; Figure 4a). Interestingly, this expansion in size is not reflected in the distribution of total brain neurons. Regardless of total brain size, cerebral cortex in all six species contains a relatively stable 18% of all brain neurons. This is in contrast to the distribution of total brain non-neuronal cells, which become relatively more numerous in the cortex as it expands in larger-brained species (Figure 4a). The long-acknowledged cortical expansion in bigger brains, thus, at least in the order Rodentia, does 'not' reflect any increasing allocation of brain neurons to the cortex, but only of non-neuronal cells, presumably glia.

3.12.4.2 Bigger Brains Have Relatively More Neurons in Cerebellum

The cerebellum represents a steady 14% of brain mass across rodent species, as reported for other mammalian orders (Clark *et al.*, 2001). Remarkably, its fraction of total brain neurons is 'not' stable: larger cerebella concentrate more and more of all brain neurons, from 59% in mouse to 72% in capybara (Figure 4b) but, in contrast to the cerebral cortex, they concentrate a steady fraction of all brain non-neuronal cells.

The increase in the relative number of cerebellar neurons can be explained through the addition of neurons to the cerebellum as a power function of the number of cortical neurons with an exponent >1.0, estimated at 1.113. The total number of neurons actually increases faster in the cerebellum than in the remaining of the brain, with an exponent of 1.181. Contrary to all expectations from volumetric data, the fact that the cerebellum gains neurons at a faster rate than all other brain structures, cerebral cortex included, and concentrates increasing fractions of all brain neurons shows that the total number of functional integrative units – neurons – increases faster in cerebellum than in cortex. This finding calls into question the validity of using surface and volume measurements as indicators of computational capacity. Given the modular structure of both cerebral and cerebellar cortices, the addition of neurons – and therefore supposedly of more modules – increases the computational capabilities of both networks, and therefore their total number of neurons should be a far more direct indicator of functional capacity than structure volume and surface, which are inflated by non-neuronal cells and connecting fibers of larger caliber. Thus, it has to be concluded that despite the volumetric expansion of the cerebral cortex as brain size increases within the order Rodentia, its

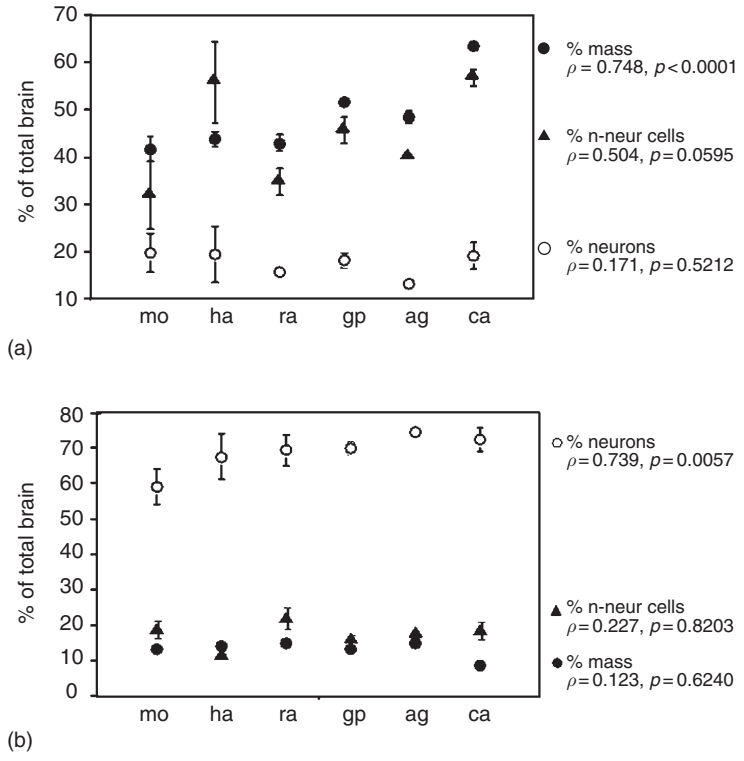


Figure 4 Cerebral cortex expands in relative volume, but retains stable relative number of neurons, while larger cerebella hold increasing relative number of neurons in an unchanging relative volume. Points represent relative distribution of mass, number of neurons, and number of non-neuronal cells in the cerebral cortex (a), and cerebellum (b) of six rodent species (mouse (mo), hamster (ha), rat (ra), guinea pig (gp), agouti (ag), and capybara (ca)). Each point corresponds to one individual animal. Correlation coefficients and *p*-values for Spearman rank correlations are given.

computational capacities may remain stable, while those of the cerebellum may actually increase. Interestingly, this does not seem to correlate with the motor abilities of the species, as, for instance, agoutis, which manipulate food with their front paws, have a richer motor repertoire than the much larger capybara, endowed with a larger relative number of cerebellar neurons. However, this may just come to show that, as noted recently (Leiner *et al*., 1991), the cerebellum is much more than a merely motor structure.

3.12.5 Cellular Scaling Rules for Rodent Brains

As shown in Figure 3, larger rodent brains contain increased number of neurons, as expected, and this is true for cerebral cortex, cerebellum, and the remaining areas separately. In striking contrast to Haug's (1987) estimate based on neuronal densities and volume (see above), the results obtained non-stereologically with the isotropic fractionator show that the power law relating cortical mass to its number of neurons has a large exponent of 1.760. This means that a 10× larger rodent cortex would have only 3.6× more neurons, or that a 10× increase in the number of cortical neurons would result in a 58× larger cortex. Other factors, such as increased neuronal size and increased non-neuronal mass, must therefore also contribute significantly to cortical expansion.

3.12.5.1 More Neurons, Many More Non-Neuronal Cells

Direct estimation of number of cells with the isotropic fractionator shows that larger brains are built with more neurons, but with even larger number of non-neuronal cells. The latter are related to the total number of neurons by a power law with exponent >1 (1.554 in cerebral cortex, 1.254 in cerebellum). This proportional expansion of the non-neuronal cell population results in the increasing g/n ratio observed both in cerebral cortex (Figure 2c) and cerebellum as these structures become larger.

Interestingly, the mass of all the structures examined increase as power laws of their respective number of non-neuronal cells with similar exponents of close to 1.0, and non-neuronal cell

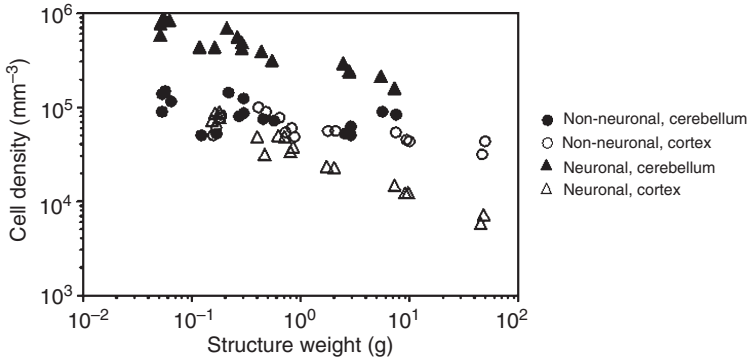


Figure 5 Cerebral cortex and cerebellum have different neuronal densities across rodent species, but similar non-neuronal cell densities. Neuronal (triangles) and non-neuronal (circles) densities are plotted on the *y*-axis against weight of the respective structure, cerebellum (filled symbols), and cerebral cortex (open symbols).

densities are strikingly similar both across brain structures and species. In fact, although across all species the cerebellar g/n ratio is much smaller than the cortical ratio, these structures share similar non-neuronal densities (Figure 5).

3.12.5.2 Neurons Become Larger As They Are Added; Glia Do Not

The different scaling of neuronal and non-neuronal cell densities with brain size suggests that these two cell types vary differently in average size: as they become more numerous, neurons must increase in size faster than non-neuronal cells.

Indeed, mathematical analysis of all the power laws obtained experimentally suggests that average neuronal size increases proportionally to the total number of neurons raised to the power of 0.760 in cerebral cortex and 0.370 in cerebellum. In comparison, the estimated variation in average non-neuronal cell size among rodents is much smaller: it follows a power law function of total number of non-neuronal cells with very small exponents of 0.114 in cerebral cortex, and 0.063 in cerebellum. This means that if the number of cortical neurons is multiplied by 100, their average size increases 33.1×, while a similar increase in the number of cerebellar neurons is accompanied by an increase in average neuronal size of only 5.5×. In contrast, 100× more numerous non-neuronal cells in the cerebral cortex will be on average only 1.7× larger, and if they become 100× more numerous in the cerebellum, their average size will increase only 1.3×.

We find that capybaras have 22× more cortical neurons than mice, and the average neuronal size is estimated to be 10× larger. The larger rodent has even more cerebellar neurons than the mouse (a 27× increase), but the average neuronal size is estimated to be only 3.4× larger. At the same time, capybaras have 153× and 82× more non-neuronal cells than mice in the cerebral cortex and cerebellum, respectively, but these cells are estimated to be only 1.8× and 1.3× larger.

Since increasing number of neurons result in larger brain size, it is expected that these neurons increase in size, with longer, highly branched processes to maintain long-distance connectivity as brain size increases, as well as larger somata to support them. However, increases in average neuronal size are observed to contribute 'less' to final structure size than changes in total number of neurons, and the 'larger' the increase in this number between two species, the 'smaller' the relative contribution of average neuronal size to final structure size. This is consistent with a strong selective pressure against increased neuronal size, lest the brain becomes too large too fast as it gains neurons (Harrison *et al.*, 2002). The faster increase in cortical neuronal size compared to cerebellum is in good agreement with the known architecture of cerebral and cerebellar cortices, the former composed of relatively large number of neurons with large cell bodies and extensively arborized processes that span long distances (Douglas and Martin, 2004), the latter composed mostly of much smaller neurons with a single, long, and comparatively local arborization (Leiner *et al.*, 1991).

In contrast, increasing number of non-neuronal cells are added to the brain in the absence of large changes in average non-neuronal cell size. Compared to neurons, glial cells act locally, so it is reasonable to expect that, as the brain grows and glial cells are added in large numbers, they retain a volume that is small enough to perform local functions of regulating the microcirculation and synaptic transmission (Nedergaard *et al.*, 2003).

It will be interesting to see whether data on neuronal and non-neuronal cell size will match our estimates as they become available by direct

measurement. According to our estimates, a rodent brain with a human-sized cerebellum would be expected to have *c.* 900× more cerebellar non-neuronal cells that are on average only 1.5× larger than in mouse cerebellum. Recent measurements of human astrocytes have shown that they are only 3× larger than mouse astrocytes (Oberheim *et al.*, 2005), which seems to be good evidence that non-neuronal cell size indeed changes very little with cell number. Similarly, Purkinje cells are 50× more numerous in human (Andersen *et al.*, 1992) than in rat cerebellum (Korbo *et al.*, 1993), and would therefore be expected to be 4.2× larger in the former, according to our estimates; in the literature, these cells have been found to have a 2.5 × bigger perykaryon (Korbo and Andersen, 1995), which falls close enough to the expected value, given that the dendritic and axonal arborizations were not considered in that study.

3.12.5.3 Glial Mass to Match Neuronal Mass: Something Remains Constant

In the midst of all the power laws that relate changing number of cells, their average volumes, and the resulting size of brain structures, it is interesting to find that one parameter remains constant: the ratio between 'total' neuronal and 'total' non-neuronal 'masses' in a given brain structure, that is, the ratio between the product of total number of neurons and their average mass, and the product of total number of non-neuronal cells and their average mass in a structure. This follows mathematically from the relationships between number of neuronal and non-neuronal cells and their relationships with structure size, and occurs simultaneously with the increased ratio between number of non-neuronal and neuronal cells (the g/n ratio) with larger brain size.

The constant total g/n mass ratio is achieved as the increased neuronal mass, resulting from larger number of neurons that increase significantly in size, is matched by the addition of much larger number of non-neuronal cells of only slightly larger size. In this way, a 2× increase in total neuronal mass is accompanied by an equal 2× increase in total non-neuronal mass, and yields a 2× increase in brain size. The overall mass constraint suggested by our data is compatible with the recent notion that glial cells serve as dynamic regulators of neuronal production, function and phenotype, and organize brain tissue into functional compartments (Nedergaard *et al.*, 2003). On the other hand, an increase in number of glial units would favor a growing participation of these cells in neural computation, as has been proposed recently (Allen and Barres, 2005; Volterra and Meldolesi, 2005), without compromising their role in regulatory and support functions. The constant neuronal/non-neuronal mass ratio also settles the apparent discrepancy between the numeric expansion of glial cells compared to neurons, while the neuronal need for metabolic support remains similar across species (Nedergaard *et al.*, 2003).

We have proposed this constant balance between total neuronal and non-neuronal mass in the brain to be a major mechanism driving changes in brain size. The constant total g/n mass ratio could be achieved economically if gliogenesis were regulated according to the number of neurons generated in each structure. This would take place during the development of each individual, as the increased neuronal proliferation that has been proposed to drive cortical growth across species (Rakic, 1995) is followed by gliogenesis, which is largely postnatal (Sauvageot and Stiles, 2002). Glial precursor proliferation is density-dependent and ceases once a steady-state glial density has been achieved, most likely by cell–cell contact inhibition (Zhang and Miller, 1996). Given the relatively invariant non-neuronal densities observed both across brain structures and species, we have suggested that continued gliogenesis until confluency is reached in a formerly purely neuronal tissue, such as newborn cerebral cortex, is a likely candidate mechanism by which the number of neuronal and non-neuronal cells are related and by which the ratio between total neuronal and non-neuronal mass could be kept constant across species.

3.12.6 Conclusions

Comparative analysis of the cellular composition of the mammalian brain is starting to confirm some trends expected from volumetric and stereological studies, and to reveal novel principles of brain scaling. Our studies with the isotropic fractionator have confirmed that neuronal density decreases with increasing size of cerebral cortex and cerebellum while non-neuronal cell density remains relatively stable; further, the g/n ratio in these structures increases with structure size. Additionally, these studies have revealed that:

1. variations in total number of neurons contribute more than variations in neuronal size towards final structure size;
2. average non-neuronal cell size changes very little across brains of different sizes;
3. the number of non-neuronal cells seems to be regulated according to the number of neurons

in the structure such that, as a result, total g/n mass ratio remains constant within a structure as its size varies; and
4. unexpectedly, the cerebellum gains neurons at a faster rate than the cerebral cortex, and concentrates increasing fractions of all brain neurons as brain size increases, despite the volumetric expansion of the cerebral cortex.

We have suggested that this latter finding is a consequence of a greater increase in average neuronal size in the cerebral cortex than in the cerebellum, matched by a corresponding increase in total non-neuronal mass. Although the cerebellum gains neurons faster than cerebral cortex, the average size of its neurons increases much more slowly, and once the non-neuronal population expands to match the total neuronal mass in these structures, the result is a much inflated cerebral cortex that still holds the same number of neurons relative to the whole brain. In this manner, volumetric expansion is dissociated from expansion of the neuronal population. This latter finding has important implications for the functional relationship and computational capacity of these structures, as discussed above.

It is important to realize that the current view of encephalization and neocorticalization as adaptive and selected traits in evolution (Jerison, 1985) are based on volumetric relationships that do not hold at the cellular level of brain composition, and therefore may not be reliable indicators of function. The very concept of encephalization carries the built-in assumption that brain size is indeed a measure of computational capacity as it puts forward the notion that a larger than expected brain size endows species with better cognitive capabilities. However, our data indicate that, at least in rodents, neocorticalization is only apparent; when it comes to number of neurons, it is the cerebellum that becomes expanded in larger brains.

It is interesting to wonder how the view of neocorticalization would be different today if studies of cellular scaling rules had been available earlier: given the increasing concentration of neurons in the cerebellum, perhaps we would deal today with concepts of cerebellarization of the brain, instead of neocorticalization as traditionally considered. Indeed, in light of the recent discoveries on the functional contribution of glial cells to intercellular signaling, in concert with neurons (Allen and Barres, 2005; Volterra and Meldolesi, 2005), there is actually the novel possibility that it is the increasing numerical predominance of glial cells in the cerebral cortex that accounts for the expected increase in computational power of larger brains.

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3.13 Scaling the Brain and Its Connections

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Glossary

area-infiltration The average percentage of neurons in a cortical area to which a neuron connects. This appears to be invariant across mammals.

area-interconnectedness The average percentage of areas in the brain to with which an area connects. This appears to be invariant across mammals.

cortical area Groups of neurons that communicate with one another largely via short-range, non-white-matter connections; whereas the connections between neurons in different areas are largely made by long-range, white-matter connections. This definition of area is related to one of the three principal experimental criteria for identifying areas, namely the pattern of connectivity to other parts of the neocortex (the other two criteria concern histology and topographic maps).

encephalization quotient, EQ Intuitively, it measures how big the brain is once we have corrected for body size. Because brain mass scales as the 3/4 power of body mass, EQ is brain mass divided by the 3/4 power of body mass.

epiphenomenal Phenomena that are not strictly functional, but are a side effect of something that is. A side effect of design features.

invariant In scaling studies, when a biological property is said to be 'invariant', it means that that property does not tend to change as a function of brain size. It does not mean that the property does not vary in other ways, e.g., from brain area to brain area.

minicolumns Thin neuroanatomical structures extending through the thickness of gray matter. The number of neurons in a minicolumn appears to be approximately invariant across mammals (namely on the order of 100). Both minicolumns and modules appear to be invariant-sized computational units of the neocortex.

modules Used to refer to a variety of neuroanatomical structures larger than minicolumns, but smaller than cortical areas, such as columns, blobs, barrels, and stripes. The number of neurons in a module appears to be approximately invariant across mammals. Both minicolumns and modules appear to be invariant-sized computational units of the neocortex.

Murray's law An empirical relationship between the diameter of a parent segment

of a tree and its daughter segments. Murray's law states that the cube of the diameter of the parent is equal to the sum of the cubes of the daughters. Evidence suggests that this law applies to neural arbors, albeit with considerable variation.

neocortical scaling — The study of the manner in which neocortical features change from small to large brains, with the aim of identifying fundamental principles governing the organization of the neocortex.

principle of economical well-connectedness — The mammalian neocortex satisfies the principle of well-connectedness, but does so in such a way that it minimizes wiring volume. Intuitively, the principle says to cheaply maintain an invariant level of interconnectivity, both at the intra- and interarea levels.

principle of invariant area-infiltration — When a neuron makes connections within an area, the number of synapses it makes is, on average, some invariant fraction of the number of neurons in the area. This invariance property is referred to as 'invariant area-infiltration', because, independent of brain size, neurons have, on average, sufficiently many synapses to 'infiltrate' an invariant fraction of the neurons in an area.

principle of invariant area-interconnectedness — The average number of area connections per area scales approximately proportionally with the total number of areas.

principle of well-connectedness — The mammalian neocortex satisfies both the principle of invariant area-infiltration and the principle of invariant area-interconnectedness.

3.13.1 Introduction

At first glance, larger brains seem more complex than smaller brains, having a greater number of synapses per neuron, greater surface convolutedness, more cortical areas, and disproportionately more white matter. However, brain size does not correlate with behavioral repertoire size as measured by ethologists (Figure 1), leading one to suspect that these seemingly increasingly complex features of larger brains are not underlying more complex computations (although see Brain Size in Primates as a Function of Behavioral Innovation for within primates). Instead, these seemingly complex features may be epiphenomenal consequences of making larger (not 'smarter') brains: it may be that a larger brain must satisfy certain invariant anatomical constraints that are fundamental to its function, and it is the satisfaction of these constraints in larger brains that leads to the seemingly complex features. In this light, the goal of studying how brains scale up in size across mammals is to identify the invariant anatomical constraints that are fundamental to brain function. Section 3.13.2 enumerates many of the neocortical changes that occur from small to large brains. Section 3.13.3 identifies key connectivity constraints that appear to be essential to brain function, no matter the size of the brain. In particular, these connectivity constraints are called 'invariant area-infiltration' and 'invariant area-interconnectedness', summed up as 'invariant well-connectedness'. Section 3.13.4 explains how brains appear to satisfy these invariant connectivity constraints in an economical fashion, and that this is what explains why brains scale up in the broad manner that they do.

3.13.2 The Empirical Scaling Relationships

In this section, I review many of the changes the mammalian neocortex undergoes from small (see Organization of a Miniature Neocortex – What Shrew Brains Suggest about Mammalian Evolution) to large brains (see the third column of Table 1 for a summary). It must be recognized that these empirical scaling relationships are only zeroth-order descriptions of the neocortex, capturing neither ecology-dependent variation in brain organization nor important architectonic differences within brains (see Principles of Brain Scaling).

3.13.2.1 Number of Neurons, Synapse Density, Number of Synapses per Neuron, Network Diameter

One of the oldest neocortical scaling results is that the number of neocortical neurons, N, scales up disproportionately slowly compared to gray matter volume, V_{gray}, and in particular, $N \sim V_{gray}^{2/3}$ (Tower and Elliott, 1952; Tower, 1954; Jerison, 1973; Passingham, 1973; Prothero, 1997b). Alternatively, $V_{gray} \sim N^{3/2}$. Although the volumetric density of neurons therefore

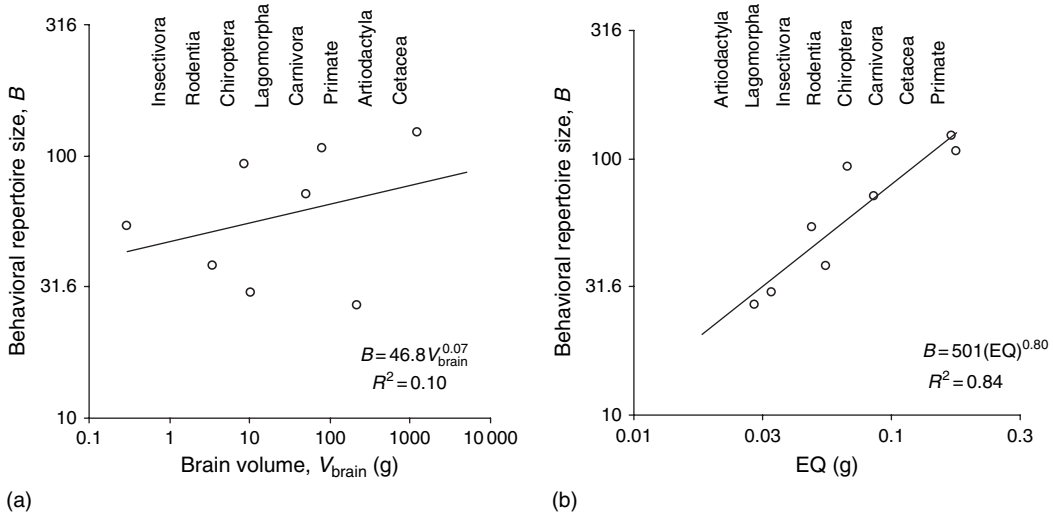

Figure 1 The neocortical scaling features are due to an increase in brain size, not due to an increase in behavioral complexity. This is because brain size does not correlate with behavioral repertoire size, as is shown in (a) which shows average behavioral repertoire sizes, B, for eight mammalian orders compiled from the ethology literature (Changizi, 2003a, 2003b) vs. brain volume, V_{brain} (averaged from animals measured in Hrdlicka (1907), Von Bonin (1937), Crile and Quiring (1940), Stephan *et al.* (1981), the Stephan Collection, Hofman (1982), Haug (1987)). There is no significant correlation between them ($df = 6$, $t = 0.82$, $p > 0.2$). As is shown in (b), however, behavioral repertoire size does increase as a function of the 'encephalization quotient', or EQ ($df = 6$, $t = 2.37$, $p < 0.05$). (EQ is brain mass (g) divided by the 3/4 power of body mass (g), which measures how big a brain is once one has corrected for how large the animal is; this is the appropriate normalization because brain mass increases as the 3/4 power of body mass (see Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals).) The moral here is that the explanation for the neocortical scaling features probably will not appeal to increasing brain 'complexity'; instead, the explanation is likely to appeal to physicomathematical constraints in making a larger – but not necessarily 'smarter' – brain.

Table 1 Empirical and predicted (via the economical well-connectedness hypothesis) scaling exponents for a variety of neocortical quantities as a function of the number of neurons, N (top), and as a function of gray matter volume, V_{gray} (bottom). The 'formula' column shows the predicted exponent, parameterized by the parameter α, where $A \sim W^{\alpha}$. The final three columns show the predicted scaling exponent for three specific values of α: $\alpha = 1$, where the number of areas scales up as fast as possible (consistent with the well-connectedness constraints); $\alpha = 0.76$, the value of α that leads to the total wire volume scaling up as slowly as possible; $\alpha = 0$, where the number of areas remains constant as a function of brain size

				Optimal		
X		*Empirical*	*Formula*	$\alpha = 1$	$\alpha = 0.76$	$\alpha = 0$
Exponent z in $X \sim N^z$						
No. of connections per neuron	δ	≈ 0.5	$1/(1+\alpha)$	0.500	0.568	1.000
Axon radius	R	≈ 0.167	$1/(3+3\alpha)$	0.167	0.189	0.333
Gray matter volume	V_{gray}	≈ 1.5	$(2+\alpha)/(1+\alpha)$	1.500	1.568	2.000
Surface area	S	≈ 1.33	$(5+3\alpha)/(3+3\alpha)$	1.333	1.379	1.667
Thickness	T	≈ 0.167	$1/(3+3\alpha)$	0.167	0.189	0.333
White matter volume	V_{white}	≈ 1.77–2	$(7\alpha+4)/(3\alpha+3)$	1.833	1.765	1.333
No. of area connections per area	D	≈ 0.5	$\alpha/(1+\alpha)$	0.500	0.432	0.000
No. of neurons per area	W	≈ 0.5	$1/(1+\alpha)$	0.500	0.568	1.000
No. of areas	A	≈ 0.5	$\alpha/(1+\alpha)$	0.500	0.432	0.000
Exponent z in $X \sim V_{gray}^z$						
No. of neurons	N	≈ 0.67	$(1+\alpha)/(2+\alpha)$	0.667	0.638	0.500
Axon radius	R	≈ 0.1	$1/(6+3\alpha)$	0.111	0.121	0.167
No. of connections per neuron	δ	≈ 0.33	$1/(2+\alpha)$	0.333	0.362	0.500
Surface area	S	≈ 0.9	$(5+3\alpha)/(6+3\alpha)$	0.889	0.879	0.833
Thickness	T	≈ 0.1	$1/(6+3\alpha)$	0.111	0.121	0.167
White matter volume	V_{white}	≈ 1.15–1.33	$(7\alpha+4)/(3\alpha+6)$	1.222	1.126	0.667
No. of area connections per area	D	≈ 0.33	$\alpha/(2+\alpha)$	0.333	0.275	0.000
No. of neurons per area	W	≈ 0.33	$1/(2+\alpha)$	0.333	0.362	0.500
No. of areas	A	≈ 0.33	$\alpha/(2+\alpha)$	0.333	0.275	0.000

decreases, the volumetric density of synapses appears to remain approximately constant as a function of gray matter volume (Abeles, 1991). Together, these two scaling results entail that the average number of synapses per neuron, δ, increases as the 1/3 power of gray matter volume; that is, $\delta \sim V_{\text{gray}}^{1/3}$ (see Figure 2). Or, as a function of the number of neurons, $\delta \sim N^{1/2}$.

Interestingly, although the number of synapses per neuron scales up considerably more slowly than the number of neurons, the scaling is sufficiently fast to maintain a low network diameter (i.e., the average number of axons that must be traversed to get from any neuron to another): assuming that the neocortex is small-world (i.e., having sufficiently many axons connecting otherwise-separated parts of neocortex that the network diameter can be approximated as that of a random network), the network diameter may be approximated as $\Lambda \approx (\log N)/(\log \delta)$, which manipulates to $\Lambda \approx 2 \times [1 + (\log c)/(\log N)]$, where c is the proportionality constant in the equation $\delta = cN^{1/2}$ (Changizi, 2001b). For sufficiently large brains, Λ approaches 2; also, estimates of c are on the order of 1, meaning that perhaps the network diameter is $\Lambda \approx 2$ in actual neocortices.

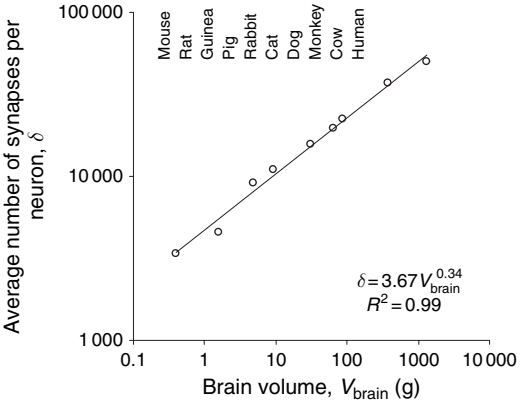

Figure 2 The average number of synapses per neuron, δ, scales as the 1/3 power of gray matter volume, V_{gray}. The plot shows the average number of synapse per neuron, δ, vs. brain volume, V_{brain}. The synapse numbers are computed using data from Tower and Elliott (1952), who present data for how neuron density decreases in larger brains (namely, it decreases as the $-1/3$ power of brain volume). Since volumetric synapse density remains invariant as a function of brain size (Abeles, 1991; Changizi, 2001b), a neuron density decrease corresponds to a proportional increase in the number of synapses per neuron. The synapse values were computed assuming that the average number of synapses per neuron in human is 50 000; this choice of 50 000 serves to set the proportionality constant, but does not affect the scaling exponent. In addition to concluding that $\delta \sim V_{\text{gray}}^{1/3}$, it also follows that $\delta \sim N^{1/2}$, where N is the total number of neocortical neurons. (This plot shows brain volume along the x-axis, not gray matter volume, but measured exponents for gray matter volume vs. brain volume are approximately 1:0.983 (Prothero, 1997a), 0.982 (Hofman, 1991), 1.054 (Hofman, 1989), 1.04 (Prothero and Sundsten, 1984), 1.06 (Frahm *et al.*, 1982), and 1.08 (Jerison, 1982). For this reason, it is empirically justified to use brain volume as a proxy for gray matter volume, and this is done in some upcoming figures as well.)

3.13.2.2 Surface Area, Thickness, Minicolumns, and Columns

The neocortical gray matter is a sheet on the outside of the brain (with white matter axons filling the center). There are neuroanatomical structures called minicolumns extending through the thickness of the neocortical gray matter from pia to white matter (Mountcastle, 1957, 1997; Tommerdahl *et al.*, 1993; Peters, 1994; Jones, 2000), and the number of neurons in a minicolumn (i.e., along a thin line through the thickness of gray matter) appears to be approximately invariant (on the order of 100) as a function of gray matter volume (Rockel *et al.*, 1980; Prothero, 1997b; Changizi, 2001b). Because of the neuron density decrease discussed earlier (namely neuron density $\rho_{\text{neuron}} \sim V_{\text{gray}}^{-1/3}$), it follows that gray matter thickness, T, must increase as the 1/9 power of gray matter volume, $T \sim V_{\text{gray}}^{1/9}$, and indeed this is what has been measured (Hofman, 1985, 1989, 1991; Jerison, 1982; Prothero and Sundsten, 1984; Prothero, 1997a). Because gray matter volume is equal to its surface area, S, times its thickness, T, it also follows immediately that $S \sim V_{\text{gray}}^{8/9}$, something also measured. Surfaces of geometrically similar objects scale as the 2/3 power of volume, and the fact that this exponent of approximately 8/9 is greater than 2/3 means that the neocortex surface cannot remain geometrically identical as the brain enlarges, but must, instead, become increasingly convoluted, as it does in larger brains. We see, then, that gray matter thickness and surface area scaling follow from (1) the neuron density decrease in larger brains, and (2) the invariant number of neurons in a minicolumn.

Minicolumns are not the only anatomical structure in gray matter that appears to have an invariant number of neurons: the number of neurons in a neocortical 'module' (such as columns, blobs, barrels, stripes) also does not appear to vary as a function of gray matter volume (see Changizi, 2003b, using data from Manger *et al.*, 1998). Such fundamental invariant-sized structures are found in a wide variety of complex network, and may be invariant in size for reasons of economical scaling (see Changizi *et al.*, 2002; Changizi, 2003b; Changizi and He, 2005).

3.13.2.3 Axon Caliber and White Matter Volume

We saw earlier that the number of synapses per neuron, δ, scales as the 1/3 power of gray matter volume (or as the square root of the number of neurons). Biological structures with more 'leaves' are almost always supported by thicker 'trunks', and we should

therefore expect that as the number of synapses per neuron increases, axon calibers and soma diameters should increase. Indeed, white matter axon caliber, R, appears to increase in size in larger brains (Harrison *et al.*, 2002), and, in particular, approximately as the 1/9 power of gray matter volume, $R \sim V_{gray}^{1/9}$ (Shultz and Wang, 2001). (Soma diameter for spinal motor neurons also scales approximately as the 1/9 power of gray matter volume (Changizi, 2001b).) It follows that axon caliber scales as the 1/3 power of the number of synapses per neuron, $R \sim \delta^{1/3}$, or $R^3 \sim \delta$. Because volumetric synapse density is invariant as a function of gray matter volume (see Section 3.13.2.1), the linear dimensions of synapses must be invariant, and the relationship $R^3 \sim \delta$ can be rewritten as $R^3 \sim \delta \times R_{synapse}^3$, where $R_{synapse}$ is the linear dimensions of a synapse. This equation is a version of Murray's law (Murray, 1926), which is an optimality principle relating a parent branch caliber to the caliber of its daughter segments, and is approximately consistent with the parent–daughter diameters of a wide variety of kinds of neural arbor (Cherniak *et al.*, 1999; Chklovskii and Stepanyants, 2003; see How to Build a Bigger Brain; Cellular Scaling Rules for Rodent Brains).

White matter volume, V_{white}, follows from the above scaling exponents as explained below. Assuming the number of white matter projecting neurons, N_{white}, scales proportionally to the total number of neurons, N, $V_{white} \sim N \times L \times R^2$, where L is the average length traveled by a white matter axon, and R is the caliber of a white matter axon. We saw earlier that $N \sim V_{gray}^{2/3}$ and $R \sim V_{gray}^{1/9}$. If L is taken to be the linear dimensions of the gray matter, then $L \sim V_{gray}^{1/3}$, and $V_{white} \sim V_{gray}^{11/9}$. If, however, L is taken to be the linear dimensions of the white matter, then $L \sim V_{white}^{1/3}$, and some algebraic manipulation leads to $V_{white} \sim V_{gray}^{4/3}$ (Changizi, 2001b). Indeed, measured exponents tend to be around 1.2–1.3 (Frahm *et al.*, 1982; Hofman, 1989, 1991; Allman, 1999; Zhang and Sejnowski, 2000; Bush and Allman, 2003). White matter volume, then, scales up disproportionately quickly as a function of gray matter volume, and this is due to white matter axon caliber increasing as it does (and this, in turn, is due to the increasing number of synapses per neuron): if white matter axon caliber were constant, then white matter volume would scale proportionally with gray matter volume (Changizi, 2001b).

3.13.2.4 Number of Areas and Number of Area Connections per Area

The neocortex is partitioned into functionally distinct 'areas', an area which is a contiguous anatomical region involved in some suite of related computations. The labs of Kaas, Krubitzer, and colleagues have determined the boundaries of many areas in a wide variety of mammals, and by compiling results from their studies it is possible to estimate the total number of areas across 19 mammals of varying brain size (see Figure 3). The number of areas, A, scales approximately as the 1/3 power of gray matter volume, $A \sim V_{gray}^{1/3}$, or $A \sim N^{1/2}$ (Changizi, 2001b, 2003b; Changizi and Shimojo, 2005). It follows that the average number of neurons per area, W, also scales as the 1/3 power of gray matter volume, $W \sim V_{gray}^{1/3}$, or $W \sim N^{1/2}$. Note that although the average number of neurons per area scales as the 1/3 power of gray matter volume, some areas such as V1 and V2 appear to violate this average tendency, scaling as the 2/3 power of gray matter volume, or proportional to the total number of neurons (Changizi and Shimojo, 2005; see Captured in the Net of Space and Time: Understanding Cortical Field Evolution, Mosaic Evolution of Brain Structure in Mammals).

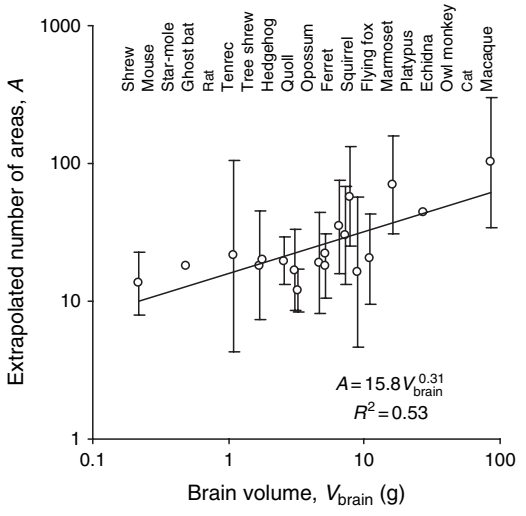

Figure 3 The number of cortical areas, A, and the average number of neurons per area, W, each scale as the 1/3 power of gray matter volume, V_{gray}. The plot shows the extrapolated number of cortical areas, A, vs. brain volume, V_{brain}. (Standard deviation bars are shown.) For each of 19 mammals studied by Kaas, Krubitzer, and colleagues, the average fraction of neocortex taken up by a cortical area was measured (Changizi and Shimojo, 2005). For example, if the average neocortical area takes up 5% of the neocortex, then the extrapolated total number of areas is 1/0.05 = 20. The 'extrapolated number of areas' is the inverse of this fraction. The data are from the following papers: Catania *et al.* (1999), Krubitzer and Huffman (2000), Krubitzer (1995), Northcutt and Kaas (1995), Krubitzer *et al.* (1995, 1997), Lyon *et al.* (1998), Beck *et al.* (1996), Manger *et al.* (2002), Kaas (1987). The best-fit scaling exponent is 0.31 (95% confidence interval is (0.16, 0.46)), or approximately $A \sim V_{gray}^{1/3}$. The average number of neurons per area, W, can be computed from this as follows: $W = N/A$, where N is the total number of neocortical neurons. The total number of neocortical neurons scales as the 2/3 power of brain volume, so $W \sim (V_{brain}^{2/3})/(V_{brain}^{0.31}) = V_{brain}^{0.36}$, or approximately $W \sim V_{brain}^{1/3}$. Note also that this means A and W each scale approximately as the 1/2 power of the number of neurons, N.

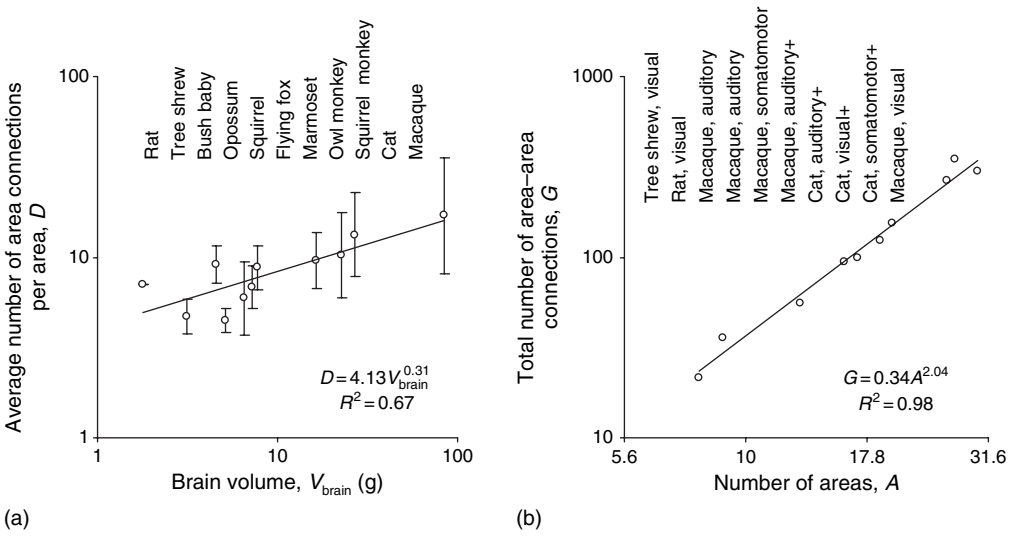

Figure 4 The average number of area connections per area, D, scales as the 1/3 power of gray matter volume, V_{gray}. a, Plot of the average number of area connections per area, D, vs. brain volume, V_{brain} (standard deviation bars shown) for 12 mammals accumulated from approximately one dozen articles in the literature (see Changizi and Shimojo, 2005). For each animal, the number of area connections has typically been measured within the literature only for a relatively small number of areas, and usually from visual or somatosensory areas. The data are from the following papers: Kahn *et al.* (2000), Beck *et al.* (1996), Lyon and Kaas (2001, 2002a, 2002b), Beck and Kaas (1998), Krubitzer and Kaas (1990a, 1990b), Collins *et al.* (2001), Lyon *et al.* (1998), Fabri and Burton (1991), Krubitzer *et al.* (1986, 1993), Kaas *et al.* (1989), Scannell *et al.* (1995), Lewis and Van Essen (2000). The best-fit scaling exponent is 0.31 (95% confidence interval is (0.145, 0.468)), so that it is approximately the case that $D \sim V_{gray}^{1/3}$ (and $D \sim N^{1/2}$). b, Plot of the total number of area–area connections, G, vs. the number of areas, A, in 'area-subnetworks' of tree shrew, rat, cat, and macaque (Changizi, 2003b; Changizi and Shimojo, 2005). These data were compiled from the following papers: Lyon *et al.* (1998), Coogan and Burkhalter (1993), Hackett *et al.* (1998), Young (1993), Kaas and Hackett (2000), Scannell and Young (1993). The best-fit scaling exponent is 2.04 (95% confidence interval is (1.807, 2.263)), or approximately $G \sim A^2$. Because the total number of area–area connections, $G = A \times D$, we can derive that $D \sim A$. Recalling that $A \sim V_{gray}^{1/3}$, we conclude again that $D \sim V_{gray}^{1/3}$ (and $D \sim N^{1/2}$).

Areas connect to other areas via white matter axons, and the average number of areas to which an area connects (or, the average number of area connections per area), D, scales approximately proportional to the 1/3 power of gray matter volume (Changizi and Shimojo, 2005), that is, $D \sim V_{gray}^{1/3}$, or $D \sim N^{1/2}$ (Figure 4). This can be shown by measurements of the average number of area connections per area across a variety of studied areas (mostly visual and somatosensory) in a variety of mammals (Figure 4a), and also by measuring the total number of area connections in 'area subnetworks' within tree shrew, rat, cat, and macaque (Figure 4b). These area and area-connection scaling relationships are consistent with the 'square-root compartment' conjecture by Braitenberg (Braitenberg, 1978, 2001; Braitenberg and Schuz, 1998; see Cortical Commissural Connections in Primates).

3.13.3 Two Fundamental Connectivity Constraints: Invariant Well-Connectedness

Two surprising and important implications concerning connectivity spring from the variety of scaling exponents discussed in Section 3.13.2.

3.13.3.1 Invariant Area-Infiltration

The first important observation concerning connectivity is that both the average number of synapses per neuron, δ (Figure 2), and the average number of neurons per area, W (Figure 3), scale approximately as the 1/3 power of gray matter volume (or as the square root of the total number of neocortical neurons). Therefore, $\delta \sim W$, that is, the average number of synapses per neuron scales approximately directly proportionally with the average number of neurons per area. When a neuron makes connections within an area, the number of synapses it makes is, on average, some invariant fraction of the number of neurons in the area (Figure 5a). This invariance property is referred to as 'invariant area-infiltration', because, independent of brain size, neurons have, on average, sufficiently numerous synapses to 'infiltrate' an invariant fraction of the neurons in an area. In humans, the average number of synapses per neuron, δ, is on the order of 10^4–10^5 and the average number of neurons per area, W, is on the order of 10^8 (given $N \approx 10^{10}$ neurons and $A \approx 100$ areas), and thus $\delta \approx cW$, where the proportionality constant is $c \approx 10^{-4}$–10^{-3}. That is, on average,

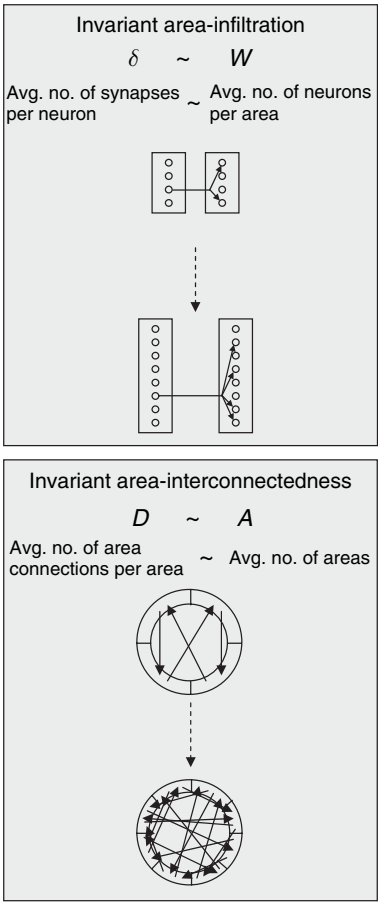

Figure 5 Two fundamental connectivity invariants found in the mammalian neocortex, summarized as 'invariant well-connectedness'. The first is 'invariant area-infiltration', which is the empirical observation that the average number of synapses per neuron, δ, scales approximately directly proportionally with the number of neurons per area, W (namely, each of these quantities scales approximately as the 1/3 power of gray matter volume (see Figures 2 and 3)). The second is 'invariant area-interconnectedness', which is the empirical observation that the average number of area connections per area, D, scales approximately directly proportionally with the number of areas, A (namely, each of these quantities scales approximately as the 1/3 power of gray matter volume (see Figures 3 and 4)).

neurons connect to on the order of 10^{-4}–10^{-3} of the neurons in an area, independent of the number of neurons in an area.

3.13.3.2 Invariant Area-Interconnectedness

The second important observation concerning connectivity is that both the average number of area connections per area, D (Figure 4), and the number of areas, A (Figure 3), scale approximately as the 1/3 power of gray matter volume (or as the square root of the total number of neocortical neurons). Therefore, $D \sim A$, that is, the average number of area connections per area scales approximately directly proportionally to the number of areas (Figure 5b). Equivalently, the total number of area connections scales approximately as the square of the number of areas (something we saw in Figure 4b). This invariance property is referred to as 'invariant area-interconnectedness'. The proportionality constant in $D \approx cA$ may be estimated from Figure 4b, which is $c \approx 0.34$, that is, each area connects to approximately 1/3 of the areas in neocortex, independent of the number of areas (although this is likely to be an overestimate because Figure 5 is for subnetworks which are probably more highly interconnected than is the entire neocortex).

3.13.3.3 Invariant Well-Connectedness

The mammalian neocortex appears to satisfy both connectivity invariances mentioned above – invariant area-infiltration and invariant area-interconnectedness – and joint satisfaction of these is referred to as invariant well-connectedness. Maintaining invariant neuron-interconnectedness for the entire neocortex (i.e., where the average number of synapses per neuron would scale proportionally to the total number of neocortical neurons) would be exorbitantly expensive (Deacon, 1990; Stevens, 1989; Ringo, 1991), and so, instead, the mammalian neocortex takes a two-tiered approach: (1) invariant neuron-interconnectedness 'within areas' (i.e., invariant area-infiltration), and (2) invariant area-interconnectedness among areas in the entire neocortex. Satisfaction of these two invariances is inexpensive relative to maintenance of invariant neuron-interconnectedness for the entire neocortex. Invariant area-infiltration appears to be due to a requirement that some minimum level of interconnectivity be achieved 'within' areas in order for areas to properly function. And invariant area-interconnectedness appears to be due to a requirement that some minimum level of interconnectivity be achieved 'between' areas in order for the entire neocortex to properly function. There is, however, currently no explanation for why mammalian brains have been selected to conform to these invariances. Nor is there an explanation for why there are two tiers, and not more.

3.13.4 Economical Neocortex

Thus far, I have reviewed the empirical scaling relationships (Section 3.13.2) and identified two important connectivity invariance principles that govern neocortical scaling (Section 3.13.3). However, I have not yet provided an explanation for why the neocortex scales up in the ways that it

does. Although, as mentioned above, I have no explanation for why the mammalian neocortex conforms to the principle of invariant well-connectedness; might it be that given that the mammalian neocortex must (for some reason) conform to this principle, then the other neocortical scaling features follow? The answer is 'no': satisfaction of invariant well-connectedness does not entail the other scaling exponents, for there are multiple possible ways of scaling a brain while satisfying invariant well-connectedness. In particular, invariant well-connectedness only states that $\delta \sim W$ (invariant area-infiltration) and $D \sim A$ (invariant area-interconnectedness); it does not say how the average number of neurons per area, W, and the number of areas, A, relate to one another.

In fact, W and A scale approximately proportionally to one another (each scales approximately as the 1/3 power of gray matter volume, or as the square root of the number of neocortical neurons), and it suffices to explain this, and the other scaling exponents then do follow. Although invariant well-connectedness does not entail the proportional relationship between W and A, if we hypothesize that the neocortex is selected to satisfy invariant well-connectedness in a volume-optimal manner – 'economical well-connectedness' – then it is possible to explain why it is approximately the case that $W \sim A$. There is considerable evidence to date that there is strong selective pressure for 'wire-optimality' in the brain (Cajal, 1995; Kaas, 1977, 1989, 1995, 1997, 2000; Cowey, 1979, 1981; Barlow, 1986; Durbin and Mitchison, 1990; Mitchison, 1991, 1992; Ringo, 1991; Ringo *et al.*, 1994; Cherniak, 1992, 1994, 1995; Young, 1992; Traverso, 1992; Jacobs and Jordan, 1992; Ruppin *et al.*, 1993; Van Essen, 1997; Cherniak *et al.*, 1999, 2004; Chklovskii, 2000; Chklovskii and Koulakov, 2000; Changizi, 2001a, 2003b, 2005; Changizi *et al.*, 2002; Chklovskii *et al.*, 2002; Klyachko and Stevens, 2003; Changizi and He, 2005; see Neural Wiring Optimization).

Changizi (2001b) presented a simple argument for why economical satisfaction of well-connectedness would explain $W \sim A$ (i.e., the number of neurons per area scaling proportionally with the number of areas). Namely, a greater number of synapses per neuron, δ, will tend to lead to a greater amount of volume devoted to 'wiring' (because more synapses per neuron requires more arborization and thicker 'trunks' supporting the arbor). Therefore, we would expect the number of synapses per neuron to scale up as slowly as possible, so long as well-connectedness remains satisfied. Because the number of synapses per neuron must scale proportionally to the number of neurons per area (i.e., $\delta \sim W$, or invariant area-infiltration), wire economy consequently expects the number of neurons per area, W, to scale up as slowly as possible. The slowest that the number of neurons per area can scale up is proportional to the number of areas, because (by invariant area-interconnectedness) each area must have sufficiently many neurons to connect to an invariant proportion of the total number of areas. So, the number of neurons per area must scale proportionally with the total number of areas, that is, $W \sim A$.

This argument above relies on the simple heuristic 'fewer synapses per neuron tends to entail lower overall wiring volume', and from it and the hypothesis of well-connectedness one can derive $W \sim A$ (and all the scaling relationships discussed earlier). However, this heuristic hides the fact the total volume of wire in the neocortex depends not only on the number of synapses per neuron, but also on how far neurons must reach. For the remainder of this section I put forth a more rigorous derivation of how wiring volume depends on different scaling strategies relating W and A. We will see that wiring optimality does indeed predict that W and A should scale approximately proportionally to one another, just as the simple heuristic argument predicted. The contents of the remainder of this section are somewhat more mathematical, and if one is content with the heuristic argument above, one may jump to the conclusion.

3.13.4.1 Economical Satisfaction of Invariant Area-Infiltration

Before explaining more rigorously why it is volume optimal for the average number of neurons per area, W, to scale proportionally with the number of areas – which is key to deriving the other scaling exponents – it is useful to see that invariant-infiltration itself is volume optimal (see Figure 6). Suppose an area needs to infiltrate the neurons in some other area. One extreme strategy – the actual strategy – is to, as the number of neurons per area, W, increases, have a fixed number of neurons carry out the infiltration, but have the number of synapses per neuron, δ, increase proportionally with W. If M is the number of neurons in the area responsible for the infiltration, then for the actual strategy, $M \sim W^0$ and $\delta \sim W$. The other extreme strategy is to keep δ invariant as W increases, and increase the number of neurons doing the infiltration proportionally with W; for this alternative strategy, $M \sim W$ and $\delta \sim W^0$. More generally, suppose that $M \sim W^\beta$, where β is a constant from 0 to 1. $\beta = 1$ corresponds to the actual scaling strategy for

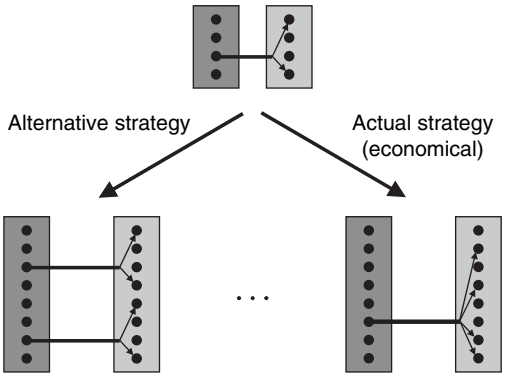

Figure 6 Illustration that the actual scaling strategy for invariant area-infiltration is volume optimal. The neocortex across mammals appears to economically maintain a constant degree of area-infiltration, no matter the size of the areas, where 'area-infiltration' is the fraction of neurons in an area to which a neuron connects. On the right is shown the actual scaling strategy, where, when the number of neurons per area is doubled, the number of neurons connecting from one area to the other remains the same, and the number of connections per neuron is doubled. The neural volume required is $V_{actual} = LR^2_{actual}$, where L is the distance between the areas, and R_{actual} is the caliber of a white matter axon. From Murray's law (see Section 3.12.2.3), $R \sim \delta^{1/3}$, where δ is the number of synapses per neuron, and so $V_{actual} \sim L\delta^{2/3}_{actual}$. Since $\delta_{actual} = 4$ in this illustration, $V_{actual} \sim L4^{2/3} = L2^{4/3}$. On the left is shown an alternative strategy, where, when the number of neurons per area is doubled, the number of neurons connecting from one area to the other doubles, and the number of connections per neuron remains the same. The neural volume required for this strategy is $V_{altern} = 2LR^2_{altern}$, where the '2' in front is due to there now being two white matter axons in the illustration, rather than one. From Murray's law, $V_{altern} \sim 2L\delta^{2/3}_{altern}$, and since $\delta_{altern} = 2$, $V_{altern} \sim 2L2^{2/3} = L2^{5/3}$. Therefore, $V_{altern} = 2^{1/3}V_{actual}$, and the actual strategy is cheaper.

infiltration, and $\beta = 0$ corresponds to the opposite extreme. To infiltrate the area, it must be that $M\delta \sim W$. It follows that $\delta \sim W^{1-\beta}$. The volume of wire required to implement the infiltration is given by $V = MLR^2$, where L is the distance required for the connection, and R is the axon caliber. As discussed in Section 3.13.2.3, $R \sim \delta^{1/3}$, and thus we have $R \sim W^{(1-\beta)/3}$. The volume of wire can be written now as $V \sim LW^{\beta}W^{2(1-\beta)/3} = LW^{(2+\beta)/3}$. Assuming that L is the same under any of the alternative scenarios, V scales up most slowly when β is minimal, namely at $\beta = 0$, corresponding to the case where $\delta \sim W$, the actual case found in the neocortex (see Section 3.13.3.1). That is, the most economical way for an area to infiltrate another is to send a constant number of neurons, and have the number of connections per neuron increase proportionally with the number of neurons in the area; not to increase the number of neurons in the area devoted to connecting to an area. Also, when combined with the invariant area-interconnectedness constraint, we can conclude that the total number of white matter neurons per area, W_{white}, is expected to scale proportionally with the number of cortical areas, A, that is, $W_{white} \sim A$. We will utilize this result below in our further theoretical development.

3.13.4.2 Economical Satisfaction of Invariant Well-Connectedness

We now consider the possible ways of parcellating the neocortex. At one extreme, there could be a fixed number of cortical areas, A, and the number of neurons per area, W, would scale directly proportionally with the total number of neurons (Figure 7, left side). In this case, $A \sim W^0$. At the other extreme, the fastest the number of areas can increase is proportionally with the number of neurons per area (Figure 7, right side), because invariant area-interconnectedness requires that there be enough neurons per area to connect to an invariant fraction of the total number of areas. In this case, $A \sim W$. Generally, this space of scaling possibilities is given by $A \sim W^{\alpha}$, where α ranges from 0 to 1 (see the x-axis of Figure 7).

Which value of α should we expect for the neocortex? The 'economical' hypothesis is that the manner of scaling up the number of areas – that is, the setting of the parameter α – is such that the total neuronal wiring volume scales up as slowly as possible (subject to the satisfaction of the invariant well-connectedness constraint). The total volume of wire, $V_{totalWire}$, can be split into the volume of intra-area wire, $V_{intraWire}$, and the volume of white matter, $V_{whiteWire}$, that is, $V_{totalWire} = V_{intraWire} + V_{whiteWire}$. The volume of intra-area wire is given by $V_{intraWire} = (N_{intra})(L_{intra})(R_{intra})^2$, where N_{intra} is the total number of neocortical neurons involved in intra-area connections, L_{intra} is the average length of an intra-area connection, and R_{intra} is the average caliber radius of the major axon for intra-area connections. Similarly, the volume of white matter is given by $V_{whiteWire} = (N_{white})(L_{white})(R_{white})^2$. We would like to write $V_{totalWire}$ as a function of the total number of neurons, N, and the parameter α, so that we may determine the value of α that minimizes the scaling rate for $V_{totalWire}$. By utilizing the two well-connectedness constraints, along with our earlier observations concerning optimal area-infiltration, we will be able to write each of the terms above – N_{intra} and N_{white}, R_{intra} and R_{white}, and L_{intra} and L_{white} – as a function of N and α.

N_{intra} and N_{white}. The total number of white matter neurons, N_{white}, is equal to the number of white matter neurons per area, W_{white}, times the total number of areas, A; that is, $N_{white} = (A)(W_{white})$.

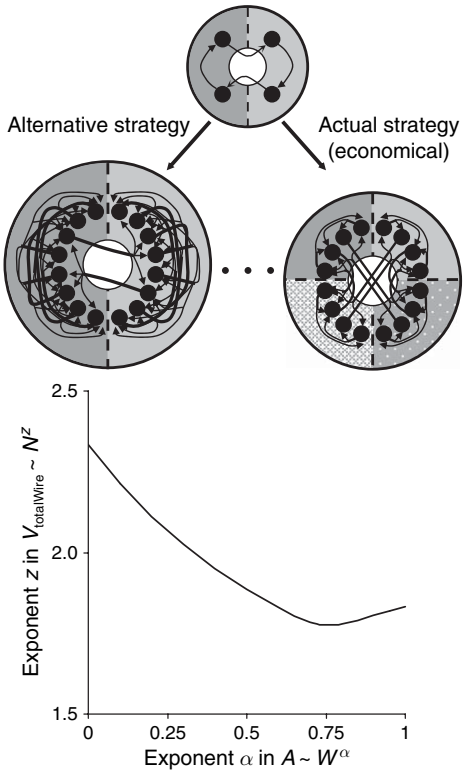

Figure 7 Illustration that the actual scaling strategy for invariant well-connectedness is near volume optimal. To maintain invariant well-connectedness, area-infiltration (the fraction of neurons in an area to which a neuron connects) *and* area-interconnectedness (the percentage of areas in the neocortex to which an area connects) must remain constant as brain size increases. On the right is shown the actual scaling strategy, where when the number of neurons in the brain, N, quadruples (from 4 to 16), the number of areas, A, approximately doubles (from 2 to 4), i.e., $A \sim N^{1/2}$. For this case, the number of areas scales proportionally with the number of neurons per area, W, which also doubles (from 2 to 4), i.e., $A \sim W^\alpha$, where $\alpha = 1$. Invariant area-infiltration is satisfied by doubling the number of neurons to which each neuron (both inter-area and intra-area) connects (from 1 to 2). Invariant area-interconnectedness is achieved by doubling the number of areas to which each area connects (from 1 to 2). This actual scaling strategy can be summarized by the exponent $\alpha = 1$, which leads (see Section 3.13.4.2) to the total volume of wire scaling as $V_{totalWire} \sim N^z$, where $z = 11/6 = 1.83$; this is shown in the plot (bottom) of the exponent z as a function of α. On the left is shown an alternative strategy, where, when the number of neurons in the brain quadruples (from 4 to 16), the number of areas remains constant (at 2). The relationship between the number of areas and number of neurons per area is therefore $A \sim W^\nu$, where now $\alpha = 0$. For this case, invariant area-infiltration is satisfied by quadrupling the number of neurons to which each neuron connects (from 1 to 4). Invariant area-interconnectedness is achieved by having each area connect to just one other area, just as in the smaller brain. This alternative scaling strategy can be summarized by this exponent $\alpha = 0$, which leads to the total volume of wire scaling as $V_{totalWire} \sim N^z$, where $z = 7/3 = 2.33$; this is shown in the plot (bottom) of the exponent z as a function of α. The exponent $z = 2.33$ for the alternative scaling strategy is very significantly higher than the $z = 1.83$ for the actual scaling strategy, and the latter exponent is near the optimal of 1.765 occurring at $\alpha = 0.76$. See Section 3.13.4.2 for the more general derivation.

By virtue of the optimality argument discussed in the previous section, the number of white matter neurons per area must scale in proportion with the total number of areas; that is, $W_{white} \sim A$. Thus, we may write $N_{white} \sim A^2$. We also know that the total number of neurons, $N = WA$ (where W is the average number of neurons per area), and along with the α power law relationship, $A \sim W^\alpha$, we have that $N \sim (A^{1/\alpha})(A)$, or $A \sim N^{\alpha/(1+\alpha)}$. Therefore, $N_{white} \sim N^{2\alpha/(1+\alpha)}$. Also, $N_{intra} = N - N_{white}$.

R_{intra} and R_{white}. We learned earlier (Section 3.13.2.3) that $R \sim \delta^{1/3}$, where R is the radius of an axon caliber. From this and invariant area-infiltration, $\delta \sim W$, we may write that $R \sim W^{1/3}$. Recalling the α power law relationship, $A \sim W^\alpha$, we have that $R \sim A^{1/(3\alpha)}$. We saw earlier that $A \sim N^{\alpha/(1+\alpha)}$, and thus $R \sim N^{1/[3(1+\alpha)]}$. We assume in this derivation that every neuron in the neocortex scales up proportionally with one another (in terms of the number of synapses per neuron), and therefore this proportionality relationship for R is valid for both R_{intra} and R_{white}.

L_{intra} and L_{white}. The average length of an intra-area connection, L_{intra}, is given by the average linear dimensions of an area, $L_{intra} \approx (V_{gray}/A)^{1/3}$, where V_{gray} is the total volume of gray matter. Gray matter volume is modeled as scaling proportionally with the total number of synapses in the network. This is, in fact, true across actual mammalian neocortices, because synapse density does not vary with neocortex size (Abeles, 1991), so doubling the gray matter volume means doubling the total number of synapses. The total number of synapses is equal to the total number of neurons times the number of synapses per neuron, or $N\delta$. Thus, $L_{intra} \sim (N\delta/A)^{1/3}$. We have seen earlier that $\delta \sim W$ (because of the invariant area-infiltration constraint), and along with the α power law relationship $A \sim W^\alpha$, we can conclude that $\delta \sim A^{1/\alpha}$. We have also seen earlier that $A \sim N^{\alpha/(1+\alpha)}$, and we may derive that $L_{intra} \sim N^{2/[3(1+\alpha)]}$. We treat the average length of a white matter axon, L_{white}, as the linear dimensions of the brain, whose volume scales approximately proportionally to gray matter volume (Changizi, 2001b), and so $L_{white} \approx V_{gray}^{1/3}$. Similar to the way we derived the relationship for L_{intra}, we get the relationship $L_{white} \sim N^{(2+\alpha)/[3(1+\alpha)]}$.

We are now in a position to write the total wire volume, $V_{totalWire}$, as a function of N and the parameter α. $V_{totalWire} = V_{intraWire} + V_{whiteWire} = (N_{intra})(L_{intra})(R_{intra})^2 + (N_{white})(L_{white})(R_{white})^2$, and plugging the values derived above, we have

$$V_{totalWire} \sim (N - N^{2\alpha/(1+\alpha)})(N^{2/[3(1+\alpha)]})(N^{1/[3(1+\alpha)]})^2 + (N^{2\alpha/(1+\alpha)})(N^{(2+\alpha)/[3(1+\alpha)]})(N^{1/[3(1+\alpha)]})^2.$$

With some algebraic manipulation, this becomes

$$V_{\text{totalWire}} \sim (N^{(4/3)/(1+\alpha)})[N + N^{(7/3)\alpha/(1+\alpha)} - N^{2\alpha/(1+\alpha)}].$$

Across the range of values for α (i.e., from 0 to 1), I numerically computed the scaling exponent of $V_{\text{totalWire}}$ as a function of N (Figure 7). The exponent is minimal – and the total wire volume scales up most slowly – when $\alpha = 0.76$. In particular, when $\alpha = 0.76$, the $V_{\text{totalWire}} \sim N^{1.7748}$. In contrast, for $\alpha = 0$, the case where the number of areas remains constant with network size, $V_{\text{totalWire}} \sim N^{2.33}$. On the other hand, when $\alpha = 1$, corresponding to the fastest that the number of areas can scale up, $V_{\text{totalWire}} \sim N^{1.833}$, which is not much faster than the optimal scaling at $\alpha = 0.76$. Accordingly, the predictions made by the economical well-connectedness hypothesis are very similar if we use $\alpha = 1$ instead of $\alpha = 0.76$.

Table 1 shows the approximate empirical scaling exponents for the variety of neocortical quantities discussed in Section 3.13.2 (both as a function of the number of neurons, and as a function of gray matter volume), along with the predictions of the above 'economical well-connectedness' model, and one can see close agreement, whether one uses the predicted exponent for the optimal $\alpha = 0.76$, or one uses the $\alpha = 1$ approximation.

3.13.5 Conclusion

Here we have summarized a variety of changes the neocortex undergoes in the transition from small to large brains (Section 3.12.2), pointed out two fundamental connectivity invariances (Section 3.12.3), and showed that the economical satisfaction of these invariances is central to an explanation of the many scaling features (Section 3.12.4). More specifically, mammalian neocortices appear to conform to two principles of connectivity, referred to as invariant well-connectedness. The first is invariant area-infiltration, which is the observation that the average number of synapses per neuron scales approximately proportionally with the average number of neurons per area. There appears to be, then, strong selective pressure across mammalian neocortices for the average number of synapses per neuron to scale up just fast enough that neuron-interconnectedness 'within areas' can remain invariant, 'and' that when an area connects to another area, the average neuron making the connection has a sufficient number of synapses to infiltrate an invariant fraction of the neurons in the area. The second principle of connectivity is invariant area-interconnectedness, which is the observation that the average number of area connections per area scales approximately proportionally with the total number of areas. There appears, then, to be strong selective pressure across mammalian neocortices for the number of area connections per area to scale up just fast enough that area networks can maintain an invariant level of area-interconnectedness. Equivalently, there is selective pressure for the total number of area–area connections to scale as the square of the total number of areas. These two principles of invariant well-connectedness – and the two hierarchical tiers of invariant interconnectedness – appear to be central to the organization of the mammalian neocortex, and the economical satisfaction of these invariances leads to the average number of neurons per area scaling approximately proportionally to the total number of areas. Since the total number of neocortical neurons is just the product of the number of neurons per area and the number of areas, it follows that the latter quantities scale as the square root of the total number of neocortical neurons. Because of invariant area-infiltration, it follows that the average number of synapses per neuron must also scale up as the square root of the total number of neocortical neurons, and a consequence of this is that neuron density decreases and axon caliber increases as discussed in Section 3.12.2 (and these, in turn, were key to understanding why surface area and white matter volume scale the way they do).

We see, then, that these scaling relationships are primarily due to selective pressure for economically satisfying invariant well-connectedness, and are thus 'epiphenomenal', that is, are not signs of more complex computations *per se*. Left unanswered is what exactly is so important about conforming to invariant well-connectedness. There is clearly a theoretical elegance to the principle, but I have no *a priori* theoretical reason for why it should be selected for, nor why there are two tiers rather than, say, three.

Also left unanswered is why brain size increases with body size, namely approximately as the 3/4 power of body mass. Given the contemporary proclivity for treating the brain as a biological computer, it is mysterious why a larger, but not more behaviorally complex, mammal should require a larger brain at all, much less one that scales so considerably with body size. If brain size did not vary with body size, these scaling problems would, of course, vanish. We are currently in the embarrassing situation of understanding why neocortical features vary with brain size as they do, but not understanding why brains vary in size so much in the first place (see Constraints on Brain Size: The Radiator Hypothesis).

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3.14 Sparse Coding in the Neocortex

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Glossary

kurtosis	The fourth statistical moment of a distribution; measures the degree to which a distribution is peaked and heavy-tailed; a Gaussian has a kurtosis of 0.
natural scene	An image of the natural world.
sparse code	A method of representing information that shows a low activity ratio; a code for which most coding units are inactive most of the time.

3.14.1 Introduction: Optimality in Biological Systems

For any biological system, an account of why the system is structured as it is requires consideration of a number of interacting forces including the uses (or goals) of the system, the environment in which the system must function, and the constraints that history and biology put on the design (see The Origin of Neocortex: Lessons from Comparative Embryology, The Evolution of Neuron Classes in the Neocortex of Mammals, Organization of a Miniature Neocortex – What Shrew Brains Suggest about Mammalian Evolution). It might seem reasonable to assume that the constraints of evolution and development play a large role in determining the design of any neural system. However, a range of recent studies have argued that neural systems have found highly efficient solutions for representing environmental information. These studies have explored topics from retinal coding (Sterling, 2004) to the computations provided on the semicircular canals with respect to head rotations (Squires, 2004) to the optimal cortical layout that minimizes neural wiring (Van Essen, 1997; see Neural Wiring Optimization). In all these studies, there remain intriguing questions regarding how close these solutions are to optimal, and how evolution and development lead to this optimality.

In this article, however, we focus on a general aspect of sensory representation called sparse coding. We argue that there is widespread evidence of such coding in neural systems across a variety of species. We look briefly at the question of what is meant by sparse coding and then ask why sensory systems would profit from performing such coding. We argue that the natural environment is inherently sparse and codes that take advantage of this structure can be both metabolically efficient and useful for learning. However, the constraints involved in producing a highly sparse code can be severe: if representing each object and pose requires a different set of neurons, the system would need a very large number of neurons indeed. We believe that many vertebrates have developed a strategy of combining high sparseness with invariance, a strategy that overcomes the combinatorial explosion of a highly sparse code. In this chapter, we address the extent to which sparseness is an optimal coding solution for natural data, and the additional processing strategies that may have shaped cortical evolution in vertebrates.

3.14.2 Defining Sparse Coding

Sparse coding generally refers to a representation where a small number of neurons are active, with the majority of the neurons inactive or showing low activity (e.g., Field, 1987, 1994; Rolls and Tovee, 1995). In his influential single neuron doctrine, Barlow (1972) suggested sparseness as one of the principles important to sensory representation. However, sparse coding in its extreme form results in a representation sometimes called a grandmother cell code. In such a code, each object in the world (e.g., your grandmother) is represented by a single cell. One might argue that a large brain with tens of

billions of neurons can certainly handle a few hundred thousand object-level neurons. And there are many studies showing that neurons exist that can be highly selective to faces and other objects (e.g., Kendrick and Baldwin, 1987; Quiroga, *et al.* 2005). However, those promoting the usefulness of sparse representations are not proposing that the ultimate goal is to have one neuron for every object – and certainly not for a particular view of every object. We believe that sparseness helps learning and prediction even at early stages of sensory processing, like those found in V1. But too much specificity or sparseness can actually make learning harder. We will explore this question later in the article.

Two lines of evidence support the notion that sparse representations are common in neural systems: the first comes from physiology, the second from computational and theoretical research. In each case, the evidence requires a definition of sparseness. There have been several definitions of sparseness and a number of ideas regarding what sparse codes actually represent.

Sparseness can be defined over a population of neurons at a given point in time (population sparseness) or it can be measured for a single neuron over some window of time; the latter is called temporal or lifetime sparseness (Willmore and Tolhurst, 2001) and it is sometimes referred to as nonparametric selectivity (Lehky *et al.*, 2005). For a given distribution of responses, we obtain a histogram of activity. One might think that the simplest definition of the sparseness of this distribution is to simply measure the proportion of active neurons, or how often a neuron is active. However, the histogram of activities is usually defined over a window of time and is therefore not binary. In response to any population of stimuli (e.g., natural scenes), one typically obtains a distribution of activities (a distribution of spike probability), so the measures of sparseness refer to the relative shape of the distribution.

Two definitions of sparseness are widespread. The first – the Treves–Rolls measure (eqn [1]) – is more appropriate for measuring the sparseness over time for real spiking neurons (Rolls and Tovee, 1995). The second definition uses kurtosis (the fourth statistical moment of a distribution) as its metric (eqn [2]) and it is more useful for modeled neurons and computational studies, where a comparison of different codes and transforms is necessary (Field, 1994):

$$S = \frac{\left((1/n)\sum_i r_i\right)^2}{(1/n)\sum_i r_i^2}, \qquad [1]$$

$$k = (1/n)\sum_{i=1}^{n} \frac{(r_i - \bar{r})^4}{\sigma^4} - 3. \qquad [2]$$

These measures are applied to histograms of responses over a population of neurons or for a given neuron over time. With the Treves–Rolls measure, the sparseness approaches zero as the neuron is either off or highly on (for a given time window). With the kurtosis measure, large values occur when a response distribution deviates maximally from the Gaussian state by being sharply peaked and heavy-tailed. Highly kurtotic behavior produces a relatively high probability of either a small or large response, and a relatively low probability of a mid-level response. Both measures can be sensitive to outliers.

3.14.3 Physiological Evidence for Sparse Coding

Much of the discussion in recent years regarding sparse coding has come from the computational and theoretical literature, but there is considerable physiological evidence for sparse representations in most biological systems. For a neuron with a full response and refractory time of 5 ms, the maximal firing rate would be 200 Hz. A system that is providing a maximal information rate would fire at approximately half the time, i.e., at 100 Hz. Although neurons may reach such rates, neurons do not maintain these rates for more than brief periods. We do not know of any neural systems that maintain such high firing rates for extended periods.

Such a high firing rate would require considerable energy resources, so much so that Attwell and Laughlin (2001) argue that the limited biochemical energy available for producing action potentials must limit the average firing rates of neurons to less than 1 Hz. Further, Lennie (2003) estimates that the limited resources imply that at any given time, only 1/50th of any population of cortical neurons can afford to show high firing rates. Therefore, for biochemical reasons alone, we should expect a considerable degree of both lifetime and population sparseness. Olshausen and Field (2005) further argue that even in areas that have been well studied – areas like V1 – these low average firing rates imply that a significant number of neurons will have such low firing rates as to be missed entirely by the typical search strategies.

As noted by Olshausen and Field (2004), there are a number of studies suggesting that many neural systems utilize highly sparse codes. DeWeese *et al.* (2003), recording from auditory neurons in the rat, have demonstrated that neurons in A1 can reliably produce a single spike in response to a sound. Evidence from olfactory systems in insects (Perez-Orive *et al.*, 2002;

Theunissen, 2003), somatosensory neurons in rat (Brecht and Sakmann, 2002), and recordings from rat hippocampus (Thompson and Best, 1989) all demonstrate highly sparse responses. Prefrontal cortex shows similar sparseness in behaving rhesus monkeys (Abeles *et al.*, 1990). As the authors of the latter study say, most areas of association cortex are "not carrying out any computations for the majority of the time."

Motor neuron representations are often described as a population code, where it is proposed that the accuracy of a movement is guided by the degree of activity of a relatively large population of neurons (see Georgopoulos, 1986). Here too, we find evidence of sparse responses. Some motor neurons in layer 6 of rabbit motor cortex will produce just one spike during some movements (Beloozerova *et al.*, 2003). And stimulation of a single neuron in the rat is sufficient to deflect a whisker (Brecht *et al.*, 2004).

With respect to sparse coding, the most widely studied sensory system is the visual system. Much of this work, which we discuss below, has been motivated by information theoretic issues. The area also contains a wealth of experimental data. In inferotemporal (IT) cortex, a wide range of studies supports the notion that neurons are selective to high-level object dimensions, and to features such as faces and hands. Such neurons are believed to show some degree of invariance over position, size, pose, brightness, etc. Nevertheless, unless we are to assume that such objects fall within the neuron's receptive field at least half of their waking life, we should expect these neurons to show a high degree of sparseness. Indeed, Baddeley *et al.* (1997) found that cells in IT show sparse responses to natural stimuli, to a similar degree as do cells in V1.

Much of the most interesting work tying together the statistics of natural signals and physiology comes from work on the responses of V1 neurons. Vinje and Gallant (2000, 2002) found that V1 neuron responses in macaque become more and more sparse as the size of a natural stimulus is increased beyond the classical receptive field. Stimulation in the classical receptive field also produced sparseness, which could reflect the rather arbitrary nature of the classical/nonclassical delineation. Moreover, stimulation in the nonclassical receptive field showed the following results:

1. increased sparseness for individual neurons during repeated presentations (lifetime sparseness);
2. increased sparseness across the population of neurons tested (population sparseness); and
3. decreased correlation in neighboring neurons, thereby whitening the response.

The results presented here provide examples of the sparse behavior of neurons in primate visual cortex under naturalistic conditions. Although we cannot argue that a sparse coding strategy is ubiquitous in the cortex, these results do support the implication that sparse coding is widespread in the nervous systems of the mammals tested. We also find evidence of sparse coding across a variety of nonmammalian species. In addition to the creatures mentioned above, the selectivity of sensory neurons has been supported by studies in amphibians (e.g., Ewert, 1980), turtles (Ammermüller *et al.*, 1995), and insects (e.g., Strausfeld and Lee, 1991; Lehrer and Srinivasan, 1992; Perez-Orive *et al.*, 2002).

Any form of selectivity implies that neurons will show a degree of sparseness in the natural environment, since selectivity by definition means neurons respond only to a portion of the possible environmental stimuli. Answering the question of why sensory systems show highly selective responses will require innovative ways of thinking about sparseness. In the next section, we consider an information theoretic approach to sparse coding and we compare this to an approach that argues for sparse coding as a result of the metabolic constraints on neural firing. We argue that both approaches will likely be needed to explain all aspects of sparse coding in neural systems.

3.14.4 Two Views of Sparse Coding

3.14.4.1 Maximizing Information Transfer

Consider a neuron, or population of neurons, that has a mean firing rate and some distribution around that mean. With a limit on the range of possible firing rates, the maximum information transfer occurs when all states of the channel are used with equal frequency: a flat distribution of firing rates. If the bound on a distribution is instead the variance of the responses (rather than the range of responses), the distribution with the greatest information rate (greatest entropy) is a Gaussian. The visual system appears to follow neither of these models. A sparse code means that neural firing rates will show a highly peaked, non-Gaussian distribution, i.e., one that does not produce maximum information transfer. Moreover, as sparseness increases, the information rate drops. Why might this be a good idea?

In general, we argue that the information rate of the system should match the information rate of the input. Natural stimuli are not random. As far as the visual system is concerned, natural scenes are not

arrays of random points of light. Rather, they are constrained by strong correlations between neighboring regions, and image discontinuities are usually defined by edges. This predictability implies that a high information rate is simply unnecessary. By taking advantage of the redundant properties of images, sensory codes can get away with sending less information and using fewer spikes.

For example, consider a collection of 5×5 pixel images that each contain one block letter of the alphabet. If we looked at the histogram of any given pixel, we might discover that the pixel was on roughly half the time. However, if we were to represent these letters with templates that respond uniquely to each letter, each template would respond just 1/26th of the time. This letter code is more sparse – and more efficient – relative to a pixel code. Although no information is lost, the letter code would produce the lowest information rate.

Moreover, a representation that was letter based would provide a more efficient means of learning about the associations between letters. If the associations were between individual pixels, a relatively complex set of statistical relationships would be required to describe the co-occurrences of letters (e.g., between the Q and U). Sparseness can assist in learning since each unit is providing a relatively complete representation of the local structure (Field, 1994).

Of course, the natural world does not consist of letters. Natural scenes are highly structured and can be modeled to a first approximation as a sparse collection of local features (e.g., edges). Early work by one of us (Field, 1987, 1994) showed that if the receptive fields of V1 neurons are modeled as a collection of linear templates, then the responses of those neurons to natural scenes are highly sparse. Furthermore, when the parameters of the modeled neurons were altered from those of V1 neurons, the sparseness dropped, suggesting that the parameters were near to optimal given the constraint of having a linear array of model neurons.

A stronger test of this hypothesis was developed by Olshausen and Field (1996), who trained a neural network to find the most sparse representation for a population of image patches drawn from natural scenes. The network was trained to develop a set of filters that would maximize sparseness and losslessness in its representation of natural scenes. Given these two criteria, the network settled on a set of filters with considerable similarity to simple cell receptive fields in V1 (Figure 1).

This basic sparse coding algorithm is quite similar to techniques that search for independent solutions by minimizing response entropy (that is, by searching for non-Gaussian response histograms). The family of these techniques has been called independent components analysis or ICA (Bell and Sejnowski, 1997). The name ICA is a bit unfortunate since the solutions are almost never independent, given natural input. But the approach has been applied to a wide range of problems and it has been employed to account for a variety of properties in early visual neurons, including spatiotemporal tuning (e.g., van Hateren and Ruderman, 1998) and spatiochromatic properties (e.g., Hoyer and Hyvarinen, 2000), and generalization of the approach has been used to model some nonlinear properties of neurons (Hyvarinen and Hoyer, 2000). In early vision, Graham *et al.* (2005) show evidence that sparseness is likely a factor contributing to the utility of center-surround receptive field organization, along with decorrelation and response gain. Furthermore, sparse codes of natural sounds have been shown to produce temporal response profiles with properties similar to those of early auditory neurons (Lewicki, 2002).

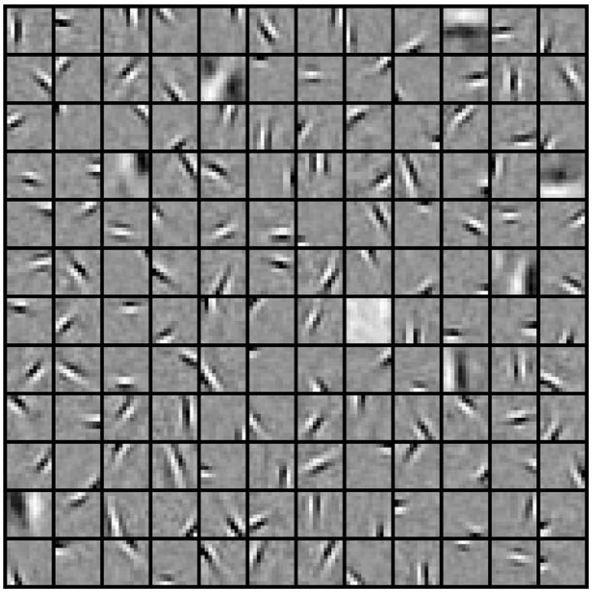

Figure 1 Results of a neural network that searches for a sparse code using filters to describe 12×12 pixel image patches drawn from a collection of natural scenes (Olshausen and Field, 1996). The collection of filters shown represents the 256 templates that the network found for describing each patch. When any given natural scene patch is multiplied by this family of filters, one finds that most of the responses are near zero and a small set of filters produces large responses (those matched to the image structure). The templates have been shown to provide a good first-order account of the responses of cortical V1 simple cells, suggesting that such simple cells are optimized to provide a sparse code for natural scenes. Adapted from Olshausen, B. A. and Field, D. J. 1996. Emergence of simple cell receptive field properties by learning a sparse code for natural images. *Nature* 381, 607–609.

This entire line of work suggests that sparseness in neural firing is primarily a result of efficiently matching the neuron's response properties with the sparse statistical structure of the environment. There remain a number of questions as to how a biological system might achieve this efficient representation. Although the particular method used by Olshausen and Field (1996) may not be biologically plausible, it is argued that learning algorithms exist that could train a system to achieve a sparse code. The advantage of learning such a code is that the properties of individual neurons need not be predetermined or coded in the genome of the organism. Rather, what is required is that the system evolve only a general sparse coding strategy. Given appropriate input, a learning algorithm would serve to produce the proper neural response properties and it would help to tile the neurons in a way that allows the correct spacing as a function of the different parameters of selectivity (e.g., position, scale, orientation). Although there is little evidence of such a learning algorithm in insects and animals with relatively simple brains, the evolution of a sparse learning algorithm may be widespread in larger brains. However, the argument is not that the system must necessarily learn from the natural world. Rather, one intriguing possibility is that the system learns from the patterned structure of the spontaneous activity in the developing organism (e.g., Wong, 1999).

It is not yet clear whether the patterns of spontaneous activity are sufficient for generating the receptive field properties found in the newborn. However, such an approach would provide a relatively simple means for producing a large number of neurons with efficient tuning. The learning algorithm also has the advantage that it can accommodate a large variation in cell number. If any evolutionary mutation or developmental change results in a larger (or smaller) brain, the learning algorithm should be capable of adapting to the system and adjusting the tiling appropriately.

Could all neurons in higher areas from V1 to IT simply be exhibiting sparse coding as a means of efficiently representing the natural world? This may be possible. A number of investigators are exploring ways to extend these ideas of efficient coding to higher levels. It is clear that these more complex representations require a form of nonlinear coding that makes the tests of efficiency considerably more difficult, though attempts to model nonlinear behavior of neurons with such efficient coding approaches have met with some success (e.g., Schwartz and Simoncelli, 2001; Wiskott and Sejnowsi, 2002).

However, there is a penalty that applies to learning if the system is too sparse. An extremely sparse code (one in which neurons are highly selective for specific objects in specific poses, lighting, etc.) would have neurons that fired quite rarely. In order to effectively learn about the world, any system must keep track of the relative probability of co-occurrences. No matter how a neural system keeps track of these co-occurrences, if they occur too rarely it would be impossible to determine whether any feature is statistically related to any other feature. We cannot learn about how 'faces' behave in particular situations if we have a neuron for every unique face. It is important that the system be invariant at some level so that we can collapse across instances of the category. Most presentations of objects or events will occur just once or not at all during development if the object is defined too precisely.

From the perspective of learning, then, a code that is too sparse becomes intractable even if there were enough neurons to accommodate it. Consequently, a system must also build invariance into the code in order to develop and function efficiently. With high-level objects such as faces, this learning constraint would require that the face-selective neurons be invariant to dimensions along which the face varies in different settings (lighting, pose, size, etc.). Thus, both invariance and selectivity are necessary for achieving an efficient, sparse representation of sparse natural input. Indeed, this invariance is a known property of visual neurons. As neurons are found to be more selective, we find greater degrees of invariance. The complex cells in V1 show selectivity to scale (spatial frequency), and orientation, and they show small amounts of invariance to position. Higher-level neurons in IT and medial temporal cortex may show much higher selectivity to faces, hands, etc. However, they also show much greater invariance to lighting, pose, and size (Rolls, 2000). In one study of human medial temporal cortex (Quiroga *et al.*, 2005), neurons were found that were selective to particular actresses (Jennifer Aniston) while invariant to pose, lighting, and position in the image.

We therefore argue that although the evolution of large brains may allow a larger number of highly selective neurons, the constraints of learning require that the selectivity go hand in hand with a greater degree of invariance. Although there have been a number of proposals regarding how invariance is achieved in the mammalian systems (Olshausen *et al.*, 1993), no firm answer has emerged. We know that some of the simpler visual systems, such as those of *Drosophila*, do not show such invariance

(Dill *et al.*, 1993). However, it remains unclear how such invariance has evolved, and what we might expect from systems that show only partial invariance.

3.14.4.2 Metabolic Constraints

We conclude our discussion by returning to the issue of metabolic constraints. Could we argue that primary evolutionary pressure driving toward sparse coding is one related to the metabolic costs of neural firing? As noted earlier, both Attwell and Laughlin (2001) and Lennie (2003) argue that there are not enough resources to achieve anything but a low-activity system. Moreover, when we find sparse activity in frontal cortex (Abeles *et al.*, 1990), it is more difficult to argue that the sparse activity must arise because it is mapping the sparse structure of the world. Even at early levels, if sparseness were metabolically desirable, there are a number of ways of achieving sparseness without matching the structure of the world. Any one of a wide variety of positively accelerating nonlinearities would do. Simply giving the neurons a very high threshold would achieve a sparse code, but the system would lose information. We argue that the form of sparse coding found in sensory systems is useful because such codes maintain the information in the environment, but do so more efficiently. We argue that the evolutionary pressure to move the system toward a sparse code comes from the representational power of sparse codes.

However, we do accept that metabolic constraints are quite important. It has been demonstrated that at the earliest levels of the visual system, ganglion cells (Berry *et al.*, 1997) and lateral geniculate nucleus cells (Reinagel and Reid, 2000) show sparse (non-Gaussian) responses to temporal noise. A linear code, no matter how efficiently it was designed, would not show such sparse activity, so we must assume that the sparseness is at least in part due to the nonlinearities in the system and not due to the match between the receptive fields and the sparse structure of the input. Since the results show sparse responses in nonsensory areas, we must accept that metabolic constraints may also be playing a significant role.

3.14.5 Conclusions

We are therefore left with a bit of a puzzle. We know that higher levels of the visual system show considerable sparseness: neurons fire at rates far below their maximal rate. However, we cannot conclude that sparseness is only a result of an efficient mapping of the sparse structure of the world. Metabolic efficiency must also be considered, independent of the statistical structure of the world. In addressing the question of why a system is sparse, we must accept that widespread sparseness in cortex is due to several factors. Many of us believe that the metabolic constraints are secondary, however, and that artificial visual systems will someday incorporate much of the coding we find in neural systems. The constraints of evolution, metabolism, anatomy, and development all play a role in determining why the nervous system is designed the way it is. But one should not presume that these constraints force the system toward some nonoptimal solution. At present, the evidence suggests that the nervous system has evolved a highly efficient learning algorithm for discovering and representing the structure in the world. And the sparse responses of neurons are an integral component of that efficient representation.

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http://www.redwood.berkeley.edu – Olhausen Lab.
http://www.pdn.cam.ac.uk – Tolhurt Lab.
http://hlab.phys.rug.nl – Van Hateren Lab (natural scene stimuli collection).

3.15 Evolution of the Somatosensory System – Clues from Specialized Species

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Glossary

areas	This term is often used to describe separate subdivisions of the brain and neocortex. In the neocortex different areas generally have a number of identifying features, such as unique appearance in histological stains (cytoarchitecture), unique connections to other areas, unique cellular responses, and result in specific deficits following damage. Some well-known cortical areas include primary somatosensory cortex (S1), primary visual cortex (V1), and primary auditory cortex (A1).
cortical barrel	A circular region of the neocortex visible in various histological stains of the somatosensory area in rodents where touch information from a single whisker projects. First recognized by Woolsey and Van der Loos (1970) in mice.
cortical magnification	The relative size of a representation, or processing area for a sensory input, in the cortical map. This generally refers to the larger representations of behaviorally important sensory inputs as compared to less important inputs. A common example in humans is the large area of cortex devoted to processing touch information from the hand relative to other, larger body parts (such are the leg or back) that have a proportionally much smaller representation in the cortex.
cytochrome oxidase	A mitochondrial enzyme. Processing brain tissue to reveal the distribution of this enzyme often reveals different subdivisions, particularly in the neocortex. Cortical barrels can be seen in the distribution pattern of this enzyme (Figure 1).
Eimer's organ	A small (40–80 μm) swelling in the nasal epidermis of talpid moles that contains and orderly array of mechanoreceptors used for tactile discriminations. Similar to a push-rod in montotremes.
electrosensory/ electroreception	Electroreception is the ability to detect weak electric fields in an aquatic environment through dedicated sensory organs (electroreceptors). This sense is sometimes used by predators (e.g., sharks) to detect the small electric fields given off by prey.
neocortex	The outer six-layered sheet of brain tissue in mammals where much of the information from sensory receptors projects. Often shortened to 'cortex' in discussions of the mammalian brain. Many investigators prefer the term 'isocortex' to avoid the implication of an invalid phylogenetic sequence suggested by the term 'neo'.
mystacial vibrissae	The large, mobile whiskers on the face of a rodent.

ocular dominance column	Stripes of cortical tissue in layer 4 of primary visual cortex that receive input from the lateral geniculate nucleus, relayed from primarily only one eye. Each stripe is generally bound by similar stripes representing the opposite, contralateral eye.
receptors	In this context, 'receptors' refers generically to the sensory organs and nerve endings that receive and communicate sensory information from the environment. More specific modality designations include mechanoreceptors, photoreceptors, electroreceptors, etc.
saccade	A saccade is a sudden, jerky movement. The term 'saccade' is most frequently used in reference to an eye movement. In the visual system a saccade is the characteristic sudden movement of the eye that positions different parts of a visual scene on the retinal fovea.
sensory representation	Generally refers to a topographic map of primary afferent inputs to the central nervous system (CNS). In the case of the somatosensory system, the sensory representations reflect the distribution of mechanoreceptors in the skin, and as such they form a 'map' of the body surface that can be identified in neocortex by recording the activity of nerve cells in response to stimulating the skin.
somatosensory cortex	The area of neocortex that receives and processes touch information from mechanoreceptors on the body.
tactile fovea	The descriptor draws an analogy between the high-resolution retinal fovea in the visual system and the high-resolution part of the star-nosed mole's nose used for detailed, tactile investigations of object of interest. A similar analogy with the visual system has been made in the auditory system of bats, where an 'auditory fovea' is said to represent the most important echolocation frequencies.

3.15.1 Introduction

The somatosensory system provides a rich source of diversity for revealing principles of mammalian brain evolution. At the same time, it is daunting to consider the number of different aspects of mammal bodies that have changed in the course of evolution and often challenging to identify examples of brain specializations that can be confidently attributed to specific sensory adaptations. Consider, for example, the vast difference in brain size between shrews – that resemble ancestral mammals in many respects – and humans, that have only recently emerged on the evolutionary landscape (Figure 1).

This comparison highlights some of the challenges to deciphering mammalian brain evolution, as the differences between shrew and human brains may parallel the differences between the small brains of early stem mammals and the larger and more complex brains found in many modern lineages. The comparison of a human brain to a shrew brain seems appropriate as an introduction because it not only illustrates a range of mammal brain sizes, but also because insectivores hold a particularly important historical position in theories of mammalian brain evolution. Fossil evidence indicates that the earliest ancestral mammals had brains and bodies similar to those of modern insectivores, particularly shrews that have little neocortex (Kielan-Jaworowska, 1983, 1984). As a result, a number of theories of brain evolution have been based on the premise that modern insectivore brains resemble those of ancestral species (Lende, 1969; Glezer

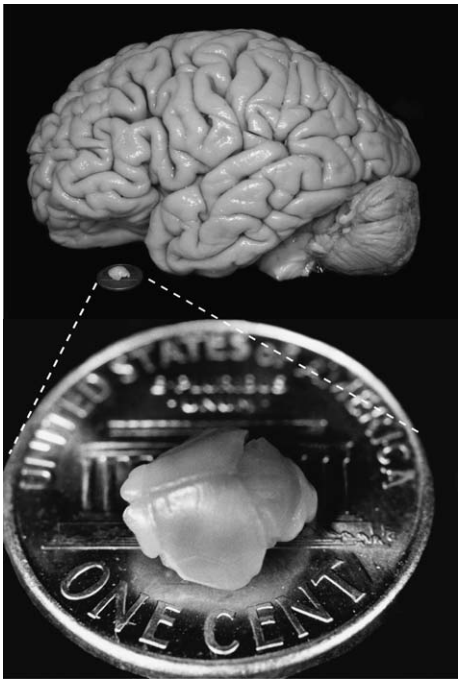

Figure 1 An adult human brain compared to the brain of a shrew. The upper panel shows the two brains at the same scale, with the shrew brain resting on a penny for scale. The lower panel shows the shrew brain enlarged.

et al., 1988; see Deacon, 1990, for review). This historical trend was bolstered by early recording experiments in hedgehogs (Lende and Sadler, 1967) and moles (Allison and Van Twyver, 1970) which indicated that insectivore cortex was poorly differentiated with overlapping cortical subdivisions. However, recent investigations of insectivores (Catania, 2000a) have revised our conception of these species as primitive mammals with poorly organized brains and thus historical theories of brain evolution based on early investigations of insectivores need to be reconsidered.

Before developing theories for how somatosensory cortex may have evolved in different lineages, it is first essential to describe what is known about the products of evolution. What are the major differences in brain organization observed across different species? What facets are unique to particular lineages, and what has been conserved across taxa? What solutions to sensory processing have recurred in the course of evolution and may thus illuminate constraints on the ways brains can be modified?

Although the brains of only a small percentage of extant mammals have been examined in detail, recent investigations of somatosensory cortex have expanded our understanding of brain organization in mammals that range from standard laboratory rats (Remple *et al.*, 2003) to monotremes (Krubitzer *et al.*, 1995; Krubitzer, 1998) and marsupials (Beck *et al.*, 1996; Rosa *et al.*, 1999; Huffman *et al.*, 1999; Catania *et al.*, 2000) representing important branches of the mammalian radiations. By considering the organization of cortex in selected species, it is possible to draw some general conclusions about how cortical organization has changed in the course of mammalian evolution.

In addition to our growing understanding of brain diversity across species, a number of recent advances in the ability to modify gene expression during the course of development have allowed investigators to mimic the process of brain evolution in the laboratory. Thus, on a small scale, some of the diversity that is observed across species can be generated within species by manipulating gene expression (Fukuchi-Shimogori and Grove, 2001). This in turn suggests potential mechanisms by which brains may have been modified in the course of evolution (Rakic, 2001).

Finally, in discussing the evolution of somatosensory areas in the brain, it is important to simultaneously consider the mechanosensory periphery. After all, the main function of the somatosensory cortex is to process information from these receptors and there is an intimate association between the sensory periphery and the central nervous system (CNS) during the course of both development and evolution.

3.15.2 How Have Brains Changed in the Course of Evolution?

3.15.2.1 Areas May Be Added to the Processing Network

There is still much disagreement and uncertainty regarding the organization of cortex and the identity of areas in many of the most intensively investigated species (see Kaas, 2005). However, it is nevertheless clear from comparative studies that larger brains differ significantly from the smaller brains in living mammals, and by extension that larger brains of modern species differ from the small brains of ancestral species that gave rise to these lineages (see Jerison, 1973; Kaas, 1987a, 1987b, 1995, 2005; Krubitzer, 2000). This is exemplified by comparing the shared cortical areas between shrews and humans (Figure 2). Shrews are particularly interesting because many of them represent the lower size range for the mammalian body and brain (see Schmidt-Neilsen, 1984). Shrews are also particularly interesting because fossil evidence indicates that early mammals also had small brains with little neocortex. Thus, understanding constraints on the organization of a small neocortical sheet may help us infer how early mammalian cortex was organized.

Shrew brains were found to have only a few cortical sensory areas with sharp borders as determined from both electrophysiological and histological evidence (Catania *et al.*, 1999). These areas include primary and secondary somatosensory cortex (S1 and S2), primary visual cortex (V1), primary auditory cortex (A1), and motor cortex (M1). Human brains also contain these same subdivisions in similar relative position in the cortex (i.e., V1 is caudal in cortex, A1 is lateral, M1 is most rostral). This comparison demonstrates two important and very general findings in mammals. First, diverse mammal species share a number of cortical areas in common. Second, larger-brained mammals tend to have more cortical subdivisions. The greater number of intervening cortical areas is not illustrated in Figure 2 for humans, but can be appreciated from the schematic in Figure 3, which illustrates the number of cortical subdivisions in a shrew compared to the estimated number of cortical subdivisions in a macaque. Whereas shrews have only five known cortical areas with little room for additional subdivisions (Catania *et al.*, 1999), macaques are thought to have over 50 different areas (see

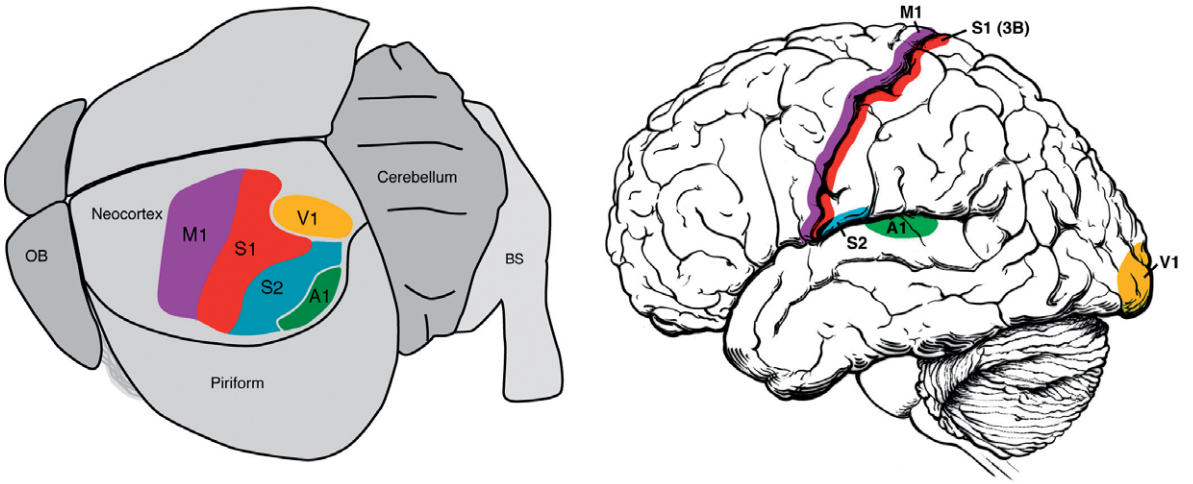

Figure 2 Shared cortical areas between a shrew and a human. Left side shows a shrew brain and cortical areas, including primary somatosensory cortex (S1), secondary somatosensory cortex (S2), primary visual cortex (V1), primary auditory cortex (A1), and primary motor cortex (M1). The same (homologous) cortical areas are depicted in the human brain on the right. Human have many additional cortical areas that are not illustrated, whereas shrews have little room for additional cortical subdivisions. OB, olfactory bulb; BS, brainstem.

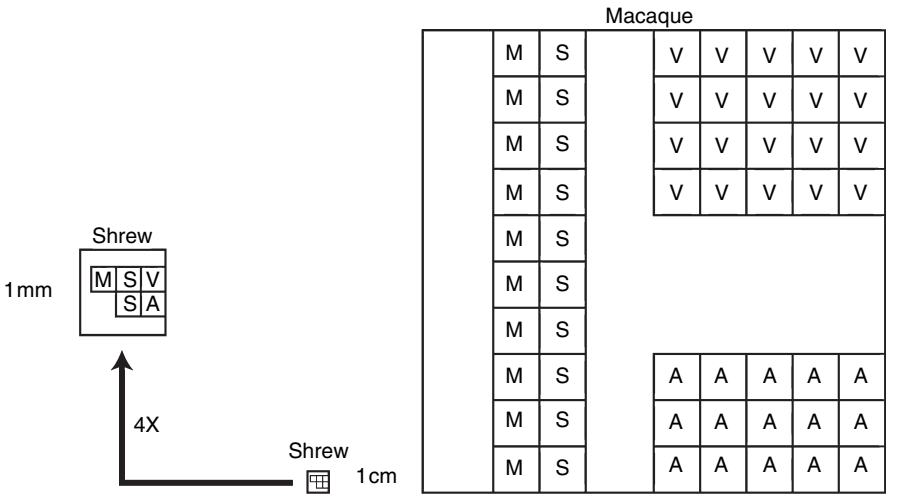

Figure 3 A schematic representation of cortical organization in a small-brained (shrew) and large-brained (macaque monkey) mammal. Shrews have as little as 0.15 cm^2 of neocortex, whereas macaques have roughly 72 cm^2 – a 480-fold difference. Humans, with approximately 800 cm^2 of neocortex, do not fit on the figure, but have neocortex with over 5000 times the surface area of a shrew. Given that shrews are similar in size and habits to ancestral mammals, there has clearly been a tremendous enlargement of cortex in many mammalian lineages. In addition to getting larger, the internal organization of cortex has changed as well. Many cortical subdivisions have been added in larger-brained mammals, and this can be appreciated by comparing the enlarged shrew brain (far left) to the macaque brain. The letters denote visual (V), auditory (A), somatosensory (S), and motor areas (M). Shrews have only a few cortical subdivisions, whereas macaques have many. The illustration is not intended to show the relative size or location of cortical areas. Reproduced from Catania, K. C. 2004. Correlates and possible mechanisms of neocortical enlargement and diversification in mammals. *Int. J. Comp. Psychol.* 17, 71–91.

Kaas, 1995 for review) and additional areas will almost certainly be identified in macaque cortex.

This observation is perhaps not surprising; however, it does raise additional questions regarding brain scaling and evolution. It is clear that large-scale changes to brain organization have occurred in many mammalian lineages – for example, in the primate and carnivore orders that have more cortical subdivisions than smaller-brained rodents and insectivores (Kaas, 1982). Greater numbers of cortical subdivisions are often considered to be an important underlying substrate for increased intelligence and behavioral complexity. Yet, it is difficult to separate factors related to brain scaling from

those related to increased processing ability. For example, as brain areas increase in size, local connections must increase in length to maintain a similar degree of global connectivity. Such increases in lengths of axons and dendrites must be accompanied by increases in their diameters in order to maintain similar conduction times between cells (Ringo *et al.*, 1994). The main point is that increasing the size of a brain and its cortical areas includes many engineering challenges and thus some cortical areas may become subdivided simply to maintain the status quo (Kaas, 2000).

In addition, it is often difficult to confidently identify a particular brain specialization related to increased behavioral complexity or processing ability when comparing distantly related species, such as insectivores and primates, as some traits may be most common in a given lineage without an obvious adaptive value. This has been termed the taxon level effect (Pagel and Harvey, 1989). One way to more confidently identify specializations related to a particular behavioral or sensory ability is to look in closely related mammals of similar brain and body size, in which only one dimension of a sensory system has changed in a particular member of the group.

3.15.2.2 The Star-Nosed Mole – A Case Study in Somatosensory Evolution

Comparing the somatosensory systems of different mole species provides what might be considered a natural experiment in the elaboration of the mechanosensory portion of the nose and corresponding representations in the brain. Unlike most other mammals, moles use the skin surface of the snout – rather than vibrissae – to explore their environment through touch. But the degree of elaboration of the nose and associated sensory organs differs greatly across species. Consider, for example, the eastern American mole (*Scalopus aquaticus*) in Figure 4. This species is a more generalized mole that resembles the kind of ancestral condition from which the star-nosed mole evolved (Catania, 2000b). How does brain organization differ between star-nosed moles and the less specialized but closely related eastern American mole?

Microelectrode recordings from the brain of *Scalopus* reveal a somatosensory cortex similar in many general respects to that found in the star-nosed mole (Figure 5a). A relatively large S1 contains a representation of the body with caudal body parts (tail and hindlimb) located medially in cortex and the face and nose represented more laterally. As in star-nosed moles, a relatively large S2 is found as a mirror image of S1 in more lateral and caudal cortex. This basic layout of two relatively large

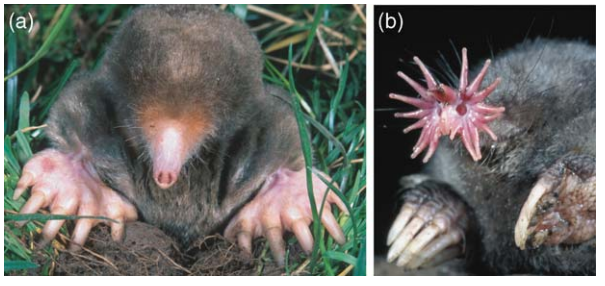

Figure 4 Comparison of two mole species. a, The eastern American mole (*S. aquaticus*) is the least specialized mole resembling the probable ancestral condition for moles (Catania, 2000b). b, The star-nosed mole (*Condylura cristata*) is the most specialized mole with a snout consisting of 22 mechanosensory appendages.

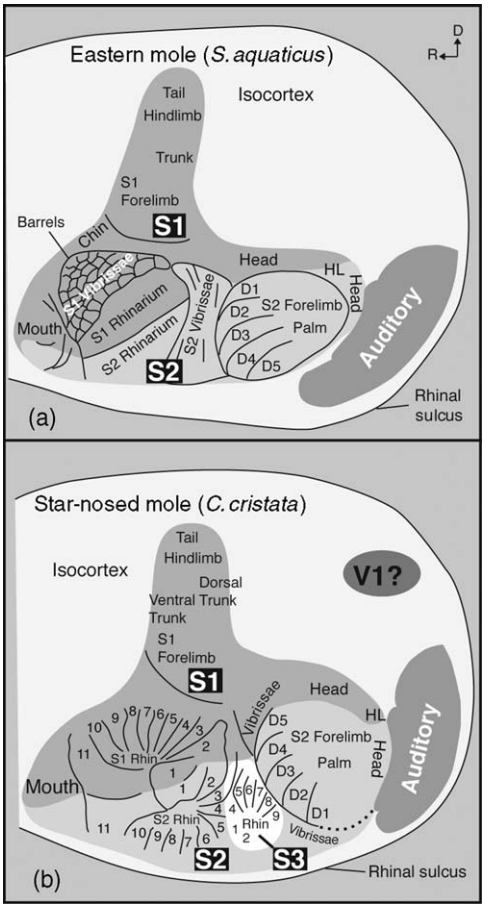

Figure 5 The results of recent investigations of cortical organization in moles. a, The eastern American mole has two somatosensory areas, primary (S1) and secondary (S2) somatosensory cortex, which include visible barrels much like those identified in rodent cortex. b, The star-nosed mole has three representations of the star (S1, S2, and S3). These areas are visibly reflected as a series of modules in flattened sections of cortex processed for cytochrome oxidase.

somatosensory areas, S1 and S2, is also found in other moles species (Catania, 2000c) and in the sister group to moles, the shrews (Catania *et al.*, 1999; see Figure 2). Thus, moles and shrews generally have two representations of the nose in lateral cortex. However, star-nosed moles have three representations of the star (Catania and Kaas, 1995) in lateral cortex (Figure 5b). The most parsimonious interpretation of these observations is that star-nosed moles have independently evolved an extra representation of the star.

This finding is from very closely related species that differ little in body weight and brain size, and it supports the conclusion that the addition of a new area to the cortical network is an important substrate for more efficient processing of sensory inputs. The most obvious difference between star-nosed moles and other moles is the elaboration of the somatosensory star with a corresponding increase in innervation density accompanied by more complex foraging behaviors (e.g., foveation movements of the star – this is discussed in Section 3.15.2.4). As a result, star-nosed moles are one of the fastest and most efficient of mammalian foragers (Catania and Remple, 2005) and the larger number of cortical representations of the star may facilitate this ability, perhaps through the parallel processing of different facets of touch information.

3.15.2.3 Behaviorally Important Areas Are Magnified in the Brain

Figure 6 illustrates cortical magnification of important sensory surfaces in the naked mole-rat and the star-nosed mole showing how the most behaviorally important sensory surfaces take up a disproportionate area of cortex. This feature of cortical maps has been documented since the pioneering studies of Adrian (1943) and Woolsey *et al.* (1942), in which it was noted that parts of the body that have the greatest tactile acuity have the largest cortical projection zones. Cortical magnifications have since been described for different sensory systems in diverse species, and this phenomenon makes for striking imagery. However, the relationship between sensory surface size and cortical representational area also raises important and fundamental questions about brain organization and evolution. Namely, how do the most important sensory surfaces acquire the largest territories in the brain?

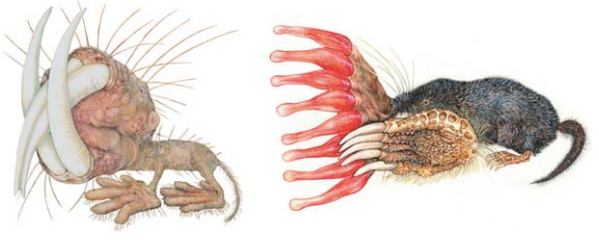

Figure 6 Cortical magnification in naked mole-rats and star-nosed moles. These schematics illustrate the relative proportions of the somatosensory cortex taken up by representations of different body parts in each species. Surprisingly, the naked mole-rat devotes much of its cortex (30% of S1) to the representation of the incisors. In contrast, star-nosed moles devote a huge portion of their somatosensory cortex to the representation of the star.

Early investigations of this relationship in rodent barrel cortex revealed a direct linear correlation between the size of a cortical barrel (the area representing a whisker) and the innervation density of the corresponding whisker (Welker and Van der Loos, 1986). This result suggested that cortical representational area could be, in general, proportional to the innervation density of the sensory surface projecting to any given area of cortex. Such a relationship would explain the expanded representations of important areas of the skin, retina, and cochlea that had been described in a number of species. At the same time, this finding suggested that there was no 'cortical component' to cortical magnification and that this parameter could be predicted without even examining the brain, simply by determining the relative innervation density of a sensory surface. Lee and Woolsey (1975) recognized this possibility and suggested that cortical representations are more appropriately described by a "peripheral scaling factor" than a "cortical magnification factor."

Of course, another possibility is that cortical representational area is not proportional to number of inputs from the periphery, and instead important sensory inputs could project to a larger area of cortex than less important inputs. This has been the subject of considerable historical debate in the visual system of primates, where some studies suggest that the large cortical representation of the retinal fovea simply reflects the number of retinal ganglion cells projecting from the retina (Drasdo, 1977; Wassle *et al.*, 1989, 1990), whereas other studies indicate that ganglion cells projecting from the fovea have a disproportionately large representation in cortex (Malpeli and Baker, 1975; Myerson *et al.*, 1977; Perry and Cowey, 1985; Silveira *et al.*, 1989). The weight of most recent evidence supports the contention that important inputs in the visual systems of primates are indeed overrepresented in the cortex (Azzopardi and Cowey, 1993). However, the few studies that have addressed this issue and the conflicting results from different studies in the

primate visual system highlight the difficulty of making these determinations in most sensory systems. This is a case where the particularly specialized sensory system of the star-nosed mole has provided new insights as a result of its anatomical specialization. But before describing how star-nosed moles can shed light on visual system organization, it is necessary to outline the parallels between the star-nosed mole's somatosensory system and the visual systems of sighted mammals.

3.15.2.4 A Somatosensory Fovea in the Star-Nosed Mole

Although the nose of the star-nosed mole is a tactile sensory surface, there are a number of behavioral and anatomical similarities between the mole's sensory system and the visual systems of other species. This is most obvious from observations of star-nosed mole behavior (Catania and Remple, 2004). The entire star is used for the detection of relevant stimuli in the environment, but once an object or food item of interest is detected, the nose is shifted in a saccadic manner for detailed investigations with the touch fovea. There are 11 finger-like appendages on each side of the star, and the ventral-most, 11th pair constitutes the fovea. The other appendages take up a much greater surface area and act as the 'tactile periphery' in a manner analogous to the peripheral visual receptors of the retina.

Because one small area of the skin surface is the behavioral focus of the star, we can address the question of whether the most important inputs from a sensory array are allocated extra territory in the cortex, or alternatively whether the sizes of each cortical representation are simply proportional to their innervation density. This question is relatively easy to answer in the star-nosed mole because of the favorable anatomical organization of the sensory system. It is possible to quantify three different parameters: (1) the number of sensory organs on the star, (2) the number of primary afferents innervating the each appendage of the star, and (3) the area of primary somatosensory cortex devoted to each appendage (Figures 7a–7c). It is also possible to accurately measure the cortical representation of the star because of the histologically visible reflection of the appendage representations as a series of modules in somatosensory cortex. This aspect of star-nosed mole brain organization is discussed in more detail in the next section.

Because these different parameters can be measured in star-nosed moles, a number of interesting comparisons can be made. First, it is possible to consider the relationship between innervation density (number of nerve fibers) and the number of sensory organs (Eimer's organs) on the skin surface of each appendage (Figure 7d). This comparison shows that the number of nerve fibers and the number of Eimer's organs co-vary almost precisely for appendages 1–9. However, for appendages 10 and 11, there are more fibers per sensory organ. This reflects the higher acuity of this behaviorally important sensory surface. But does this account for the cortical magnification of the fovea, as suggested by studies in rodent barrel cortex? Figure 7e shows this comparison (average area of cortex per afferent for each appendage of the star) clearly indicating that the higher innervation for the fovea area of the star does not account for the cortical magnification of the fovea. Instead, star-nosed moles devote a greater average area of cortex to the most important afferents from the 11th appendage of the star (the tactile fovea) and conversely a smaller average area of cortex to the representations of the afferents from remaining 10 appendages (Figure 7e). Thus, the favorable anatomy of star-nosed mole's sensory system has allowed for the quantification of variables that are difficult to measure in many species and these findings may reflect a common relationship between sensory surfaces and the cortex in mammals. For example, the degree of cortical overrepresentation of the inputs from the fovea of the star is similar to the degree of overrepresentation of the retinal fovea in primates (Catania, 1995; Azzopardi and Cowey, 1993).

Finally, the subdivision of the star-nosed mole's sensory system into fovea and periphery is a remarkable example of the convergent evolution of similar features across disparate sensory systems. It suggests this organizational scheme is a general solution to designing a high-resolution sensory system. The most familiar and common example of a fovea-periphery organization is of course found in many visual systems of diverse mammals; however, auditory systems can have an acoustic fovea as well. This has been demonstrated in a number of studies by Suga and colleagues (Suga and Jen, 1976; Suga, 1989) for mustached bats (*Pteronotus parnellii*). Mustached bats emit an echolocation call that includes a narrow frequency range around 60 kHz that is particularly important for detecting the acoustic evidence of wing-beats caused by flying insect prey. A large proportion of the hair cells in the bat's cochlea are tuned to this important echolocation frequency and a large territory of the bat's A1 is devoted to processing sounds at this frequency. Thus, mustached bats have an acoustic fovea, and they have the acoustic equivalent of a saccade as well. This is necessary because returning

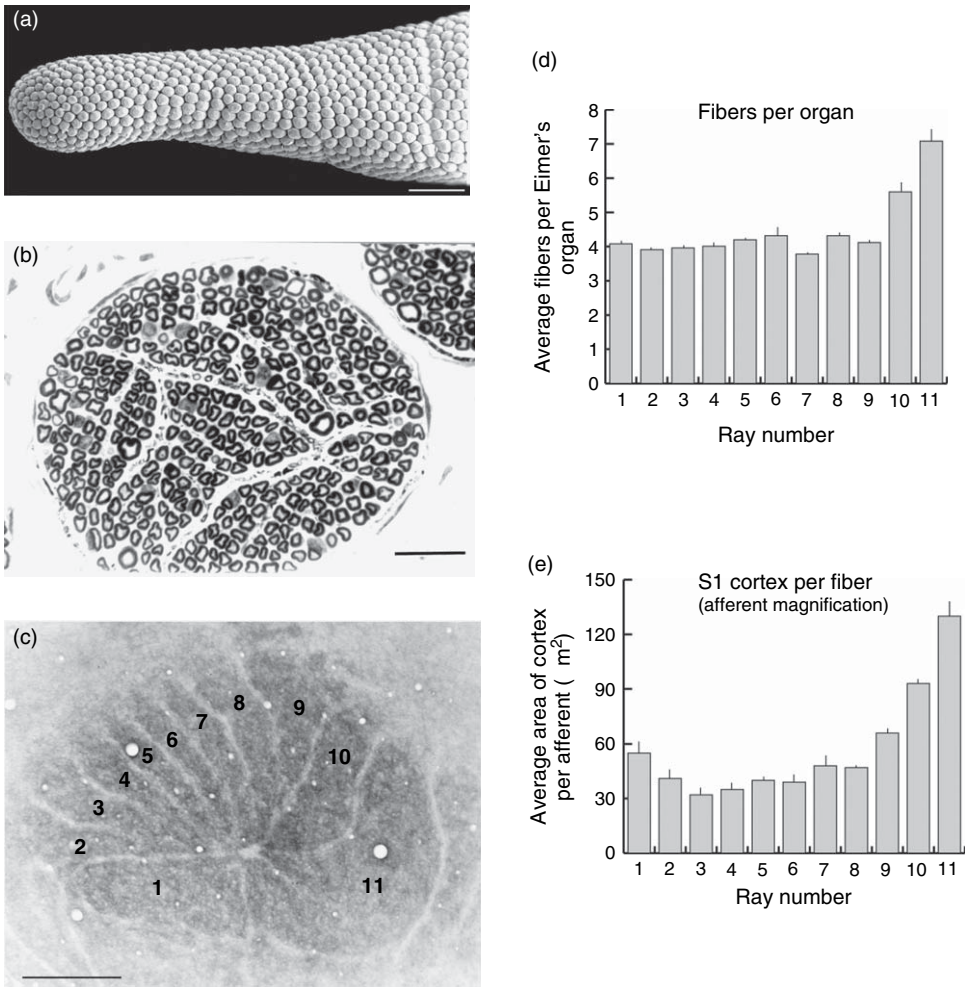

Figure 7 Quantification of the number of sensory organs, innervating nerve fibers, and representational area of the star in primary somatosensory cortex. a, A single appendage of the star under the scanning electron microscope showing the many visible sensory organs (Eimer's organs) covering the skin surface. b, A thin section of tissue showing a small portion of the many myelinated afferents supplying an appendage of the star. c, A potion of the cortex of a star-nosed mole that has been flattened and processed for cytochrome oxidase to reveal the primary somatosensory representation of the star. The area representing each appendage is visible as a separate subdivision. d, A graphic representation of the ratio of fibers (afferents) innervating each appendage per sensory organ on each appendage (or ray) of the star. e, The average area of cortex devoted to the primary afferents for each appendage (ray) of the star. Scale bars: a, 250 μm; b, 20 μm; c, 500 μm. Reproduced from Catania, K. C. and Kaas, J. H. 1997c. Somatosensory fovea in the star-nosed mole: Behavioral use of the star in relation to innervation patterns and cortical representation. *J. Comp. Neurol.* 387, 215–233.

echoes are often Doppler shifted to different frequencies depending on the speed of the bat and its target. To compensate for these Doppler shifts, bats are constantly shifting the frequency of their outgoing pulses to 'focus' the returning echo on the high-resolution area of the acoustic fovea. This behavior, called Doppler shift compensation (Schnitzler, 1968), is surprisingly similar to a saccade in the visual system.

The most well-developed visual systems, somatosensory systems, and auditory systems, all exhibit a fovea-periphery organization. An obvious benefit of this design is the conservation of neural processing area in the brain and innervating nerve fibers at the level of the sensory periphery. For example, making the entire sensory system high resolution would require a massive enlargement of the nerves carrying information to the brain, and a corresponding enlargement of the cortical areas processing the inputs. The ultimate result would be a staggering increase in brain size. It is far more efficient to devote a large part of the computational area of the brain to a small part of the sensory system (the retinal, tactile, or acoustic fovea) and then move that area around like a spotlight to analyze important stimuli (or in the case of bats, move the frequency of echolocation

pulses to ensure that Dopplar shifted echoes can be analyzed by the fovea).

3.15.2.5 Modules Represent Sensory Surfaces in Diverse Species

Woolsey and Van der Loos (1970) made the discovery of modules in the somatosensory cortex that represented the important facial vibrissae, or whiskers, in mice. They described cylindrical groupings of cells that were most easily seen in sections of the cortex cut parallel to the cortical surface. Electrophysiological recording of neuronal responses revealed that each barrel corresponded to the cortical representation of a single whisker on the face. This finding was remarkable because it revealed a visible reflection of a somatosensory map and at the same time provided a useful model system for exploring many details of mammalian brain organization and development. Cortical barrels were also considered to provide anatomical support for the columnar hypothesis of cortical organization, which suggests that cylindrical columns of interconnected neurons are the fundamental organizational unit of neocortex.

From the time since cortical barrels were first described, a number of investigations of cortex have revealed cortical subdivisions, or modules, related to sensory specializations in diverse species. Star-nosed moles provide one of the more dramatic examples of this relationship. Figure 8 shows details of star-nosed mole cortex. In the case of star-nosed moles, the receptors represented in cortex are part of an elongated skin surface, rather than a hair surrounded by a ring of mechanoreceptors as found in rats and mice (see Rice *et al.*, 1993). As described previously (Figure 5b) electrophysiological recordings from the cortex of star-nosed moles reveal three representations of the star in lateral cortex. When sections of the flattened cortex are cut parallel to the cortical surface and processed for cytochrome oxidase (Wong-Riley and Carroll, 1984) three different maps of the star are visible (Figure 8b). Each of these maps represents the entire contralateral star and each cortical module representing an appendage takes the from of elongated wedge.

The representations of the appendages of the star-nosed mole differ from cortical barrels of rodents in a number of ways. First, the representations of the appendages consist of elongated stripes of cortical tissue, rather than circular barrels. Second, the representation of the tactile fovea is greatly expanded in cortex relative to the size of this appendage on the star. As outlined above, the representation of this appendage reflects the behavioral importance of the fovea, rather than the innervation density of the sensory surface. Finally, in the star-nosed mole's cortex multiple maps of the sensory surface are uniquely visible. Three different somatosensory areas, S1, S2, and a new area we

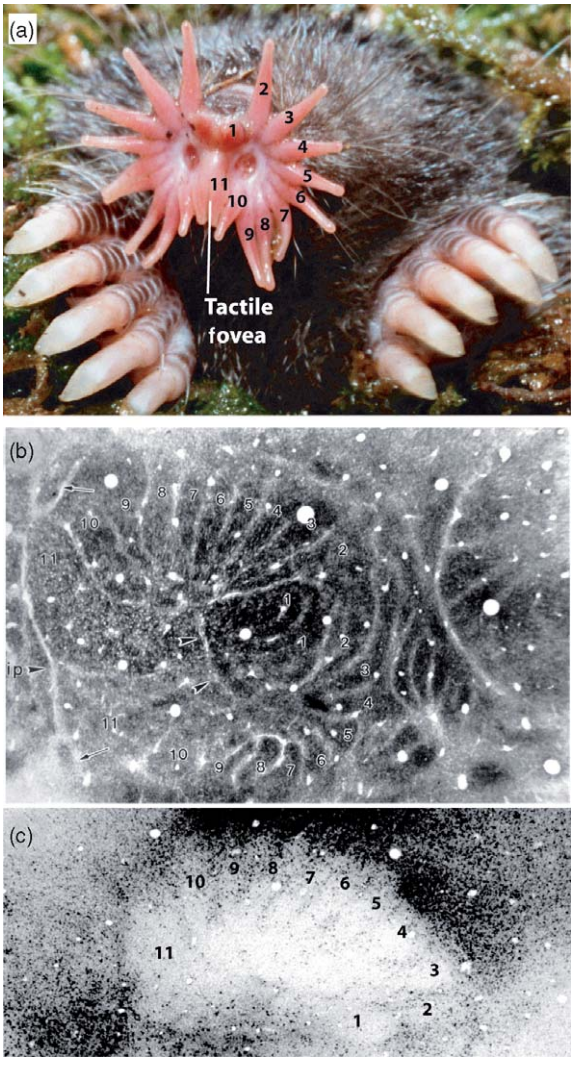

Figure 8 The unusual mechanosensory star and its corresponding cortical representation in the star-nosed mole (*C. cristata*). a, A star-nosed mole emerges from an underground tunnel showing its large forelimbs and the 22 fleshy appendages that surround each nostril. The 11th appendages on each side act as the somatosensory fovea and are used for detailed tactile investigations. b, A section of flattened cortex revealing all three cortical representations of the star (S1, S2, and S3 – see Figure 5b) visible as a series of modules, each representing an appendage from the contralateral star. c, An example of the specificity of callosal connections around the S1 star representation. Cells and terminals are concentrated in the septa between appendage representations and surrounding the star representation but are absent from the centers of each cortical stripe. Reproduced from Catania, K. C. and Kaas, J. H. 2001. Areal and callosal connections in the somatosensory cortex of the star-nosed mole. *Somatosens. Mot. Res.* 18, 303–311.

have termed S3, contain modules representing individual appendages.

From these observations, one can conclude that cortical modules are not constrained to form traditional columns, as suggested from barrels. It is also clear that insectivores may have exceptionally well-organized and complex cortical representations. This result is in stark contrast to results from early investigations of insectivores, including moles (Allison and Van Twyver, 1970), which suggested they had overlapping cortical areas with poorly defined topography. In this regard it is significant that the modules in the star-nose mole's cortex have different connections than the septa between modules. For example, tracer injections into the cortex reveal that callosal connections terminate selectively in the septa between cortical modules, whereas intercortical connections terminate primarily within modules (Figure 8c).

A different kind of modular representation of a sensory surface is found in the eastern American mole (*S. aquaticus*). This mole has an unusual and sensitive forelimb consisting of an oval palm and heavily clawed digits. In S2 of this species cytochrome oxidase processed sections of cortex reveal a modular reflection of the forelimb. This cortical pattern appears just like the large clawed hand that it represents (Figure 9). Tracer injections into the spinal cord of the eastern American mole show that the modular forelimb representation is also the location of dense areas of corticospinal projecting neurons (Catania and Kaas, 1997a). These examples of different kinds of connections to different parts of cortical modules in moles support the general conclusion that different parts of cortical modules may be the selective substrate for the distribution of specific cortical circuitry (Chapin *et al.*, 1987; Koralek *et al.*, 1990; Fabri and Burton, 1991; Hayama and Ogawa, 1997; Kim and Ebner, 1999).

In addition to cortical modules discussed above in rodents and insectivores, investigations of cortical organization in the duck-billed platypus have provided a different example of modules representing

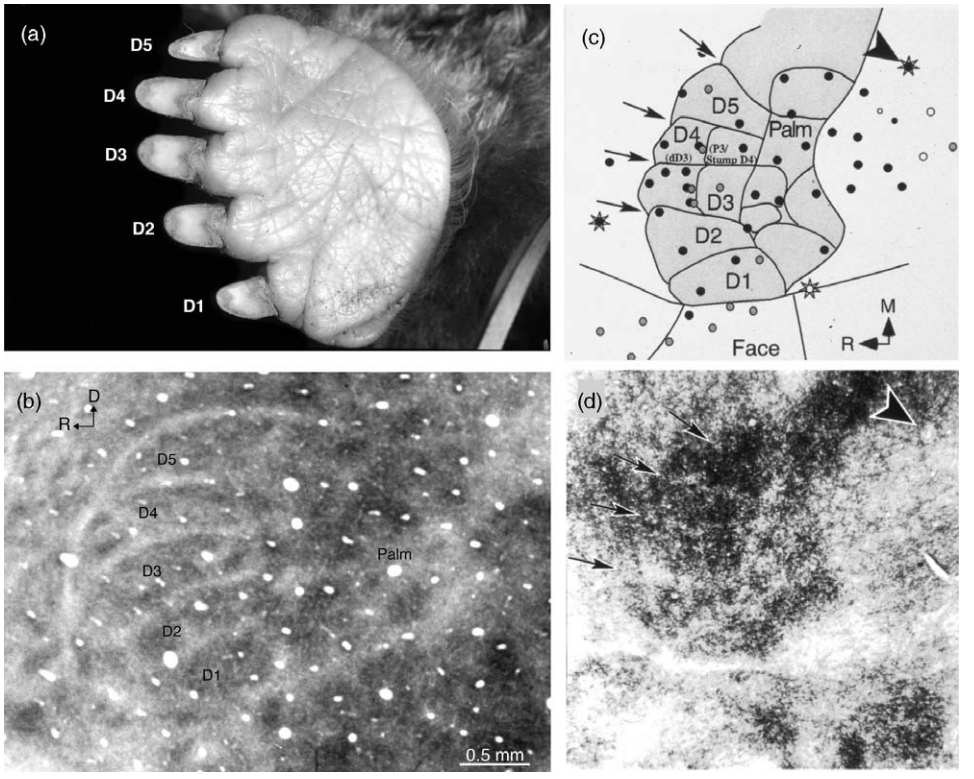

Figure 9 The representation of the eastern mole forelimb (*S. aquaticus*) and the hand of an owl monkey (*Aotus trivirgatus*). a, The large clawed forelimb of the mole. b, The cortical representation of the mole's forelimb as revealed by sections processed for cytochrome oxidase. The representation appears just like the large clawed hand it represents. c, A reconstruction of cortical recordings from an owl monkey showing the relative location of areas that responded to the digits (D1–D5) and the palm. d, Histological sections from the corresponding area of S1 (area 3B), in the same owl monkey, showing the cortical modules that represent the fingers and palm of the hand (arrowhead marks microlesions). b, Reproduced from Catania, K. C. and Kaas, J. H. 1997a. The organization of somatosensory cortex and distribution of corticospinal neurons in the eastern mole (*Scalopus aquaticus*). *J. Comp. Neurol.* 378, 337–353. d, Reproduced from Jain, N., Catania, K. C., and Kaas, J. H. 1998. A histologically visible representation of the fingers and palm in primate area 3b and its immutability following long-term deafferentations. *Cereb. Cortex* 8, 227–236.

sensory surfaces. The platypus bill contains tens of thousands of mechanoreceptors and electroreceptors (Manger and Pettigrew, 1996). Microelectrode mapping of the platypus somatosensory cortex has revealed a large S1 representation of the bill in lateral cortex (Krubitzer *et al.*, 1995). Flattened sections of cortex processed for cytochrome oxidase reveal alternating stripes of cortical tissue with dark and light regions representing higher and lower amounts of chronic neuronal activity. The dark areas represent the projection zones for mechanosensory information whereas the light zones represent the projection zones for combinations of mechanosensory and electrosensory information. Thus, S1 in the platypus contains receptor specific subdivisions very similar to the alternating bands of cortex representing rapidly adapting and slowly adapting mechanoreceptors in S1 of primates (Sur *et al.*, 1981), cats (Stretavan and Dykes, 1983), and raccoons (Rasmusson *et al.*, 1991). This anatomical arrangement of different sensory inputs in the platypus cortex is also reminiscent of ocular dominance columns representing inputs from the different eyes in primate area 17 (Hubel *et al.*, 1976).

The examples of cortical modules described above are from a range of particularly specialized mammals, and this raises the question of how common such representations of tactile sensory surfaces are across species and whether such findings have implications for primate and human brain organization. Relatively recent findings in primates suggest there are similar organizing principles for mechanosensory inputs across these diverse species. Jain *et al.* (1998) examined flattened cortex of three different primate species processed for myelin (Gallyas, 1979) and identified myelin-dense cortical modules representing the mechanoreceptors of the digits and palm in S1 (Figures 9c and 9d). Thus, the cortical representation of the primate hand, like the representation of rodent whiskers and the mole's star, is visibly reflected in flattened sections of cortex (see also Qi and Kaas, 2004). These findings indicate that large- and small-brained mammals share common developmental principles that segregate maps in similar ways. They also suggest there is a ubiquitous instructional role for the sensory periphery in guiding the formation of central representational maps.

3.15.2.6 The Sensory Periphery Guides Aspects of Cortical Development

The finding of histologically visible cortical maps of sensory surfaces that reflect the details of mechanoreceptor topography raises the question of how somatosensory areas become matched to the sensory periphery. Because the development of the somatosensory system begins with the skin surface and ends at the cortex (see Killackey *et al.*, 1995) there is opportunity for the sensory surface to instruct the cortex. Evidence for such an instructive role of the sensory periphery comes from the somatosensory system of rodents where it has been shown that early damage to a whisker disrupts the formation of the corresponding cortical barrel (Andres and Van der Loos, 1985; Woolsey, 1990).

A different but related kind of evidence comes from strains of mice bred for variations in the whisker pattern. Van der Loos and Dorfl (1978) noted that strains of mice born with extra whiskers on the face also developed extra barrels in the cortex in the appropriate topographic location (see Epigenetic Responses to a Changing Periphery – Wagging the Dog). They argued that it was unlikely for a single mutation to have simultaneously altered the entire sensory system from whisker to barrel, but rather a mutation acting at the level of the early developing skin surface was more likely to have been communicated to the subcortical nuclei and then to the developing cortex. Similar results have more recently been reported in star-nosed moles, where wild-caught animals have an unusually high rate (5%) of extra or missing nasal appendages. The different nose configurations are invariably reflected in the cortical maps (Catania and Kaas, 1997b).

Although Van der Loos and Dorfl made a compelling argument, they could not entirely rule out the possibility of a single genetic modification simultaneously and independently altered the brain and the whiskers of mice. Recently, however, their interpretation of an instructive role for the skin surface has received strong support from investigations in which altered whisker patterns were induced during embryonic development by transfecting the epidermis of mice with a virus containing the patterning gene Sonic hedgehog (Shh). This manipulation resulted in the formation of extra whiskers on the face, and later extra barrels in the cortex (Ohsaki *et al.*, 2002). However, in this case the genetic change was clearly restricted to the skin surface, supporting the hypothesis that the skin surface instructs the later-developing cortex.

Another possible role for the periphery in guiding the formation of cortex may be found in the timing of developmental events. For example, the retinal fovea in primates develops earlier than the peripheral retina, and inputs from the fovea have a preferentially magnified representation in cortex (as previously described). Similarly, the tactile fovea in star-nosed moles develops earlier than the more peripheral parts of the star. This can be

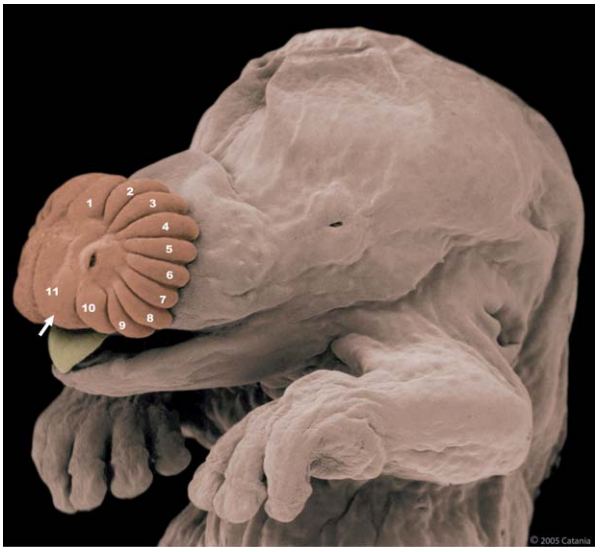

Figure 10 An embryonic mole showing the developing star. The appendages are numbered 1–11, as in adults. Note however, the relatively much larger area of the star taken up by the 11th, foveal appendage (arrow) at this early stage of development compared to appendage 11 in adults (see Figure 8a). Examination of this development sequence (Catania, 2001) reveals that the fovea leads the development of the star, and this may allow afferents from the developing fovea to capture a larger area of cortex (e.g., Figure 7e) in a competition for cortical territory. Photo copyright 2005 Catania.

appreciated by examining embryonic (Figure 10) and adult (Figure 8a) star-nosed moles and comparing the size of the 11th appendage (the tactile fovea) at these different stages. The 11th appendage takes up a far greater proportion of the star in embryos than it does in adults. More detailed investigations of this relationship (Catania, 2001) reveals that the tactile fovea leads the development of the star, such that it grows large early, has the largest innervated sensory surface in embryos, and develops sensory organs (Eimer's organs) before the peripheral appendages of the star (appendages 1–10). Yet later in development the peripheral appendages grow larger than the fovea, until in adults the 11th appendage is dwarfed by the rest of the star (Figure 8a).

The early development of these important sensory surfaces may give them an advantage in a competition for cortical territory during development. Evidence for this possibility comes from studies of the primate visual system. When one eye is sutured shut and deprived of visual input during critical periods of development, ocular dominance columns related to that eye are greatly reduced in size compared to the open eye (Hubel *et al.* 1977). Activity dependent expansions have also been documented for the somatosensory system, where the most active regions of barrel cortex undergo the greatest amount of growth during development (Riddle *et al.*, 1993; Purves *et al.*, 1994). These studies suggest that the most active inputs during critical periods of development have a competitive advantage in capturing representational space in the cortex.

So far, I have highlighted some of the evidence for changes that may have commonly occurred in the course of the evolution of the somatosensory system. These include the magnification of behaviorally important areas of sensory maps, the addition of new areas to cortical networks, the formation of a fovea-periphery organization for high-resolution sensory systems, and the subdivision of areas into modules representing segregated sensory surfaces in the periphery. In this last section I will outline some ideas for potential mechanisms by which some of these changes may occur. Additional details may be found in Epigenetic Responses to a Changing Periphery –Wagging the Dog.

3.15.3 What are the Mechanisms of Evolutionary Change?

3.15.3.1 Levels of Organization

The examples outlined above for the somatosensory system suggest two different levels of organizational change in the evolving neocortex that may be altered by two different mechanisms. The first level involves alterations of details of cortical representations that stem from the developmental link between the sensory periphery and the brain. Evidence for this possibility comes from a number of sources as outlined in the previous sections, including surgical alterations to the whiskers that change barrel patterns in mice, strains of mice bred with supernumerary whiskers that have extra barrels in the cortex, wild-caught star-nosed moles with extra appendages on the star and extra representational stripes in the cortex (indicating this occurs in natural populations), and evidence that changes in the timing of developmental events at the sensory surface may have an important impact on cortical development.

A second level of organizational change is the addition of completely new areas to the cortex, in the form of new maps of the sensory periphery. Evidence for this kind of change comes from comparative studies that illustrate the variation in numbers of cortical subdivisions in differently specialized species. The extra somatosensory area in star-nosed moles (Figure 5) compared to other mole species provides one example that can be

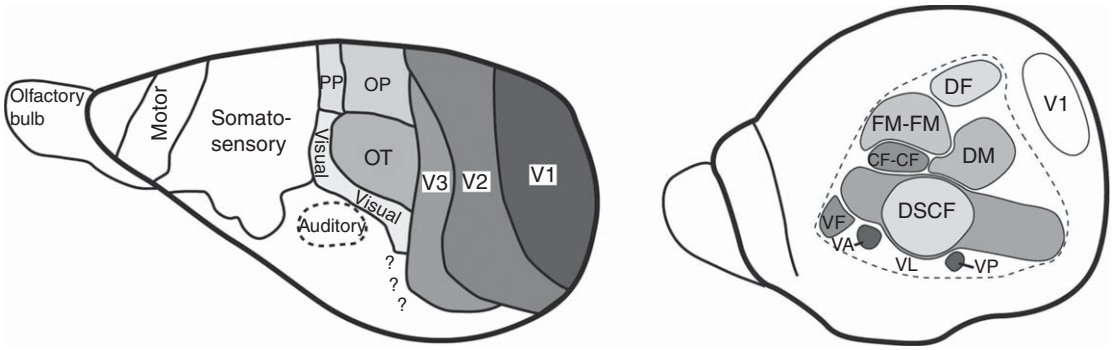

Figure 11 Cortical organization in megachiropteran and microchiropteran bats, demonstrating visual and auditory specializations, respectively. a, Summary of cortical areas in the megachiropteran flying fox (*Pteropus poliocephalus*). This fruit-eating species relies heavily on vision and this is reflected in the proportion of cortex devoted to vision and the number of corresponding visual areas. Roughly half of the cortex is taken up by a series of at least six visual areas (shaded areas) and a number of additional areas are likely to be found in more rostral-lateral cortex. b, Summary of cortical areas in the microchiropteran mustached bat (*Pteronotus parnellii*). In contrast to megachiropteran bats, microchiropteran bats have reduced visual systems and depend heavily on echolocation to navigate and locate flying prey. This is reflected in the organization of their neocortex which is dominated by a network of eight or more auditory areas (shaded areas) that largely process information in the frequency range of returning echolocation pulses. These closely related species provide an example of how cortex has evolved in parallel with the more complex visual and auditory abilities of each respective species. a, Data from Rosa, M. G., Krubitzer, L. A., Molnar, Z., and Nelson, J. E. 1999. Organization of visual cortex in the northern quoll, *Dasyurus hallucatus*: Evidence for a homologue of the second visual area in marsupials. *Eur. J. Neurosci.* 11(3), 907–915. b, Data from Suga, N. 1989. Principles of auditory information-processing derived from neuroethology. *J. Exp. Biol.* 146, 277–286.

confidently attributed to the elaboration of the somatosensory system. Other examples include comparison of the many visual areas in highly visual megachiropteran bats to the few visual areas in echolocating microchripteran bats, and conversely comparison of many auditory areas for processing echos in the microchiropteran bats to the few auditory areas in nonecholocating, megachropteran bats (Figure 11).

3.15.3.2 Potential Mechanisms of Change

As described above, the intimate developmental relationship between receptor arrays and cortical maps suggests that many changes to cortical areas in the course of evolution may initially occur simply by altering the body. It seems likely that cortical and subcortical areas of the brain are flexible enough to accommodate changes to the sensory periphery that may provide a selective advantage. For example, the expansion of a sensory surface allows for a greater area of the environment to be investigated per unit time. This is presumably the selective pressure that drove star-nosed moles in the direction of enlargement of their mechanosensory snout relative to other species. In support of this possibility, star-nosed moles eat relatively small prey items compared to other species of moles, and this requires locating more prey per unit time to satisfy metabolic requirements (Catania and Remple, 2005). The evidence of supernumerary appendages in some moles suggests a simple mechanism by which such an expansion of the sensory surface can occur – that is, change the star locally and the sensory processing areas will accommodate the alterations through a developmental cascade.

Yet changes to cortical areas to accommodate different configurations of a sensory surface may have important, negative consequences for sensory processing (Figure 12). In this respect, a cortical area may be challenged in the same general ways that have been outlined for increasing the size of the entire brain (Deacon, 1990; Kaas, 2000). One problem is the lengths and numbers of interconnections within a cortical area. As neurons become more widely separated, the diameters of their axons and dendrites must become greater to maintain similar conduction times between neurons (Ringo *et al.*, 1994). This in turn typically requires increases in the size of the supporting neuronal cell body in order to supply the metabolic requirements of the neurites. In addition, as the number of neurons in larger areas increases, the number of connections between neurons must increase drastically to maintain a similar degree of global connectivity between neurons within the area (Deacon, 1990). All of these changes require more space in the cortex, which compounds the problem. Thus, increasing the size of a cortical area could result in a suboptimal processing area and set the stage for the adaptive benefits of adding a new area to the cortex.

Figure 12 provides a schematic outline for how some of these changes may occur. The progressive

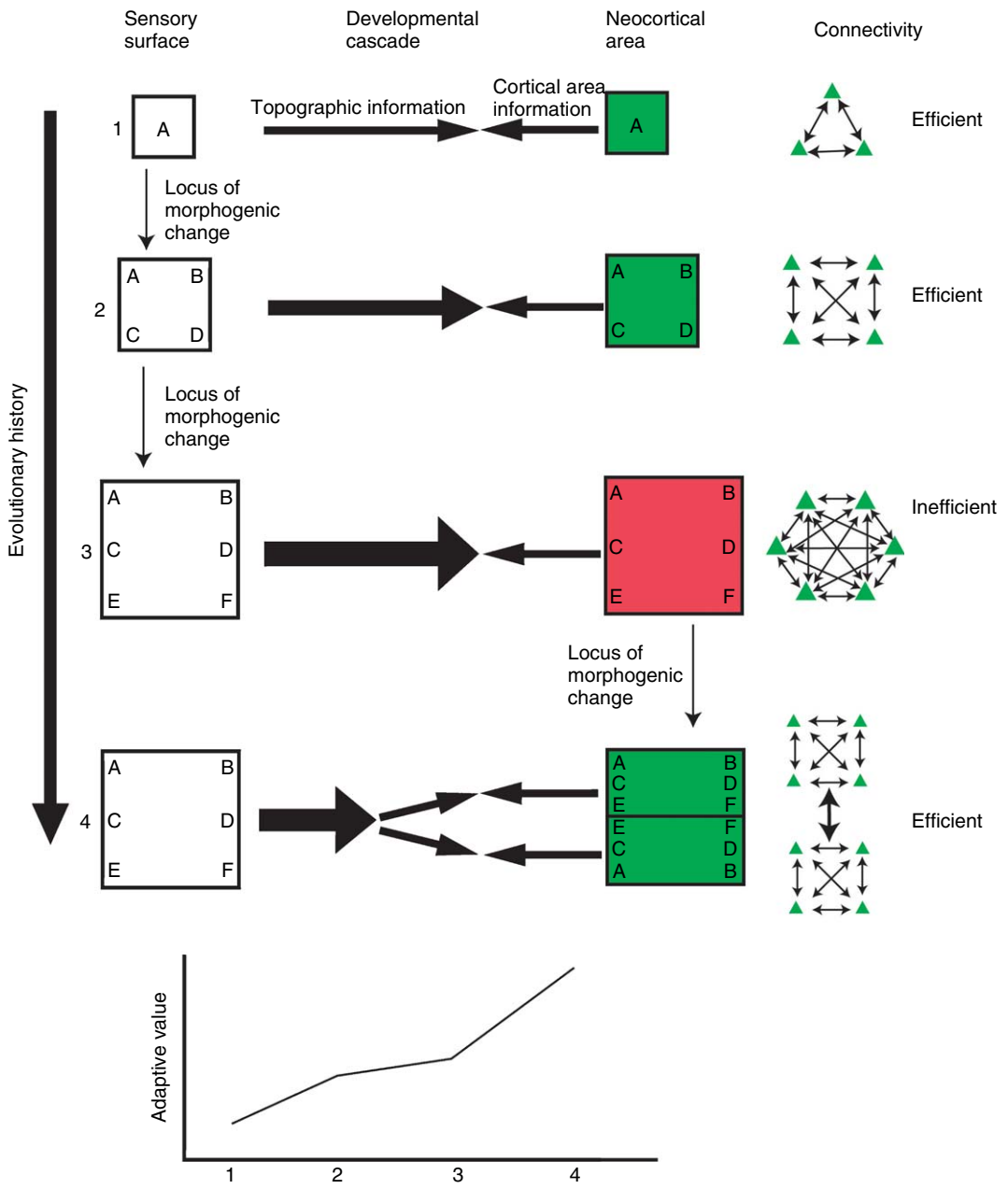

Figure 12 Schematic illustration of possible steps in the progressive evolution of a more complex cortex with new areas. Steps 1–4 represent a progression of changes in the species over successive generations. The graph at the bottom represents the proposed adaptive value of each evolutionary change for steps 1–4. In this proposal, the sensory surface leads the evolutionary process of brain reorganization through a cascade of developmental events in steps 1–3. This begins with the expansion of the sensory surface and a corresponding expansion of the representation of the sensory surface in cortex. The far right side represents the level of connectivity between neurons needed for sensory processing. Although each step is presumed to provide a net advantage (lower panels), by step 3, the cortical processing area is strained and no longer processing the information at peak efficiency. This sets the stage for step 4, during which the cortical area is duplicated (through developmental mechanisms centers in the cortex – see text for example) allowing for the two smaller areas to efficiently process information. Although not illustrated, the two areas are now free to specialize in processing different facets of sensory information and this is considered to be part of the adaptive value of this step (lower panel).

evolution of a sensory system is illustrated from top to bottom of the figure. The initial stages (1–3) reflect the progressive elaboration of the sensory surface (left side) and the corresponding expansion of the representation in cortex (right side) through a developmental cascade. The graph at the bottom illustrates the proposed adaptive value of each change. Initially the developmental changes to the sensory surface are accommodated by the later-developing brain, and there is a steep rise in adaptive

value (1–2). However, at some point (3) the cortical area is no longer at an optimal size for processing information from the sensory surface (as illustrated in red). Although the expansion of the sensory surface has still resulted in an increased net adaptive value for the sensory system as a whole (bottom panel), the stage is now set for the addition of a cortical area to optimize sensory processing. With the addition of a cortical area (4) there is another steep rise in adaptive value of the sensory system (4). Although not illustrated, the ability for the two daughter areas to specialize for processing different facets of sensory information may provide the most important advantaged for sensory processing.

There seems to be ample evidence from both experimental manipulations of development and naturally occurring variants that steps 1 and 2 can occur. That is, changes to the sensory system localized to the sensory periphery may cause alterations of the representations in the CNS. However, evidence for variation in the number of cortical areas is much less obvious, and must usually be inferred and reconstructed from comparative studies across species. Although there may be some ongoing variations in numbers of cortical areas in a given species, so few brains are processed and examined in detail for any species that the chances of such variants being identified are small. This can be contrasted with variants in body parts and sensory systems that can be readily identified by simply examining an animal's body (e.g., Van der Loos and Dörfl, 1978). As a result, the potential mechanisms for altering cortical area number are most readily deduced from laboratory investigations of patterning-gene expression.

Recent investigations and manipulations of gene expression patterns in developing mouse cortex suggest some of the mechanisms that control cortical area position, orientation, and number (Cecchi, 2002; Fukuchi-Shimogori and Grove, 2001; Ohsaki *et al.*, 2002; O'Leary and Nakagawa, 2002). These investigations have revealed graded expression of patterning proteins in the developing cortex that can be manipulated to cause predictable alterations in the positions of entire cortical subdivisions. One growth factor in particular – FGF8 (a member of the fibroblast growth factor family) – has been the focus of a number of recent studies. FGF8 is normally expressed at the rostral pole of the developing neocortex. In a landmark experiment, Fukuchi-Shimogori and Grove (2001) introduced a second source of FGF8 at the caudal pole of developing mouse neocortex. When they later examined the adult somatosensory cortex in these mice, some individuals had generated a partial mirror-image duplication of the S1 barrel field (Figure 13) that presumably was supplied by its own set of thalamocortical axons (O'Leary and Nakagawa, 2002).

This experiment has profound implications because the generation of a new, mirror-image representation of a sensory surface has clearly occurred many times in the course of mammalian brain evolution. Thus, addition of a new FGF8 source to developing cortex produces a phenotype in the laboratory that mimics a common product of cortical evolution.

Long before genetic manipulation of patterning genes was possible, previous investigators of mammalian cortical diversity had suggested that sudden duplications of cortical areas might occur as

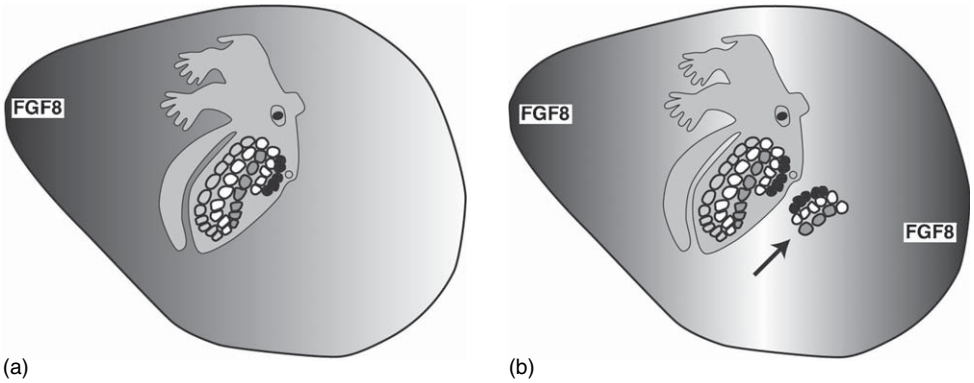

Figure 13 Schematic illustration of recent experiments that have induced the partial duplication of the cortical barrel field by adding a new source of FGF8 to the caudal part of developing cortex. a, FGF8, a member of the fibroblast growth factor family, is normally expressed rostrally in developing cortex. b, When a second source of FGF8 was introduced by electroporation during fetal development, adults were later found to have a partially duplicated barrel field (arrow). This results suggests a mechanism by which mirror image duplications of a cortical area might occur in the course of mammalian evolution. Reproduced from Fukuchi-Shimogori, T. and Grove, E. A. 2001. Neocortex patterning by the secreted signaling molecule FGF8. *Science* 294, 1071–1074.

mammalian brains evolved (Allman and Kaas, 1971; Kaas, 1982). The idea was that a new area could then become specialized to perform new functions while releasing the original area from some of its functions. There are a number of attractive features to this theory of cortical elaboration. Meristic changes – or alterations to a standard part – are a common mechanism of evolutionary change that has been well documented from the level of genes (see Ohno, 1970) to entire body parts (Raff, 1996). That this can occur for cortical areas seems likely in light of the recent findings of Fukuchi-Shimogori and Grove (2001).

In addition to this recent evidence from FGF8 expression, there are a number of considerations related to cortical area organization that suggest duplication of an area may be an efficient mechanism for expanding cortical functions. For example, such a mechanism (dependent on chemical gradients) would likely result in mirror-image maps (see Figure 13b and Catania, 2004), as observed for the supernumerary barrel representation in mice with an extra FGF8 source. Most adjacent cortical areas are mirror images of one another and share a congruent border (Kaas, 1982). This results in areas that are more topographic as a group, than non-mirror-image areas (i.e., neighbor relationships are maintained at, and across the congruent border between areas). It seems likely that such topographic representations are a particularly efficient configuration of cortex. Such an organization groups neurons that interact together, reducing fiber lengths and minimizing conduction delays. Topographic representations may also facilitate detection of movement and the refinement of acuity through center-surround receptive field configurations.

An alternative possibility for cortical elaboration is that cortical areas slowly fission by gradual separation. However, this seems less likely, as the result would be two daughter areas with the same (non-mirror image) orientation – and this is seldom observed. In addition, areas that gradually separate from one another would pass through a very nontopographic and presumably less efficient intermediate stage. Finally, if chemical gradients play a major role in the positioning and orienting of cortical areas during development, gradual separation of two areas may be difficult to achieve and the resulting, non-mirror-image representations may be difficult or impossible to code with chemical gradients (Catania, 2004).

3.15.4 Conclusions

Investigations of specialized mammals reveal a number of clear trends in mammalian brain evolution. This includes the expansion of the representations of behaviorally important sensory surfaces, the subdivision of cortical areas into modules representing parts of a sensory surface, and the addition of entirely new cortical areas to the processing network. Surgical alterations of sensory surfaces during development and the discovery of natural variations in sensory arrays suggests that many of the changes to the representations in the cortex may occur simply as a result of changes to the sensory surface that are communicated centrally by a developmental cascade. However, larger-scale changes in brain organization, such as the addition of new cortical areas to the processing network, require alterations of gene expression that are centered in the developing brain. The most recent advances in manipulating the expression of patterning genes in the cortex suggest mechanisms by which areas may be added to the cortex. These findings support some long-standing theories for how the brains of ancestral mammals may have evolved to produce the diversity of cortical configurations observed in modern mammalian lineages.

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3.16 Somatosensory Specializations in the Nervous Systems of Manatees

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Glossary

oral disk	The expanded mystacial region of the sirenian face that contains bristle-like hairs used in tactile investigation.
oripulation	Use of the perioral bristles for prehensile grasping and directed movements.
perioral bristles	Prominent facial vibrissae used in feeding and investigative behavior.
Rindenkerne	Aggregations of neurons found in layer VI of some cortical areas; may be analogous to barrels associated with vibrissae in other taxa.

3.16.1 Introduction

Florida manatees are large-bodied, fully aquatic herbivorous mammals that are members of the order Sirenia, which has a fossil record that can be traced back to a four-legged ancestor that lived approximately 50 Mya (Domning, 1994, 2001a). Within the Sirenia, the trichechids (manatees) diverged from the dugongid lineage (dugongs) 25–40 Mya. Manatees in their modern form are a relatively recent offshoot, having evolved in the Amazon basin about 2 Mya (Domning, 2001b). They are currently represented by three species, found in Florida and the Caribbean (*Trichechus manatus*), South America (*Trichechus inunguis*), and West Africa (*Trichechus senegalensis*).

Because they are herbivorous and are not preyed upon, manatees lack the rapid movements and complex behaviors associated with predation and predator avoidance (Hartman, 1979). They are relatively slow-moving, usually reside in turbid, shallow water habitats, have greatly reduced visual systems (Marshall and Reep, 1995), poor visual acuity (Bauer *et al.*, 2003), do not appear to utilize echolocation (Gerstein *et al.*, 1999), and exhibit reduced chemosensory systems (Reep *et al.*, 1989). Under these circumstances, details of the near-field environment become significant, and it can be advantageous for an aquatic animal to maximize somatosensory acuity (see Evolution of the Somatosensory System – Clues from Specialized Species). Behavioral and anatomical evidence suggests that sirenians have done just that, as have the platypus (Manger and Pettigrew, 1995) and river dolphin (Layne and Caldwell, 1964).

Manatees eat a wide variety of aquatic vegetation. Food is gathered via modified perioral vibrissae that are used in a prehensile grasping fashion to oripulate food and bring it into the oral cavity. This behavior may be optimized to increase the efficiency of food gathering, because manatees forage for a significant amount of time daily and consume an amount equivalent to as much as 10% of their body weight per day. This manner of feeding and the use of vibrissae to grasp objects are both unique to sirenians (Marshall *et al.*, 1998a).

Manatees perform tactile investigations of novel objects or potential food items using the vibrissae of the oral disk, the expanded central mystacial region of the face (Figure 1) (Hartman, 1979; Marshall *et al.*, 1998a; Bachteler and Dehnhardt, 1999). Interestingly, prior to contact, manatees often close their eyes, perhaps to improve tactile discrimination. Tactile acuity using the oral disk vibrissae is comparable to that of the Asian elephant using its trunk (Bachteler and Dehnhardt, 1999).

3.16.2 Tactile Hair

Among the many unusual anatomical features of sirenians, one of the most significant for

Figure 1 Manatee face. The oral disk (od) is flared and its bristle-like hairs are everted in this side view of a manatee face. The large perioral bristles of the U2 field on the upper lip (U2) are withdrawn into their fleshy pad.

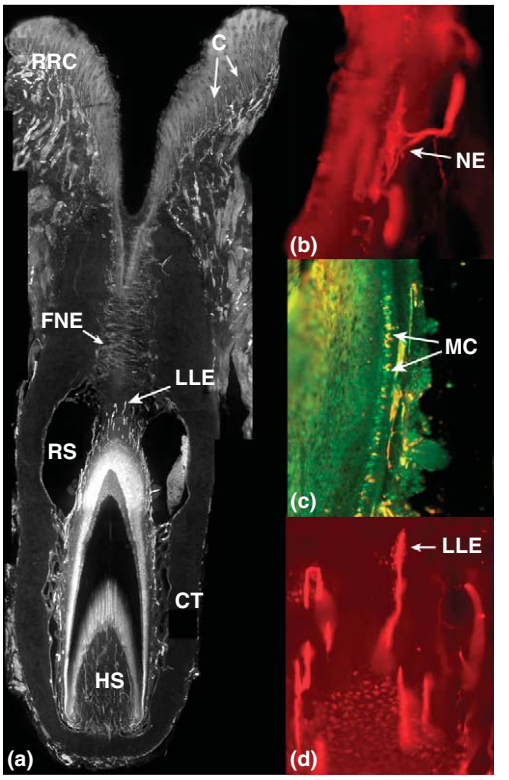

Figure 2 Follicle morphology and innervation. Panel a presents a longitudinal section of a U2 follicle, illustrating the prominent ring sinus (RS), connective tissue capsule (CT), and base of the hair shaft (HS). Dermal papillae underlying the epidermis of the circumferential rete ridge collar (RRC) are innervated by C fibers (c). At the inner conical body level, circumferential free nerve endings (FNEs) are present, as are novel endings (NEs in panel b). At the level of the RS, dense aggregations of Merkel cells (MCs in panel c) and longitudinal lanceolate endings (LLEs in panel d) are evident.

neurobiological function is the presence of true vibrissae (tactile hair) on the entire body, and the absence of pelage hair (Dosch, 1915; Reep *et al.*, 1998, 2001, 2002). Sirenians are the only mammals known to exhibit this condition, although fossorial naked molerats possess nonvibrissal hairs that mediate orientation to tactile stimuli (Crish *et al.*, 2003). Manatees possess approximately 5300 vibrissae, distributed about 30 times more densely on the face than on the postcranial body. The face contains approximately 2000 vibrissae, innervated by approximately 110 000 axons, whereas the postcranial body contains roughly 3300 smaller vibrissae innervated by approximately 100 000 axons.

The facial region of sirenians is highly modified in association with its use in feeding behavior (Reep *et al.*, 1998; Marshall *et al.*, 1998a, 1998b). The facial vibrissae are organized into nine distinct fields that vary according to location, size, and stiffness. Six of these fields comprise the perioral bristles on the upper and lower lips (~224 total), utilized during feeding behavior. These are the largest and stiffest vibrissae (hence the term 'bristle', coined by Murie, 1872), and are also the most heavily innervated (61–254 axons per follicle). The oral disk (Figure 1) contains approximately 600 bristle-like hairs, vibrissae that are intermediate in stiffness and innervation (49–74 axons per follicle) between the perioral bristles and postcranial vibrissae. The oral disk is usually flaccid, but in preparation for tactile scanning it flattens and expands, causing the bristle-like hairs to evert (Figure 1). The remainder of the face (chin and supradisk region around the nostrils) is invested with finer vibrissae that are not actively moved or everted, and these are similar in stiffness and innervation (~34–48 axons per follicle) to the postcranial hairs.

All facial follicles have the anatomical attributes of follicle–sinus complexes, including a circumferential blood sinus, dense connective tissue capsule, and substantial innervation. In addition, the facial follicles possess a ring sinus, defined as a distinctly enlarged nontrabeculated region located between upper and lower trabeculated cavernous sinuses. Preliminary analysis of a facial vibrissa immunolabeled for PGP 9.5 (protein gene product 9.5, a universal cytoplasmic protein) and NF200 (a 200 kDa neurofilament subunit) revealed that the dermal papillae of the rete ridge collar were densely innervated by C fibers (Figure 2a). This innervation may facilitate accurate assessment of water temperature, which is critical for the Florida manatee's survival in winter. Immunofluorescence also

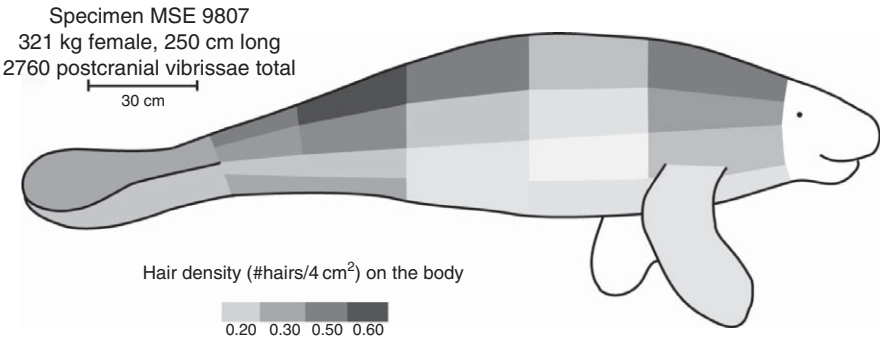

Figure 3 Distribution of postcranial vibrissae. Vibrissae are present over the entire body (facial distribution not shown in this figure). Growth produces an expansion of girth in the midsection, which results in lower density of vibrissae in this region. Adapted from figure 2 of Reep, R. L., Marshall, C. D., and Stoll, M. L. 2002. Tactile hairs on the postcranial body in Florida manatees: A mammalian lateral line? *Brain Behav. Evol. 59*, 141–154.

revealed that the inner conical body region in the manatee follicle lacks transverse lanceolate endings thought to be associated with whisking behavior in rats (Ebara *et al.*, 2002), but is supplied with novel large endings (Figure 2b). At the level of the ring sinus, the outer root sheath of the follicle was richly supplied with Merkel cells and longitudinal lanceolate endings (Figures 2c and 2d). Merkel cells represent slowly adapting, low-threshold receptors that, in the rat and cat, appear to provide directional sensitivity at the level of the ring sinus due to the terminal arbor from each afferent being restricted along one margin of the follicle (Ebara *et al.*, 2002). Finally, whereas each follicle–sinus complex is penetrated by a single deep vibrissal nerve (DVN) in rats and cats (Ebara *et al.*, 2002), manatee vibrissae appear to be innervated by several nerve bundles branching from the DVN (Reep *et al.*, 2001).

Vibrissae of the postcranial body (Figure 3) are 2–9 mm in length and are distributed fairly sparsely (~25 mm apart), except in specialized regions such as the edge of the fluke and the perivulvar area (Reep *et al.*, 2002). Unlike in the face, there are no obvious regional differences in postcranial follicle structure, suggesting that to a first approximation these follicles represent a distributed system of repeated modules. Postcranial follicles are markedly smaller than those of the perioral bristles and bristle-like hairs of the face, and are similar in size and innervation to those of the chin and supradisk region. They exhibit an elongated circumferential blood sinus, dense connective tissue capsule, and substantial innervation (21–47 axons per follicle). Rather than a ring sinus, most postcranial follicles exhibit a dorsal enlargement in the sinus, but retain connective tissue trabeculae that span the width of the sinus.

The presence of vibrissae on the entire body constitutes a distributed three-dimensional somatosensory array potentially capable of encoding the intensity and direction of water displacements and low-frequency vibrations associated with significant environmental stimuli, such as approaching conspecifics and other animals, water currents, and tidal flows. Therefore, this system may be used for touch at a distance, analogous to the lateral line system in fish. Gerstein *et al.* (1999) proposed that this system of hairs might also detect low-frequency acoustic energy in the form of near-field particle displacements. In a psychophysical study of manatee hearing, they noted that improvement in detection at low frequencies occurs within the range of frequencies (0.1–0.2 kHz) that correspond to lateral line detection in fish.

3.16.3 Central Somatosensory Regions

The brainstem, thalamus, and cerebral cortex all exhibit anatomical features suggestive of specializations associated with processing the large amount of information emanating from approximately 210 000 axons innervating the vibrissae on the body of a manatee (see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates).

3.16.3.1 Brainstem

The brainstem nuclei most relevant to the manatee somatosensory system include Bischoff's nucleus, the trigeminal nucleus, and the cuneate–gracile complex. Bischoff's nucleus is present in many tailed animals (Ariens Kappers *et al.*, 1936; Johnson *et al.*, 1968), and through electrophysiological experiments it was shown to represent the tail and to project heavily to the somatosensory thalamus in raccoons (Ostapoff and Johnson, 1988). This distinct group of cells at the midline of the caudal medulla has also been identified in the manatee (Figure 4) and constitutes the presumptive fluke representation. In manatees, fine movements of the fluke are used to adjust the position of the body while moving, and presumably this

involves a significant amount of sensory feedback via Bischoff's nucleus.

Manatees have large trigeminal nerves and well-developed trigeminal and somatosensory nuclei, but reduced visual thalamic and brainstem nuclei (Welker *et al.*, 1986; Johnson *et al.*, 1986; 1987; Reep *et al.*, 1989). These anatomical observations are also reflected in sirenian behavior, particularly in the case of the trigeminal nerve system, which is extensively involved when the face is used for tactile exploration. Given its level of behavioral importance, it is no surprise that recent analysis of the trigeminal nucleus has revealed an organized, parcellated nucleus (Figure 5). This may indicate a topographical relationship linking each subdivision to functionally significant regions of the face such as the nine follicle fields mentioned above, and may be comparable to the barrelettes discovered to correspond to individual vibrissa follicles in the principle sensory nuclei of other species. The cuneate–gracile complex, which represents the forelimb flippers as well as the upper and lower body of the manatee by extrapolation from other species, also exhibits notable parcellation indicative of functionally segregated and organized cutaneous inputs (Figure 5). Presumably, a large proportion of the compartmentalization seen in the trigeminal and cuneate–gracile nuclei represents the segregated processing of inputs from the tactile hairs.

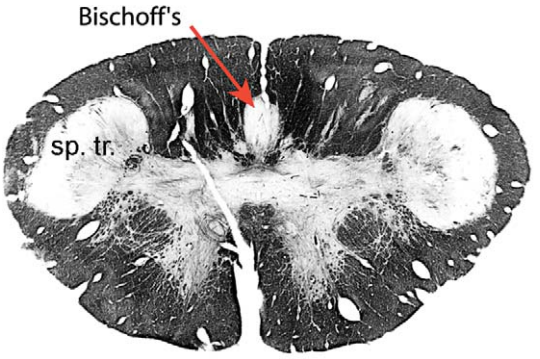

Figure 4 Bishcoff's nucleus. This site of representation of somatosensory afferents from the fluke is visible in the caudal brainstem as a prominent midline structure. Spinal trigeminal nucleus = sp. tr. Myelin stain, coronal section.

3.16.3.2 Thalamus

The principle somatosensory nucleus in the thalamus is the ventroposterior (VP) nucleus, with a ventral posterolateral (VPL) component representing the body and a ventral posteromedial (VPM) portion representing the face and most of the head. Given the manatee's reliance on haptic input, VPL and VPM would be expected to constitute a large proportion of the thalamus, and this does appear to be the case. Nucleus VP stains positively and rather uniformly for cytochrome oxidase (Figure 6). Acetylcholinesterase histochemistry produces a dark reaction product in the lateral portion of VP, suggesting that this corresponds to VPL, whereas the lighter-stained medial component corresponds to VPM, as in some other taxa (Bennett-Clarke *et al.*, 1999). The large size of the presumptive VPM suggests that the face and head are represented by a disproportionately large region within VP, consistent with the fact that manatees make extensive use of the bristles and bristle-like hairs of the face and oral region during a variety of behaviors. Cytochrome oxidase staining has not revealed the presence of barreloids in the manatee VPM. These are the thalamic counterparts to brainstem barrelettes, and are

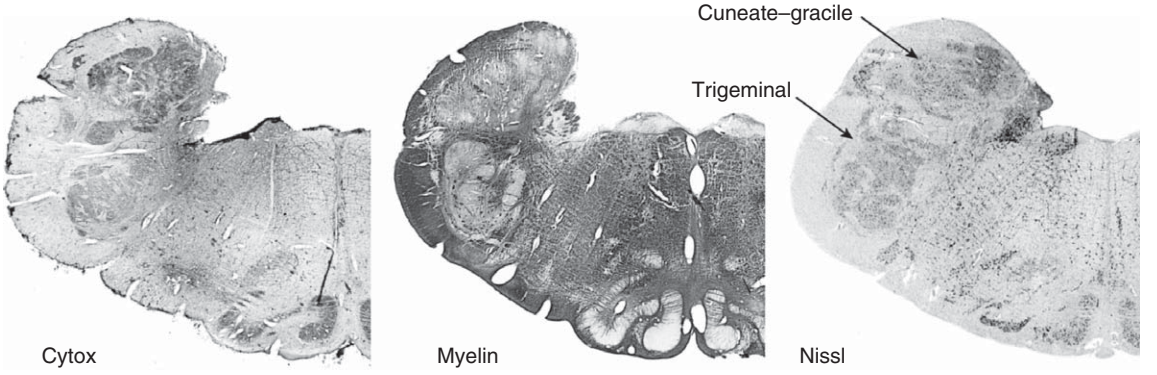

Figure 5 The brainstem contains large, partitioned somatosensory nuclei. Three coronal sections at approximately the same level reveal prominent bands in the cuneate–gracile complex, indicating a detailed organization of cutaneous inputs from the forelimb flippers as well as the upper and lower body. The trigeminal nucleus exhibits a similar parcellated organization, presumably related to the well-developed tactile sensitivity of the manatee face. Sections were stained for cytochrome oxidase (cytox), myelinated axons (myelin), or cell bodies (Nissl).

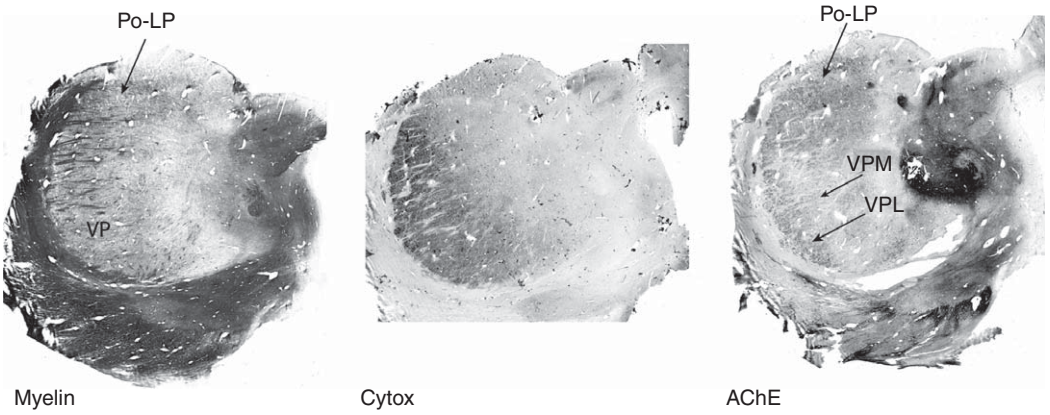

Figure 6 The thalamus exhibits large somatosensory nuclei. The general location of the VP nucleus is apparent on sections stained for myelinated axons (myelin) or cytochrome oxidase (Cytox), and VP is bounded dorsally by the posterior nucleus (Po-LP). Acetylcholinesterase staining distinguishes the VPL subnucleus as a dark-staining region, and the VPM subnucleus as a zone of light staining. The VPL–VPM complex is relatively large, consistent with the importance of somatic sensation in manatee behavior.

somatotopically organized regions related to vibrissal input in some other taxa (Jones, 1983).

3.16.3.3 Cerebral Cortex

The presumptive somatosensory cortex consists of areas CL1, CL2, DL1, and DL2. It was first identified on the basis of its location and cytoarchitectural characteristics (Reep *et al.*, 1989; Marshall and Reep, 1995). More recently we have discovered that portions of this region stain positively for cytochrome oxidase and are richly supplied with myelinated axons (Sarko and Reep, 2004), as is the case for somatosensory cortex in other taxa (Krubitzer, 1995). This can best be seen in flattened cortex preparations that reveal four distinct patches in the neonate (Figure 7), corresponding to the presumptive forelimb flipper, face, body, and tail representations (Sarko and Reep, 2003). There are only three distinct patches in juvenile and adult specimens (Sarko and Reep, 2004), and this may represent modification and refinement of sensory inputs as manatees develop.

As first noted by Dexler (1913), some areas of the sirenian cerebral cortex contain Rindenkerne (cortical nuclei), clusters of neuron cell bodies in layer VI (Figure 8). Rindenkerne may be specializations akin to the barrels seen in layer IV of the primary somatosensory cortex of other taxa having specific cortical representations for vibrissae (Rice, 1995). Like barrels, Rindenkerne react positively for cytochrome oxidase and some also stain positively for acetylcholinesterase (Reep *et al.*, 1989; Marshall and Reep, 1995). The presumptive face representation consists of the far lateral areas CL1 and CL2 (Figure 7). Area CL1 contains the largest

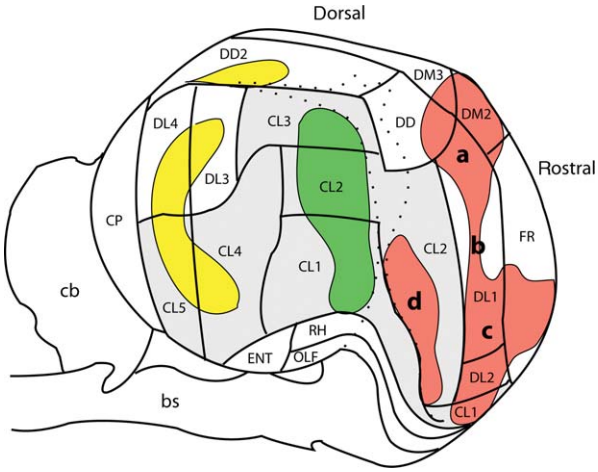

Figure 7 Sensory areas of cerebral cortex. Overlay of cytochrome oxidase staining pattern onto a cytoarchitectural map of the right hemisphere of specimen TM 0310, viewed laterally. Colored areas represent presumptive sensory cortex: orange, somatosensory; green, auditory; yellow, visual. Cortical areas containing Rindenkerne are shaded gray. Within somatosensory cortex are indicated the presumptive representations for the fluke (a), forelimb flipper (b), perioral face (c), oral disk and postcranial body (d). Dotted lines represent the vertically oriented lateral fissure; bs, brainstem; cb, cerebellum.

Rindenkerne (0.4–1.0 mm in diameter), whereas those found in area CL2 are about half this size and are often located more superficially in layer VI (Reep *et al.*, 1989). We hypothesize that the large Rindenkerne in area CL1 are related to the perioral bristles, whereas the smaller Rindenkerne of area CL2 are related to the bristle-like hairs of the oral disk and the postcranial vibrissae. This hypothesis is consistent with the finding that in mice the largest vibrissae are represented by the largest barrels (Woolsey and Van der Loos, 1970). However, if

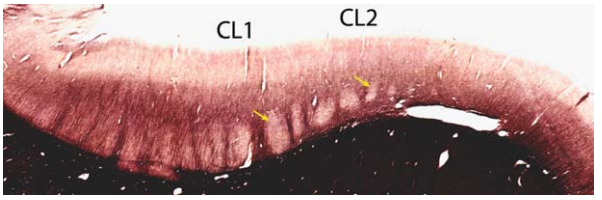

Figure 8 Rindenkerne in the CL1/CL2 transition area. Rindenkerne are clusters of neuron cell bodies that appear as light ovoid regions (arrows) in layer VI in this myelin-stained section. Larger clusters define cytoarchitectural area CL1, whereas smaller clusters mark the transition into area CL2.

the presence of Rindenkerne in the CL fields implies vibrissae representations, why does presumptive primary auditory cortex (see Figure 7) occupy territory in the CL fields? One possibility is that the somatosensory and auditory representations overlap. This would be consistent with a functional continuity between the perception of sound by the auditory system and processing of hydrodynamic stimuli by the vibrissae.

Marshall and Reep (1995) identified three additional cortical areas (CL3–CL5) containing small Rindenkerne. These areas may represent an expanded somatosensory representation for the postcranial vibrissae, but they overlap with the presumptive auditory and visual representations (Figure 7). Furthermore, their location caudal to the lateral fissure (i.e., temporally, given the ventralward rotation of the sirenian brain) argues against this. If these small Rindenkerne do represent vibrissae, then areas CL3–CL5 may be multisensory areas analogous to PV (parietal ventral area) or VS (ventral somatosensory area) (Kaas and Collins, 2001).

3.16.4 Summary

The somatosensory specializations present in manatees appear to be associated with adaptation to aquatic herbivory. Similar specializations are present in the other extant sirenians (Marshall *et al.*, 2003) and have likely been present for millions of years, at least since the divergence of the manatee and dugong lineages. Pronounced somatosensory specializations also exist in the platypus, an aquatic monotreme, and the star-nosed mole, a fossorial insectivore. Unusual cases such as these are important because they represent the range of variation present in sensory systems and their central representations, and thus the known extent of evolutionary potential.

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3.17 Somatosensory Adaptations of Flying Mammals

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Glossary

boundary layer	In reference to the airflow around a wing or airfoil, the thin layer of air immediately adjacent to the surfaces where air speed is reduced by surface drag.
camber	Cross-sectional profile of an airfoil with a convex curve to upper surface that provides lift.
homunculus	Schematic, two-dimensional presentation of body surface representations mapped in mammalian somatosensory neocortex.
interfemoral membrane (IFM)	The segment of wing membrane between a bat's legs.
neuroethology	The study of the nervous system in context with the natural behaviors and functional requirements of an organism.
radiohumeral membrane (RHM)	The segment of wing membrane in front of a bat's arm and forearm.

3.17.1 Introduction

As bats represent the only mammals with true powered flight, most of this article considers the bat somatosensory system. Although bat flight has long been observed and studied, surprisingly few data exist on sensory adaptations for flight. The oversight may result from the fact that the bat's somatosensory system has seldom been considered from an ethological viewpoint and almost never considered in terms of the unique capabilities and limitations of the bat hand-wing or how the somatosensory system might support bat flight or flight-related behaviors.

Bat flight has a highly acrobatic quality based on a wing that is largely made up of an elaborated hand (Chiroptera, the order of bats, translates as hand-wing). Figure 1 shows the basic pattern of the bat wing with a membrane of thin skin stretched between body wall with elongated forelimb arm and digits that act as wing-supportive struts. In most bats, a continuation of the wing membrane stretches between the legs (the interfemoral membrane or IFM). In the transformation into an airfoil, the bat hand has become functionally closer to a quadriped's hand than a biped in the sense that the hand-wing is almost entirely used to support the body (at least during flight) with little apparent ability to grip, carry, or manipulate objects. In other mammals, dense or specialized tactile innervation is generally associated with dexterity and a need for feedback in the fine control of manipulation and grip. Surprisingly, there is considerable evidence (from studies beginning over 140 years ago) that the bat wing is densely innervated with a number of potential tactile specializations. Only in the past 20 years, however, have these features been considered in terms of their role in a hand that is specialized for flight rather than manipulation. Before reviewing the earlier and recent studies of the bat somatosensory system, it is useful to consider some of the unique characteristics and challenges of flight and foraging with a hand-wing and specific roles for tactile feedback from the wing (Zook and Fowler, 1982, 1986; Zook, 1985, 2005, 2006; see The Evolution of Neuron Classes in the Neocortex

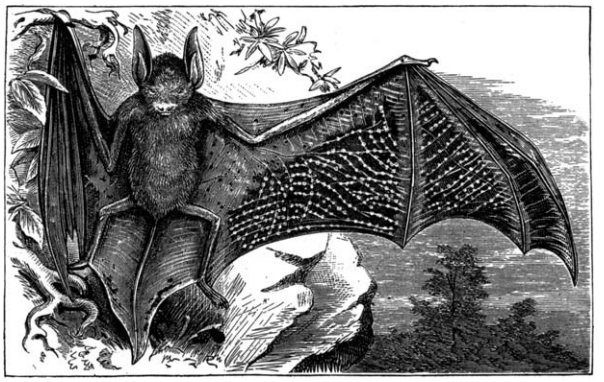

Figure 1 Nineteenth-century lithograph of *Myotis welwitschii*. Reproduced from Maxim, H. 1912. The sixth sense of the bat. The possible prevention of sea collisions. *Sci. Am. Suppl. V* 74, 148–150, with permission.

of Mammals, Evolution of the Somatosensory System – Clues from Specialized Species, The Evolution of the Basal Ganglia in Mammals and Other Vertebrates, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?).

3.17.1.1 Wing Camber, Boundary Flow, and Lift

Although the dexterity of the bat hand-wing is limited, the bat's limbs and hands retain sufficient joint mobility for a flying bat to adjust the overall shape of the hand-wing airfoil, or even the shape of separate wing segments. With this articulated airfoil, a flying bat can continually adjust its wings to fine-tune the lift properties of wing to enhance control of wing aerodynamics, providing for a level of maneuverability unmatched by most birds.

The shape of an airfoil is critical for generating and sustaining lift properties (specifically the camber of the airfoil, the cross-sectional contour of the wing between leading and trailing edges). (For more complete treatments of bat-wing aerodynamics, see Rayner, 1987; Norberg, 1990; Altringham, 1996.) Figure 2a shows a simplified pattern of the airflow around a cambered airfoil. The parallel lines above and below the wing represent the pattern of laminar flow found within the boundary layer of air that lies next to the wing. Lift is based on the fact that air in the boundary layer must travel farther and thus faster over the top of the airfoil than below the airfoil. With this velocity difference, air pressure above the airfoil is reduced relative to below, and lift is created. When other factors are constant (air speed, angle of wing attack, drag), lift can be amplified by increasing airfoil curvature (camber), but only as long as boundary layer flow is able to follow the curved surface. Figure 2b shows the condition of boundary layer

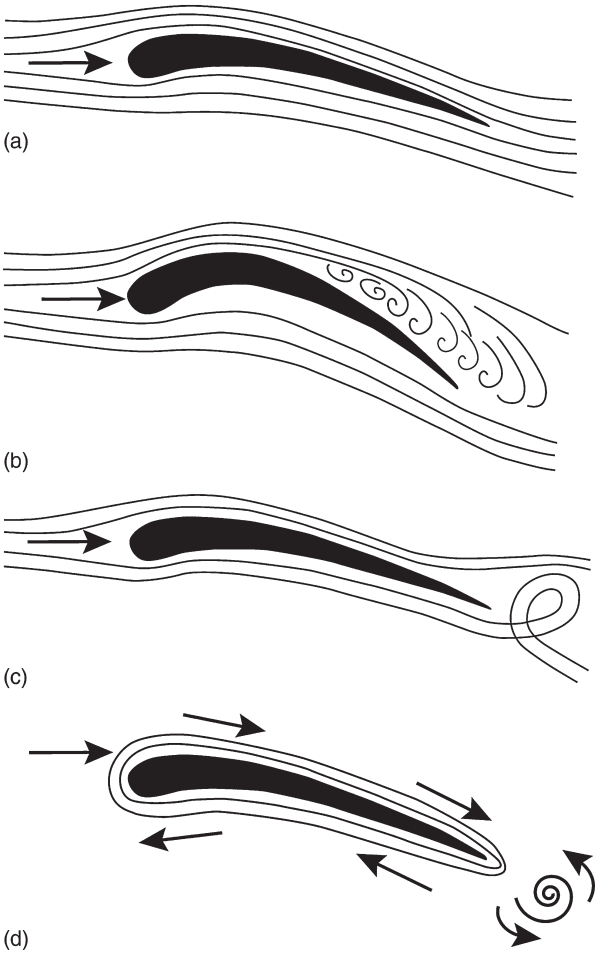

Figure 2 Boundary layer airflow patterns. The curved profiles represent a cross-sectional shape of a typical airfoil (camber), leading edge to left, trailing edge to right. a, Parallel lines represent the typical pattern of laminar airflow in the boundary layer next to wing surfaces. b, Boundary layer separation and boundary turbulence result when airfoil curve or camber is increased past the point where boundary flow can follow the curve. c, Flapping is thought to set up vortex wake patterns which develop and are shed from wing tips or from the trailing edge as shown here. d, During the wing-beat cycle, vortex theory suggests that boundary layer flow will circulate around the wing.

separation, where camber has been increased beyond the point where boundary flow can no longer follow the airfoil surface. Separation results in local turbulence in the flow and a sudden loss of lift (the wing stall point). The bat's control of wing shape allows it to adjust camber to optimize lift (and reduce stall speed). Unfortunately, the complex variables that affect boundary flow in flapping flight make estimation of boundary flow, camber, and stall difficult to judge or predict (Norberg, 1990; Altringham, 1996).

In flapping flight, the wings must provide propulsion as well as lift through changes in wing shape and angle of attack throughout the wing-beat cycle.

Vortex wake theory applied to a flapping airfoil predicts that the boundary layer flow will be pulled into the wing. Rotational currents are set up along wing surfaces, generating local vortex eddies which tend to be shed off the trailing edge of the wing (Figure 2c) or off the wing tips (Rayner, 1987; Altringham, 1996). During all or part of the wing-beat cycle, pressure gradients cause boundary flow to circulate around the wing (Figure 2d) which affect ongoing lift, drag, and propulsive forces acting on the wings (Rayner, 1987; Altringham, 1996; Norberg, 2002). The proposed somatosensory specializations of the bat wing may provide necessary feedback regarding wing lift by monitoring boundary layer flow, turbulence, circulation, and wake patterns (Zook, 1985, 2005; Zook and Fowler, 1986). The basic data to support this theory are reviewed here along with recent physiological and behavioral experiments from our lab (Zook, 2005, 2006).

3.17.1.2 Wing Capture of Active Prey

Although well adapted for flight (Swartz, 1998), the hand-wing's dexterity is limited by the extreme attenuation of its membrane-bound digits as well as the reduced strength and number of intrinsic muscles (Swartz *et al.*, 1996; Norberg, 2002). This functional tradeoff between flight and manipulation abilities presents a real challenge for most foraging behaviors and especially for the midair capture of flying insects. Bats may occasionally use their jaws to snatch insects out of midair, but such direct mouth capture is seldom a real option, given the size of a typical bat's mouth, the size of most prey, and the relative three-dimensional motion between pursuer and evasive prey (Webster and Brazier, 1965; Kalko and Schnitzler, 1998). While wing digits cannot be used to pick insects out of the air, the wings can be cast like nets to sweep a targeted insect out of the air (Vaughan, 1970; Kalko, 1995; Kalko and Schnitzler, 1998).

Once an insect is swept from air to the wing surface, there is still the challenge of transferring prey from the wing to the bat mouth without the ability to grasp the struggling insect during transfer (Webster and Griffin, 1962; Webster and Brazier, 1965; Zook, 2006). Furthermore, since capture generally takes place at night, and wing-gathered insects are too close for effective echolocation, transfer from wing to mouth must take place without either visual or echolocative cues (Fenton, 1990). Despite these challenges, foraging can be quite efficient, with some estimated capture rates greater than 90% (Kalko, 1995; Acharya and Fenton, 1999; Rydell *et al.*, 2002; Surlykke *et al.*, 2003). Feedback from wing cutaneous receptors could play a major role in this remarkable foraging efficiency (Zook, 2005, 2006).

3.17.2 Innervation of the Bat Wing

3.17.2.1 Early Neurohistology

As appropriate neurohistological techniques became available around the turn of the nineteenth century, a number of studies focused on the innervation of the wing (Schöbl, 1871; Sabussow, 1910; Ackert, 1914; see Griffin, 1958; Quay, 1970b, for reviews). These early studies and all that followed noted a particularly dense innervation of wing membranes, with nerve fibers concentrated along the bundles of elastin–collagen fibers that form a weblike network of prominent bands within all membranes, as shown in Figures 1 and 3 (Gupta, 1967; Holbrook and Odland, 1978). Most observers have commented on the regular series of raised domes distributed in the skin over the membrane bands. As shown in Figures 3 and 4, each dome is marked by a central hair follicle that is surrounded

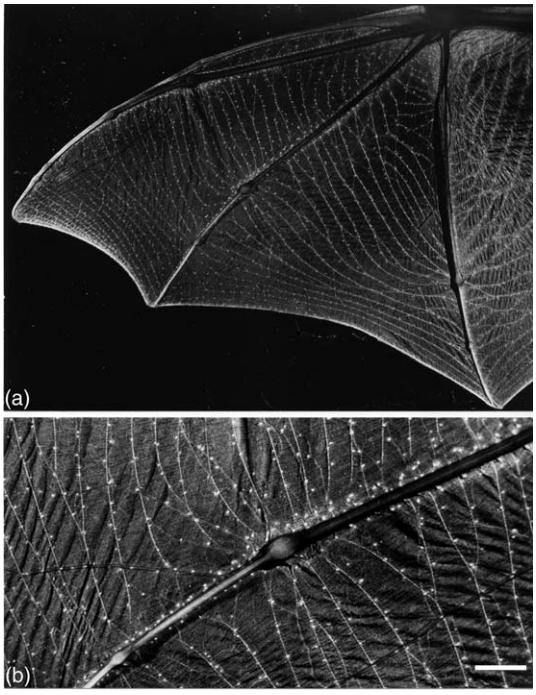

Figure 3 Photographs of transilluminated wing membranes from an *Antrozous pallidus* wing. a, Low-magnification photograph of the distal wing segments. b, Close-up view of the finger joints of the fourth digit shown in (a). Note the weblike pattern of membrane elastin–collagen bands that span the wing with a tendency to converge at wing joints. Both photographs also show the typical wing pattern of single domes spaced along the bands as well as the tendency for domes to be concentrated near digits and joints. Scale bar: 2 mm. Reproduced from Zook, J. M. and Fowler, B. C. 1986. A specialized mechanoreceptor array of the bat wing. *Myotis* 23, 31–36, with permission.

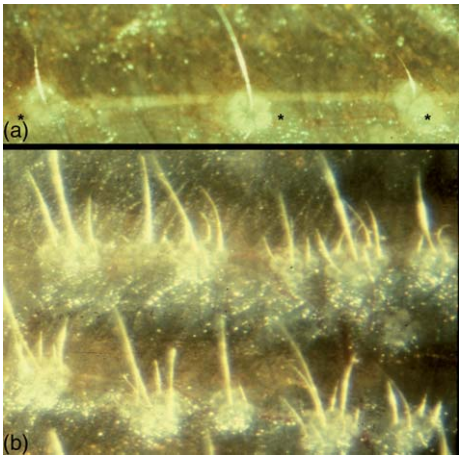

Figure 4 High-magnification photomicrographs of wing membrane domes from an *Antrozous pallidus* wing. a, A close-up view of the main wing pattern of single domes spaced along a membrane band. Asterisks mark the shadows of transilluminated domes positioned on the opposite membrane surface. b, A view of band-associated arrays of dome clusters found on both surfaces of the IFM. The average diameter of domes are 100 μm (a) and 250 μm (b).

by prominent sebaceous and apocrine glands (Schöbl, 1871; Ackert, 1914; Gupta, 1967; Quay, 1970a; Zook and Fowler, 1986; Crowley and Hall, 1994). Studies with a neurohistological focus consistently noted concentrations of nerve fibers and endings within the domes' dermis, basil epidermis, and at the hair follicle (Schöbl, 1871; Ackert, 1914; Quay, 1970b). In several microchiropteran species, free nerve endings and a number of possible terminal specializations were also reported in association with wing domes (Sabussow, 1910; Ackert, 1914).

Largely for historical reasons, none of the wing studies published before the 1980s had made an association between the rich innervation of the bat wing and possible sensory demands of flight and wing foraging. Before 1930, studies of the bat wing were dominated by the curious, but prevailing, theory that bats were able to navigate and forage in the dark due to a special tactile sixth sense of their wings. This tactile-navigation theory had been promoted by Georges Cuvier and others from the end of the eighteenth century. Touch remained the predominant explanation for the bats' blind-navigation skills for over a century, even though the Italian scientist, Lazzaro Spallanzani, had reported compelling experimental evidence of the role of bats' ears in distance navigation as early as 1795 (for reviews, see Dijkgraaf, 1949; Griffin, 1958; Neuweiler, 2000). The wing tactile theory persisted largely because, before the discovery of ultrasound and echolocation, no one could see how the ears could provide the distance sensing necessary for navigation.

In 1908, Walter Hahn came closest to associating wing tactile receptors with flight (and bat ears with navigation) when he observed that bats with grease-coated wings had trouble flying while bats with earplugs could fly but had trouble avoiding obstacles (Hahn, 1908). Hahn's mixed results did not deter Maxim (1912), who, in an attempt to improve ship navigation, came up with the most concrete mechanism for a tactile-based navigation sense. Maxim proposed that the bat's own wing beats generated a series of propagating, low-frequency waves – waves that could be reflected back from obstacles and processed by cutaneous receptors of the bat's wing (and face). The bat illustrated in Figure 1 appeared in Maxim's (1912) *Scientific American* article, where he introduced his design for a ship-based iceberg detector derived from the bat's supposed tactile navigation. Maxim's original caption noted: "This bat furnishes us with a very good illustration of the sensitive wing that enables a bat to send out vibrations and to receive the echo. The spots on the wing probably represent nerve centers." Although his first conjecture was off the mark, his second observation has proven quite prophetic.

Prior to these early wing studies, Merkel (1875) and Pinkus (1905) had separately described raised dome structures with a potential sensory function in other mammalian skin. These touch domes were named for their concentration of nerves and specialized terminal cells, Merkel cells, in the dome's basal epidermis (Iggo and Muir, 1969; Halata *et al.*, 2003). Merkel cells and their associated nerve terminals have come to be recognized as one of the four basic classes of mammalian tactile receptors (Smith, 1967; Iggo and Muir, 1969; Johnson, 2001; Halata *et al.*, 2003). Although not noted until later (Zook and Fowler, 1982), the bat domes bear a close resemblance to a less common form of touch dome–Merkel cell complex, a Haarscheibe, distinguished from other touch domes by a central or peripherally placed hair follicle (Pinkus, 1902; Smith, 1967).

Following the discovery of echolocation, the bat's wing received less attention, considered mainly in terms of the thin membrane's value for study of neurovascular, glandular, or lymphatic systems (reviewed in Quay, 1970a). Although the few additional histological studies mentioned the innervation of wing in passing, none directly addressed the possibility of a sensory role of the wing in flight and flight behaviors (Schumacher, 1932; Gupta, 1967; Quay, 1970b). It was not until almost 50 years later, in light of new attention on bat somatosensory neocortex in the 1980s, that wing innervation and wing domes were to be

considered in terms of flight (Zook and Fowler, 1982; Calford *et al.*, 1985).

3.17.2.2 Studies of the Somatosensory Central Nervous System

These cortical studies began with electrophysiological mapping of bat wing and body representation in primary somatosensory cortex (S1), and were mainly driven by evolutionary, comparative interests (Calford *et al.*, 1985; Wise *et al.*, 1986; Krubitzer, 1995). Most have been extensively reviewed elsewhere (Krubitzer *et al.*, 1993; Krubitzer, 1995) and will be summarized here only to the extent that they are relevant for flight adaptations. The majority of these studies were undertaken in species from the nonecholocating, frugivorous megachiropteran suborder of bats. The megachiropterans possess well-developed visual and olfactory systems useful for diurnal fruit foraging. As fruit eaters, their flight is not as finely tuned as it is in Microchiroptera, and tactile specializations may be less elaborate, particularly in comparison to insectivorous species which must actively pursue evasive prey in the night air. It is worth noting here that all of the primary studies reported large central neural representations of the hand-wing in Megachiroptera (Calford *et al.*, 1985; Krubitzer *et al.*, 1993; Martin, 1993; Manger *et al.*, 2001a, 2001b) as well as Microchiroptera (Zook and Fowler, 1982; Wise *et al.*, 1986).

3.17.3 Somatosensory Receptors of the Wing

3.17.3.1 Surface Features

Re-examination of the somatosensory periphery initially focused on the wings of the microchiropteran bat, *Antrozous pallidus*, and quickly began to reveal new details of the wing somatosensory system (Zook and Fowler, 1982, 1986). As *A. pallidus* is specialized as a gleaning/terrestrial foraging insectivore, two additional species were added to these studies, *Pteronotus parnellii* (an aerial/gleaning feeder) and *Eptesicus fuscus* (an aerial feeder).

The domes found on the bats' wing membranes were both more numerous and more highly organized than the Haarscheibe observed in other species (Smith, 1967). Similar to the earlier wing descriptions, domes in these species were distributed in regular arrays spaced out along wing elastin–collagen bands (Figures 3 and 4a), with additional concentrations grouped along the edges of wing bones (Figure 3). Individual domes were often spaced along the leading and trailing edges of the wing and scattered over limb bones and digits. In all three species, the density of domes per unit area varied between wing segments with a general increase toward the leading and trailing edges as well as wing tips. While dome distribution patterns were similar across the wings of all species, there is variation in dome diameter (100–400 μm), hair size, and hair length as well as specialized regional distributions (see discussion of the IFM, below). The largest domes were found in *A. pallidus*, most likely a byproduct of the exceptionally large, dome-associated glands in this desert-dwelling species.

In all species examined, domes appear to be distributed over the two sides of each wing membrane in almost mirror-image patterns. This can be appreciated in Figure 4a (*A. pallidus*), where each dome and hair projecting from the ventral surface is almost always closely opposed to a dome on the dorsal side (asterisks in Figure 4a).

The IFM was distinguished by a very different pattern, with small clusters of domes spaced out along the elastin–collagen bands of the membrane. This cluster pattern was particularly distinct over the dorsal and ventral surfaces of the IFM in *A. pallidus* (Figure 4b). Light microscopy and scanning electron microscopy showed that each IFM cluster consisted of a large, central dome with an unusually long hair surrounded by five to six smaller domes, each with a shorter, smaller-diameter hair (Zook and Fowler, 1982, 1986). The IFM clusters were also seen in the other species examined, but these were generally more randomly organized.

In their histological examination of the megachiropteran wing, Crowley and Hall (1994) found similar dome structures on the wing surfaces of several megachiropteran species. The megachiropteran domes differed in size of dome and diameter of dome hairs (respectively 2× and 5× larger) and in dome distribution across wing membranes. Although the megachiropteran wing membranes have similar elastin-band arrays, Crowley and Hall reported that megachiropteran domes were neither specifically associated with wing elastin bands nor regularly distributed across wing surfaces. There did not appear to be any special dome clustering or dome distribution on the megachiropteran IFM. Crowley and Hall did note that megachiropteran domes were particularly concentrated in lines running over elevated or projecting features of the wing, such as the larger membrane blood vessels, membrane-bound muscles, and wing bones. These particular locations may improve the mechanical isolation of domes from the surrounding wing membranes or could raise domes and dome hairs away from the wing surfaces to sample better specific regional boundary layer flow patterns.

3.17.3.2 Dome Neurohistology and Primary Afferent Recordings

Preliminary studies of bat wing with modern histological techniques (Zook and Fowler, 1982, 1986) revealed a large population of presumptive Merkel cells (arrowheads, Figure 5) concentrated at the basement membrane along the dome surface and surrounding the hair follicle. Following the classic description of Merkel cells (Pinkus, 1905; Smith, 1967; Halata *et al.*, 2003), these dome cells were typically large, clear cells with lobulated nuclei restricted to the epidermal basal lamina. This identification has been supported by more recent histology (Zook, 2005) showing a positive staining of these cells with both Merkel-specific quinacrine fluorescence (Nurse *et al.*, 1983) and a cell-specific antibody to the cytokeratin protein, CK20 (Moll *et al.*, 1995). Nerve fibers within the dome complex can be traced to individual Merkel cells, although free nerve endings and as yet unidentified specialized nerve terminals were identified around the dome hair follicle.

Preliminary recordings of wing dome primary afferent nerves have been made in the microchiropteran species, *A. pallidus* and *E. fuscus* (Zook and Fowler, 1986; Zook, 2005). These studies used suction and hook electrodes to isolate and record single afferent fiber activity from either a main limb nerve or from a smaller membrane nerve bundle isolated within the proximal wing membranes. Recorded afferents generally showed low-threshold responses to light touch or air-puff stimuli and could be characterized as either slowly adapting (SA) or rapidly adapting (RA) units. Both SA and RA responses could be associated with tactile stimulation of specific domes or wing regions. Afferent SA responses could be elicited by direct contact with an individual dome or from a small cluster of domes. Responses generally could not be elicited from light touch of the wing membrane surrounding a responsive dome or in between domes. Such response patterns are typical of Merkel cells in other mammals which are distinguished by their mechanically isolated, SA response patterns (Iggo and Muir, 1969; Halata *et al.*, 2003). In some wing afferent recordings, SA responses could be elicited by contact or movement of the dome hair. Still other dome-associated afferents showed primarily RA responses to hair movement. These latter afferents were particularly sensitive when air puffs were used to move dome hairs.

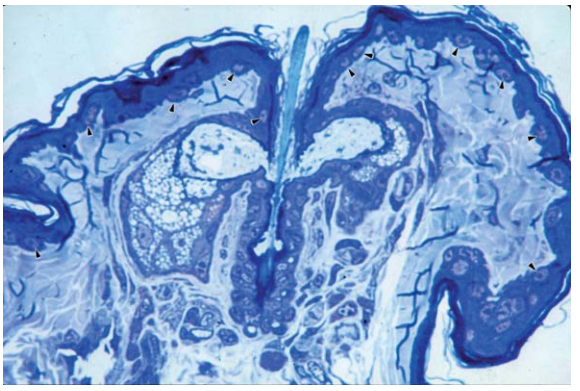

Figure 5 A high-magnification photomicrograph of a wing membrane dome in cross section. Methylene blue stain from *A. pallidus*. The darkly staining epidermis contains a number of clear cells, Merkel cells, along the boundary with the lighter-staining dermal layer. Concentrations of Merkel cells are also found encircling the centrally placed hair follicle above and below the dome's large sebaceous glands. The cross-sectional width of this dome is 120 μm.

The large-nerve recordings revealed another population of less selective RA afferents with a generalized response to membrane contact or membrane stretch. These afferents were characterized by medium to large receptive fields involving substantial portions of a membrane segment and often including a portion of a neighboring arm or digit. In many cases, these stretch-responsive afferents showed eccentric receptive fields with a point of greatest sensitivity near the convergence of multiple elastin bands at joints of the digits, wrist, or arm with a larger, less sensitive response field extending out from the side of the joint toward the middle of an adjacent wing membrane (Zook, 2005, 2006). Primary afferents serving cutaneous receptors in the IFM have not been examined.

Perhaps these studies' most interesting observation was the degree to which responses to threshold stimulation were confined to one or the other surface of a wing membrane. In other words, a given afferent might respond vigorously to the slightest contact with the dorsal membrane surface while remaining silent with even major deflections of the opposing ventral membrane surface. This surface-specific response was quite unexpected, given the extremely thin wing membrane skin that ranges in thickness from less than 0.03 mm in aerial-feeding insectivores to around 0.06 mm in many terrestrial-feeding microchiropteran species (Studier, 1972). megachiropteran species can have somewhat thicker membranes up to around 0.25 mm (Crowley and Hall, 1994). Given these dimensions, it is difficult to see how these receptors on opposing wing surfaces could be both sensitive to surface contact yet mechanically isolated from stimulation of the opposing surface.

The surface-specific response phenomenon was most dramatic in the case of the broadly sensitive,

large-field, stretch receptor population. Although afferents associated with stretch receptors responded to the slightest surface contact within its receptive field, almost all were markedly insensitive to gross stimulation of the opposite membrane surface, including gross physical deformations. The smaller receptive fields associated with dome and dome hair receptors were also more mechanically isolated from stimulation of the membrane surface directly opposing the dome than to the surrounding membrane on the same side as the dome. This uncoupling of membrane surfaces could be useful for selective discrimination of different airflow patterns over dorsal and ventral surfaces or may even be useful for improving the detection and localization of insect prey in contact with a wing surface.

The structural and physiological bases of this surface-specific receptor sensitivity have yet to be explored. It would seem likely that the limited mechanical isolation between surfaces could be enhanced by some form of mutual, contralateral inhibition within the local receptor population (Zook, 2005). Among the topics yet to be explored are the suggestion of afferent responses to directional stimulation of dome hairs and dome clusters, and the possibility of selective response of dome hairs to specific airflow or turbulence patterns.

Different types of wing hairs and pelage hair distributions have been observed over the wings of some microchiropteran species. While the forearm bones in most bats project above the membrane surface, such drag-inducing protrusions may be smoothed out by a tapered pattern of surrounding pelage hairs observed in some species, most notably in the swifter-flying Microchiroptera, where drag is a major factor (Vaughan and Bateman, 1980). A similar tapered extension of pelage hairs may smooth the transition between body and dorsal IFM (Vaughan, 1970). For slow-flying species, Vaughan and others have suggested that the projecting forearm bones or other hair patterns might be used to induce a measured degree of turbulence into the boundary air layer as a means of reducing flow separation from the wing reducing drag (Vaughan, 1970; Hill and Smith, 1984; Norberg, 2002).

3.17.3.3 Possible Roles of Tactile Receptors in Flight

The observed wing stretch receptor population has the most obvious potential role as a means of monitoring wing and wing membrane strain during sudden turns or extreme flight maneuvers (Zook, 2005). A number of studies have noted the fine balance struck by the wing between the need to conserve flight mass and the requirements for structural support (Studier, 1972; Swartz *et al.*, 1996; Swartz, 1998; Norberg, 2002). The ability to gauge relative strain on delicate wing elements during flight would seem crucial for all bats. A possible secondary role for stretch receptors in foraging (Zook, 1985, 2005, 2006) will be covered in the next section.

Domes and dome hairs show a number of characteristics that would make them suitable for monitoring boundary layer airflow, such as their ordered distribution across all wing surfaces as well as their mechanical isolation from the membrane and sensitivity to air stimuli. A preliminary survey across species suggests that the greatest variation in dome distributions may be found between species specialized for either slow or fast flight, which may have different requirements for monitoring boundary flow or wing vortex patterns (Zook and Fowler, 1986).

One preliminary behavioral study explored the potential role of dome hairs in flying bats (Zook, 2005). Several bats of the species *A. pallidus* and *E. fuscus* were flown in a lab setting after a depilatory cream was used to remove dome hairs from all wing surfaces. Flying bats without dome hairs showed no obvious difficulties during straight flight or when performing shallow turns. Flight performance, however, was clearly affected during sharp-angle turns. Although there were wide variations between tests and among bats, all showed abnormally large changes in flight elevation during most turns, often taking the form of an oscillating pattern of elevation changes. Following regrowth of dome hairs, normal turning behavior was re-established in each test bat, marked by a gradual rise during entry into the turn and a smooth transition through the turn (Aldridge, 1987).

Sharp turns are among the more complex of flight maneuvers, involving sudden braking, loss of air speed, and major changes in wing position and conformation (Norberg, 1985, 1990; Aldridge, 1987). Although preliminary, these hair ablation experiments suggest that dome hairs may be particularly involved in complex flight maneuvers that require rapid and precise adjustments of wing camber to maintain optimal lift. Similar wing-spanning sensory arrays would presumably be useful for birds, but in a feathered wing, sensory feedback for lift control may be obtained from extracutaneous receptors, specifically from muscle spindle populations in the avian forearm (Brown and Norberg, 1997). In bats, similar proprioceptive-based feedback could be provided by the muscle spindle afferents of the intrinsic membrane, striated muscles found within the proximal wing of many bats (Schumacher, 1932; Gupta, 1967; Crowley and

Hall, 1994). While the main role of these membrane-bound muscles may be for shaping or tensioning the membrane during flight (Swartz *et al.*, 2004), their spindle afferents might also signal local membrane disturbances, such as the ripples or fluttering that would result from boundary layer turbulence (similar to the lufting motion of a poorly trimmed sail).

Apart from a potential role in monitoring boundary flow, the clustered domes of the IFM are particularly intriguing in light of the proposed roles of this membrane in flight and aerial stability. Although the IFM is not present in all species and thus may not be essential for flight (Vaughan, 1970; Hill and Smith, 1984), it does add an additional lift surface and may aid in flight maneuvers such as an air brake during turns or as a flap for adjusting longitudinal stability (Vaughan, 1970; Norberg, 1985). Since bats have a short fuselage and lack long tails, pitch control may have to be actively maintained, possibly by curving the IFM either upward or downward to counterbalance shifts between a bat's center of mass and center of lift during different parts of the wing-beat cycle (Vaughan, 1970; Rayner, 1987). The IFM's elaborate dome arrays may provide feedback to optimize vestibulomotor reflexes controlling longitudinal stability (Zook, 2005). Wing dome hair arrays and their regional patterns need to be explored in terms of their potential interaction with vestibular and motor control of flight and flight stability in all dimensions (Horowitz *et al.*, 2004).

3.17.3.4 Possible Roles for Wing Receptors in Foraging

All or part of the wing receptor array could be used for locating, tracking, and assisting transfer of wing-gathered prey from initial contact point to mouth. Given their distribution, response patterns, and mechanical isolation, wing domes and dome arrays could provide fairly accurate positional feedback to aid in locating prey during manipulation and transfer (Zook, 2005, 2006). Insect movement and direction of movements across the wing surfaces could be tracked from a sequential stimulation of individual domes, dome hairs, or dome hair arrays.

The IFM is a favored site for capture in all kinds of foraging (Webster and Brazier, 1965; Arlettaz, 1996; Kalko and Schnitzler, 1998). While the IFM can be cupped more than the other wing membranes, struggling prey still cannot be pinned in place. Tactile feedback from IFM dome clusters might provide even more feedback on prey contact points or the direction of prey movement than the spaced dome arrays of the main wing. The main wing membranes of the forearm are the second most common sites for prey contact during aerial foraging. While the forearm and digits provide a longer reach for sweeping in prey than would be possible with the IFM, the forearm wing cannot be cupped for containing prey to the degree possible with the IFM (Arlettaz, 1996; Zook, 2005, 2006).

Forelimb sweeping motions may offer another advantage during capture. By continuing the wing's sweeping motion through the contact point, a bat may use the wing's acceleration and the insect's inertia to stick the insect to the wing membrane. Such an inertia-pinned insect would be easier to control during the short but critical transfer from wing to mouth. While the large receptive fields of the stretch receptors may not be useful for precisely locating wing-gathered prey, they could provide a means to monitor where the insect first contacts the wing membrane (Zook, 2005, 2006) and how well the insect is pinned to the wing contact point.

3.17.4 Central Somatotopic Representation of the Bat Wing: A Neuroethological Perspective

The observed peripheral tactile specializations provided an impetus for examining neocortical somatotopic representations of the wing in *A. pallidus* (Zook and Fowler, 1982). At the same time, a series of other studies were begun that mapped somatotopic representations of wing and body in several megachiropteran species, focusing primarily on the comparative and evolutionary implications of these cortical maps (Calford *et al.*, 1985; Wise *et al.*, 1986). As with most central sensory maps, these somatotopic maps generally reflect the relationships and proportions of the related sensory surface, in this case skin tactile receptors, with a disproportionate representation of peripheral structures that have particularly rich innervation. Figure 6 shows comparable schematic representations (homunculi) of bat somatotopic maps from primary neocortical area S1. Figure 6a is a homunculus redrawn from Zook and Fowler (1982), while Figures 6b and 6c represents homunculi respectively from a different microchiropteran species and a megachiropteran species (Wise *et al.*, 1986). A rat homunculus is included for comparison (Figure 6d).

Focusing on the wing representations, all four bats showed an enlarged representation of the hand with a disproportionate representation of the thumb, the only free digit of the hand-wing. All the bat maps contain representations of the remaining digits and

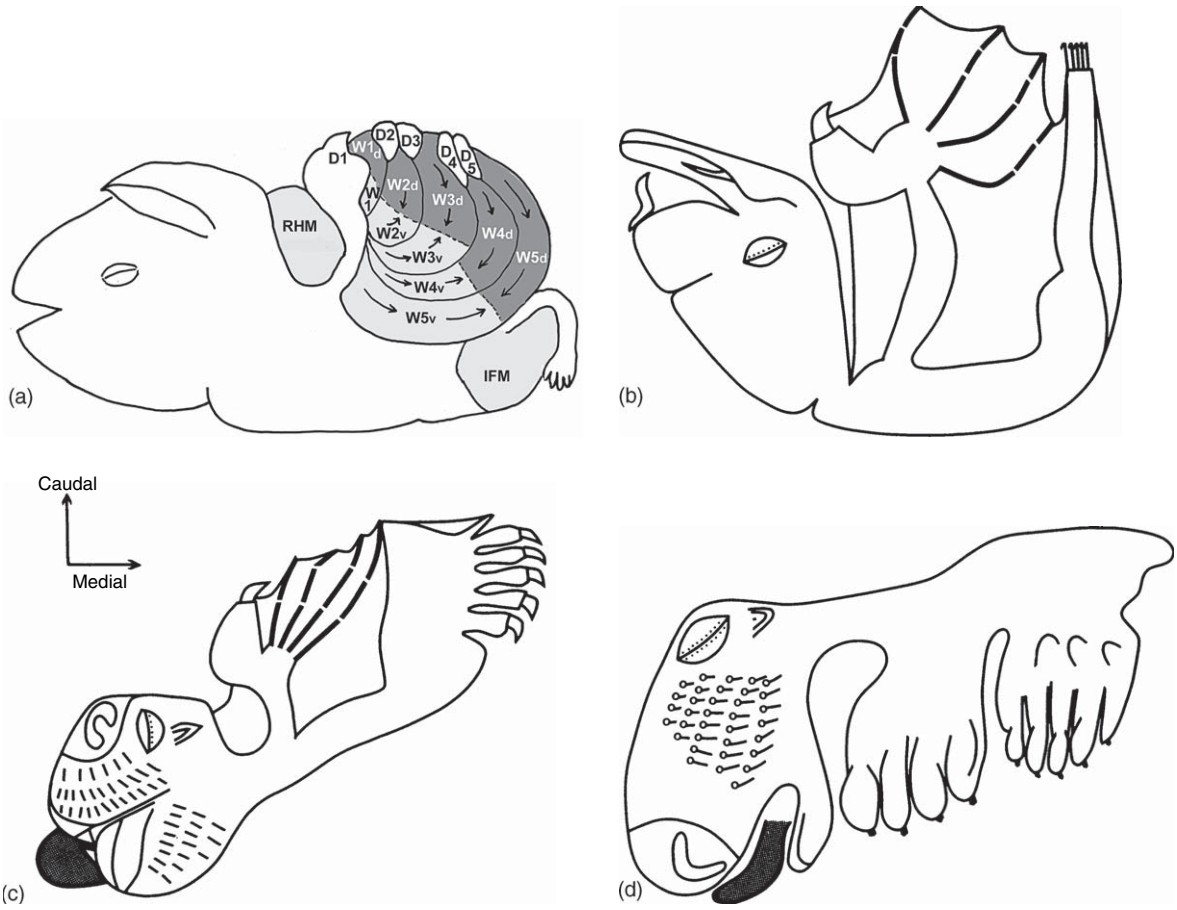

Figure 6 Schematic representations of the body surface (homunculi) from mapping studies of primary somatosensory cortex (S1) from two microchiropteran bats: a, *Antrozous pallidus* data (partly) from Zook and Fowler (1982); b, *Macroderma gigas* from Wise *et al.* (1986); and c, the microchiropteran bat *Pteropus poliocephalus* from Calford *et al.* (1985). A homunculus from the rat is shown in (d). Maps are not to scale, but are all oriented to match the position of the original cortical recordings on the surface of the cortical hemisphere. As indicated by the marker, the right side of each map is closest to the cortical midline, the left (face) side of each map is toward the lateral side of the hemisphere, while the top of each map is oriented toward the caudal pole of the brain. The bottom of each map is oriented toward the rostral pole of the brain. In map (a), the wing membranes (dark and light gray shading, labeled W1–W5) were represented somewhat separate from the digits (digits 1–5 labeled D1–D5) with a separate representation of each membrane surface (ventral=light gray, dorsal=dark gray). Each membrane surface representation was internally consistent, but flipped along the trailing edge (dashed lines). The arrows point the membrane maps' progression in the representation from leading to trailing edges. The ventral surface leading edge begins near the palm/D1 while the dorsal surface leading edge begins near the digits D2–D5, with both representations converging into the single trailing edge representation. RHM, radiohumeral membrane; IFM, interfemoral membrane. b–d, Reproduced from Somatosensory cortical representation in the Australian ghost bat, *Macroderma gigas*, *J. Comp. Neurol.*; Wise, L. Z., Pettigrew, J. D., and Calford, M. B.; Copyright © 1986, Wiley-Liss. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

wing membranes. In the Zook and Fowler map, however, membrane segments are partially segregated from the digits and include separate representations of the dorsal and ventral forelimb membrane surfaces. The other bat-wing representations report a single wing surface with digits positioned between wing segments. It is not clear whether surface-specific responses were encountered in these latter studies.

The difference between results in the different bats may be a matter of the different techniques or species used. Most somatotopic mapping studies depend almost exclusively upon surface-stimulating probes to elicit a standard response over all skin regions and across species. In order to elicit surface-specific responses from the wing membranes, the Zook and Fowler studies used a combination of tactile probe and air-puff stimulation. Air puffs may have selectively stimulated a subset of skin tactile receptors, imposing a modality bias on the cortical maps. In other species, when the different submodalities of tactile sensation are independently mapped on cortex, the somatotopic patterns can be quite different from the more standardized, probe-based map (Dykes, 1983; Friedman *et al.*, 2004). It is also

possible that the dual-surface pattern is unique to a subset of microchiropteran bats with specific flight or foraging requirements. Of the two microchiropteran species represented in Figure 6, *A. pallidus* is a gleaning and terrestrial insectivore known for the use of passive sound localization and highly maneuverable flight (Fuzessery *et al.*, 1993), while *Macroderma gigas* is an exceptionally large carnivorous bat with a well-developed visual system and less acrobatic flight (Wise *et al.*, 1986).

One of the most striking aspects of the surface-specific membrane representation in *A. pallidus* is the internal consistency within the two membrane surface maps. The ventral surface map reflects the hand and membrane pattern seen in the other bats in Figure 6, with the membrane segments extending caudally from the hand and thumb (D1) representations. At the trailing edge of the ventral surface representation, the membrane map flips to begin representing the dorsal surface, progressing from trailing to leading edge (arrows in the two membrane surface maps begin near the representation of the leading edge and point toward trailing edge representation where the surface maps converge). The fact that peripheral separate-surface response patterns are retained to the level of S1 may indicate a degree of independent processing of the two surfaces' receptor populations.

These wing-specific features aside, the general pattern of cortical body maps were otherwise quite consistent among these bat species (Wise *et al.*, 1986; Krubitzer, 1995). The greatest variation appears in the hindlimb placement, which may reflect the difficulty in mapping this underrepresented area (Calford *et al.*, 1985). Compared to S1 cortical maps in other mammals, however, the mapped position of the bat forelimb was strikingly different. In the general mammalian pattern, the representation of the limbs projects rostrally in the brain (see the orientation bars in Figure 6) and is mapped on the homunculus ventral from the trunk and below the head with the digits projecting rostrally (Figure 6d). In the bats (Figures 6a–6c, the forelimb and digital representation extended toward the caudal brain so that the homunculi map's arm and wings project dorsally away from the trunk and above the head. This partial map reversal was first noted by Calford *et al.* (1985) and has been consistently observed in all species examined (Zook and Fowler, 1982; Calford *et al.*, 1985; Wise *et al.*, 1986; Krubitzer *et al.*, 1993). Comparisons of bat and general mammal somatosensory pathways and subcortical maps have shown that the limb reversal is set up through a series of transformations between the sensory periphery, dorsal column nuclei, and somatosensory thalamus (Martin, 1993; Manger *et al.*, 2001a, 2001b).

Calford and others have suggested that the digital reversal may be a cortical reflection of the manner that bats' hands are held at rest with digits positioned caudal to the hand and arm (Calford *et al.*, 1985; Wise *et al.*, 1986). While there is no direct evidence to support this habitual position theory, it is intriguing, as it implies a major proprioceptive influence in the ordering of these somatotopic maps. Joint proprioceptive, muscle spindle, and other extracutaneous somatosensory modalities have not been examined in bats but may all play a role in facilitating the exquisite vestibulomotor control exhibited by night-flying and night-foraging bat species (Horowitz *et al.*, 2004). Proprioceptive somatosensory modalities are likely to play a central role in night navigation, and may be critical for the bat's extraordinary ability to memorize and navigate complex flight paths without echolocation or other sensory guidance (Möhres and Oettingen-Spielberg, 1949; Griffin, 1958).

3.17.5 Future Prospects for Somatosensory Study of the Bat Wing

The study of the somatosensory system in mammalian flight has a history of misdirection, neglect, and lack of clear focus. Hopefully this article has given a sense of the possibilities for future study and the potential of the neuroethological approach that has proven so successful in the exploration of bat echolocation. While the focus here has been on the bat wing, cutaneous receptors of the head and face may offer an even greater range of specializations, for example, the thermal and tactile receptors in vampire bats (Kürten and Schmidt, 1984; Kürten, 1985). With regard to the wing, more data are needed, focusing on a comparative neurohistology of the chiropteran wing and a closer examination of cortical representation of the wing in a broader range of representative species. With regard to broader comparisons, there may be some interesting similarities between bat dome arrays and the manatee's array of tactile body hairs (see 00068). The manatee hairs appear to function as a mammalian lateral-line system for the detection of water currents and other near-field phenomena (Reep *et al.*, 2002). In a manner similar to Maxim's proposed tactile sixth sense, a manatee's tactile hairs could serve as part of an active, remote-sensing system capable of detecting and interpreting echo patterns from pressure waves set up by the manatee's own movements.

Further study of both peripheral and central adaptations needs to establish behaviorally

appropriate stimuli to explore proprioceptive as well as cutaneous somatosensory receptor populations in terms of flight and flight-related behaviors. Although interesting in themselves, the wing dome arrays and the uncoupling of wing membrane responses and representations suggest that the main somatosensory adaptations for flight may lie in the population responses, the interplay of feedback from cutaneous and extracutaneous receptors, and their integration within the motor and vestibulomotor reflexes that optimize flight control (Horowitz *et al.*, 2004). This broader perspective of the role of somatosensory specializations in mammalian flight could lead to a greater appreciation of the sensorimotor mechanisms involved in vertebrate flapping flight as well as the role of feedback in the design and optimal control of micro air vehicles (Shyy *et al.*, 1999; Norberg, 2002).

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3.18 Evolution of Pain Pathways

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Glossary

ACC	The anterior cingulate cortex (a portion of limbic motor cortex), where the behavioral motivations associated with homeostatic emotions are represented.
dpIns	The dorsal posterior insular cortex (a portion of limbic sensory cortex), where the representations of pain, temperature, itch, and other interoceptive sensations are located in primates.
homeostasis	The ongoing dynamic neural processes that maintain the physiological condition of the body.
insular cortex	The viscerosensory or limbic sensory cortex, which is called the insula, or island, because in the human brain it is buried inside the folds of the lateral sulcus.
interoception	Used here to mean the sense of the physiological condition of the body.
lamina I	The most superficial layer of the spinal (and trigeminal) dorsal horn.
MDvc	The medial thalamic nucleus that relays lamina I spinothalamic projections to anterior cingulate cortex in primates.
spinothalamic tract	The ascending pathway from the spinal cord to the thalamus.
VMpo	The lateral thalamic nucleus that relays lamina I spinothalamic projections to insular cortex in primates.

3.18.1 Introduction

Pain has traditionally been classified as an aspect of somatic sensation, for the obvious reason that pain is, first of all, a physical sensation we feel from our bodies. Accordingly, it was thought for many years that pain is represented within the same somatosensory system that represents the exteroceptive sense of touch and the proprioceptive sense of limb movement (the dorsal column – medial lemniscal system). The highly organized somatotopic maps of the body in the somatosensory cortices of mammals (see Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications) were thought to be necessary to explain the fact that pain, like touch, is a discriminative sensation that can be localized quite well on the skin surface.

Nevertheless, as we all recognize, pain can originate from any part of the body (that is, not just skin) and it has a strong emotional quality – a motivational affect – and other mystifying qualities (sensitization, radiation, wind-up, persistence, and so on) that make it quite different from touch and other classical senses like hearing and vision. Furthermore, the presumption that the somatosensory cortices have a critical role in pain is strongly contraindicated by the repeated observations that lesions and stimulation of the somatosensory (or perhaps better, sensorimotor) cortices, which definitively alter mechanoreception and proprioception, rarely have any affect on pain, temperature, itch, and other feelings from the body.

Although many textbooks today still describe pain as an aspect of the somatosensory system, recent functional anatomical evidence demonstrates that human pain sensation is subserved by a novel, unforeseen sensory pathway that is a phylogenetically distinct extension of an ancient, hierarchical

system that conveys ascending afferent activity associated with homeostasis – the maintenance of the physiological condition of the body. In primates, this system provides the basis for a high-resolution, modality-specific sense of the condition of the body (interoception redefined). In humans, higher-order re-representations of this system provide a substantive basis for the subjective awareness of the material me, that is, how you feel. These re-representations, as it turns out, are involved in all subjective feelings and emotions, consistent with the James–Lange theory of emotion and Damasio's somatic marker hypothesis of consciousness.

The homeostatic afferent (or interoceptive) system conveys afferent activity associated with all feelings from the body other than discriminative mechanoreception (touch) and proprioception (limb movement); these feelings include hot, cold, and mechanical pain, innocuous thermal sensations (warm, cool), itch, muscle burn, ache and cramp, visceral movement and cramp, visceromotor flush, and even sensual (C-fiber-mediated) touch. The essential psychophysiological distinction between these somatic feelings and the sensations of mechanoreception and proprioception is that these feelings all relate the physiological condition of the body, which is reflected in their association with an immediate affect (pleasantness, unpleasantness) that is the perceptual correlate of the homeostatic behavioral motivation they drive. For example, the sensation of muscle burn relates the deficient metabolic condition of the muscles (hypoxia, hypercapnia, and increased lactate) to the forebrain limbic behavioral control systems. The essential neurobiological distinction between these somatic feelings and the sensations of touch and limb movement is that they are all subserved by small-diameter afferent fibers and provide the afferent information required for homeostatic control of smooth muscle, whereas mechanoreception and proprioception are subserved by large-diameter afferent fibers that provide afferent information required for control of skeletal muscle. This fundamental functional distinction is made early during the ontogeny of the dorsal root ganglia and the spinal cord, and in primates this fundamental distinction is maintained in distinct patterns of thalamocortical organization. Thus, the cortical representation of these feelings from the body is located in a region associated with autonomic control rather than musculoskeletal control.

The evidence for these concepts has been described in detail in several recent reviews (e.g., Craig, 2002, 2003). In the following, I describe the organization of the human pain pathway, I discuss the comparative anatomy of the pain pathway in subhuman mammals, and then I address the evolutionary pressures that can be inferred to have led to the development of this system in the human brain. Because this system is more accurately termed a homeostatic afferent pathway, it should only nominally be called a pain pathway.

3.18.2 The Human Pain Pathway

Considerable clinical evidence documents the statement that lesions that alter or interrupt pain sensation occur in the anterolateral spinal cord, the posterolateral thalamus, the posterior parietoinsular cortex, or the anterior cingulate cortex (ACC). Similarly, stimulation in each of these regions can cause pain. Activation in the posterior parietoinsular and ACCs is also found in all evoked potential and functional imaging studies of pain. The anatomical substrate responsible for these findings is the lamina I spinothalamocortical pathway, as described in the following text.

3.18.2.1 Primary Afferent Connections

Small-diameter (A-delta and C) primary afferent fibers that report all aspects of the physiological status of all tissues of the body (which include nociceptors, thermoreceptors, osmoreceptors, and metaboreceptors from skin, muscle, bone, and viscera) terminate monosynaptically on neurons in lamina I of the spinal (and trigeminal) dorsal horn. Lamina I neurons provide the output of the superficial dorsal horn, which otherwise contains local interneurons. During development, the large-diameter primary afferents from mechanoreceptors and proprioceptors arrive in the dorsal horn first and contact the deep dorsal horn cells. Then the entire dorsal horn rotates ventromedially, and the small-diameter primary afferents arrive in a second wave that is genetically coordinated with the arrival of lamina I neurons. The lamina I neurons originate from progenitors of autonomic interneurons (in the lateral horn) and migrate to the top of the dorsal horn (carried along by its rotation), arriving precisely when the small-diameter afferents arrive. This exquisite developmental coordination indicates that together the small-diameter afferents and the lamina I neurons form a coherent system for homeostatic afferent activity. Small-diameter afferents that innervate visceral organs by way of the cranial parasympathetic nerves terminate similarly in the nucleus of the solitary tract in the medulla.

3.18.2.2 Lamina I Spinal and Bulbar Projections

The projection targets of lamina I neurons in the spinal cord and brainstem are all associated with autonomic control, indicating that lamina I provides the central continuation of the small-diameter afferent pathway conveying homeostatic sensory activity. The lamina I neurons project strongly to the autonomic cell columns of the thoracolumbar and sacral spinal cord, where sympathetic and parasympathetic preganglionic motor neurons are located. This provides the substrate for spinospinal somatoautonomic reflexes. In the brainstem, lamina I neurons project to all of the major homeostatic integration sites, which also receive parasympathetic afferent activity by way of the solitary nucleus, and are heavily interconnected with the hypothalamus and amygdala; these sites include the caudal and rostral ventrolateral medulla, catecholamine cell groups A1–2 and A5–7, parabrachial nucleus, and periaqueductal gray. These brainstem regions contain premotor autonomic neurons that have descending projections to spinal autonomic regions, as well as neurons with descending projections that modulate activity in the dorsal horn. The lamina I projections to the brainstem provide the substrate for the modality-selective somatoautonomic spinobulbospinal reflexes activated by spinal small-diameter afferents, which are critical mechanisms for homeostatic function. Lamina I also receives descending modulation not only from these brainstem preautonomic sources, but also selectively from descending fibers from the hypothalamus. Thus, the hierarchical lamina I spinal and bulbar projections provide the long-missing central afferent limb of the efferent autonomic nervous system. As far as I am aware, the homeostatic afferent pathway formed by small-diameter afferents and lamina I neurons is present in fish, lizards, birds, and mammals.

3.18.2.3 Lamina I Projections to the Forebrain

In subprimates, the integration of ascending homeostatic afferent activity from lamina I occurs mainly in the brainstem. There, the parabrachial nucleus at the junction of the pons and midbrain receives dense input from both lamina I and the nucleus of the solitary tract, and it projects densely to the hypothalamus and the amygdala. The parabrachial nucleus is thus the main homeostatic afferent integration site of the supraspinal autonomic control regions. In rats and cats, there are additional, weak rostral projections from lamina I that can be regarded as primordial evolutionary attempts at more direct forebrain integration; these include direct spinal projections to a variety of preautonomic (limbic) cortical regions, as described in the section on comparative anatomy below. By contrast, in primates there are direct, well-organized homeostatic afferent projections to sensory relay nuclei in the thalamus, and thence to cortex, from both lamina I and the nucleus of the solitary tract. The homeostatic afferents from parasympathetic nerves, which terminate in the nucleus of the solitary tract of the brainstem, and the homeostatic afferents from sympathetic and somatic nerves, which terminate in lamina I of the spinal (and trigeminal) dorsal horn, activate dorsal posterior insular cortex (dpIns) by way of direct ascending projections to the basal (parasympathetic) and posterior (sympathetic) parts of the ventromedial nucleus of the thalamus (that is, VMb and VMpo). These are modality-specific, topographically organized projection pathways that are phylogenetically distinct to primates and enormously well developed only in humans. The dpIns contains representations of all interoceptive afferent activity from lamina I and the solitary nucleus. (For illustrations of these projections, see Craig, 2002, 2003, 2004a.)

In humans, this interoceptive cortical field engenders several sensations, or feelings, associated with the homeostatic condition of the body. Functional imaging studies provide convergent evidence confirming the role of dpIns as the primary sensory cortex involved in pain, temperature, itch, muscle burn, sensual touch, hunger, thirst, cardiorespiratory activity, etc. (see Craig, 2002, 2003). Notably, these include well-localized, discriminative sensations that traditionally have been associated with the classical somatosensory system.

For example, dpIns is the only site in the contralateral cortex that evinces linear activation correlated with the temperature of a graded innocuous cooling stimulus. This activity corresponds directly with the linear activation of thermoreceptive-specific lamina I spinothalamic neurons. The dpIns is also the only cortical site where lesions produce thermanesthesia, and only stimulation in VMpo and dpIns can produce graded, well-localized sensations of cooling, whereas lesions or stimulation of parietal somatosensory areas do not affect thermal sensation. Thus, this cortical site is the representation of discriminative innocuous thermal sensation. This conclusion is confirmed by recent functional imaging evidence that thermosensory activation of dpIns is somatotopically organized (Figure 1; Hua *et al.*, 2005). The available anatomical tracing and functional imaging data indicate that the representation of painful stimuli in dpIns is also graded and somatotopographically organized; thus,

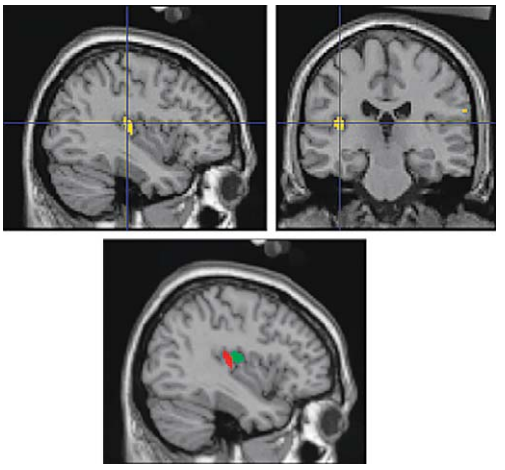

Figure 1 A composite showing the localization in dpIns of activation by graded innocuous cooling stimulation obtained using fMRI. The top panels show the location of activation correlated with stimulus temperature on the hand, and the bottom image shows the anteroposterior somatotopographic organization of activation from the hand (red) and the neck (green). Reproduced from Hua, L. H., Strigo, I. A., Baxter, L. C., Johnson, S. C., and Craig, A. D. 2005. Anteroposterior somatotopy of innocuous cooling activation focus in human dorsal posterior insular cortex. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R319–R325, with permisson from the American Physiological Association.

it can subserve the haptic aspects of temperature and pain sensations that were formerly considered exteroceptive aspects of somatosensation.

The organization of the topographic maps in dpIns provides strong support for the fundamental distinction between these interoceptive cortices and the exteroceptive (or, sensorimotor) cortices that receive lemniscal input. In contrast to both S1 and S2, which are organized mediolaterally, the interoceptive cortical areas are organized in the orthogonal, anteroposterior direction. Thus, in S1, the foot is represented in the medial postcentral gyrus and the hand and face are represented progressively more laterally, and in S2, the foot is represented in the medial part of the parietal operculum and the hand and face are represented progressively more laterally (see Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications). In stark contrast, the anatomical tracing and the functional imaging evidence indicate that in both the cooling thermosensory representation and the noxious heat (pain) representation in dpIns, the foot is represented posterior and the hand and face are represented progressively more anterior (Figure 1; Hua *et al.*, 2005; Brooks *et al.*, 2005). This matches the orthogonal neighborhood relationship of the interoceptive and exteroceptive maps in the thalamus (that is, between VMb/VMpo and VPM/VPL), which are joined at the representation of the mouth, the embryological junction of the inside and the outside of the body. This organization validates the neuroanatomical and neurochemical distinctness of interoceptive modalities, important for homeostasis and autonomic activity, from the exteroceptive and proprioceptive modalities of touch and limb position, important for somatic motor control.

The ascending lamina I pathway in primates also activates the ACC (i.e., limbic motor cortex) by way of the ventral caudal portion of the medial dorsal thalamic nucleus, or MDvc. In addition, it activates a portion of sensorimotor cortex, area 3a, that is intercalated between the primary somatosensory area and the primary motor area by way of a collateral projection from VMpo. The anterior cingulate projection is directly associated with the affective motivation, the unpleasantness, of pain. The area 3a projection is likely involved with cortical control of the reflex motoric action of pain, that is, sensorimotor integration. This is consistent with the projection of vagal and other visceral afferent activity to anterolateral area 3a. Nevertheless, a role for this area 3a input in perception (e.g., the exteroceptive capacity of cutaneous pain) should still be considered. (Area 3a is the probable source of activation often interpreted as S1 in functional imaging studies of pain. A similar source of confusion in many functional imaging studies of pain is the nomenclative misidentification of dpIns as S2.)

3.18.2.4 Significance of the Homeostatic Afferent Representation for Emotion

In macaque monkeys, the interoceptive representation in dpIns projects directly to the orbitofrontal cortical regions that are associated with hedonic evaluation and emotional regulation of behavior (Carmichael and Price, 1996). In humans and humanoid primates, on the other hand, there are multiple re-representations of interoceptive afferent information, first in the middle insula (which also aggregates activity from the amygdala and from other cortical sensory regions) and then in the most anterior insula. The anterior insula seems to provide a meta-representation of the state of the body that is associated with subjective awareness of the material self as a feeling (sentient) entity across time, that is, emotional self-awareness, consistent with the ideas of James and Damasio (Craig, 2002, 2004b). These pathways become clearly lateralized as they progressively activate higher-order homeostatic afferent re-representations in more anterior portions of the human insula. This lateralization in humanoid primates makes the relevant homeostatic afferent

representations coincide with the asymmetric sympathetic and parasympathetic efferent control regions on the right and left sides of the forebrain. In other words, the feelings represented in the right anterior insula seem to be associated predominantly with sympathetic activity, and thus with arousal, danger, negative affect, withdrawal (aversive) behavior, and individual-oriented (survival) emotions. The feelings represented in the left anterior insula seem to be associated predominantly with parasympathetic activity, and thus with nourishment, safety, positive affect, approach (appetitive) behavior, and group-oriented (affiliative) emotions. This neuroanatomical model can explain the wealth of psychophysiological evidence indicating that the forebrain representation of emotion and affective style is asymmetric (Craig, 2005). The phylogenetic emergence of these anatomical pathways in humanoid primates corresponds well with the stark qualitative distinction between humanoid and subhumanoid primates in terms of self-recognition and emotional communication; thus, humanoid primates recognize themselves in the mirror test, whereas other primates do not, and humanoid primates use intentional gestures for communication, whereas other primates do not (MacPhail, 1998; De Waal, 2003). This phylogenetic progression also matches well the unique appearance of large spindle neurons in anterior insula and anterior cingulate cortices of humanoid primates (Craig, 2004b; Allman *et al.*, 2005).

3.18.2.5 Physiological Identification of the Lamina I Homeostatic Afferent Pathway as the Pain Pathway

Spinal and trigeminal lamina I neurons comprise several modality-selective, morphologically distinct classes of neurons that each receive selective input from particular subsets of afferents. These classes correspond with distinct feelings from the body (including first (sharp) pain, second (burning) pain, cool, warm, itch, sensual touch, muscle burn, etc.). The lamina I spinothalamic tract (STT) neurons project their axons to contralateral thalamus in the lateral STT, precisely where cordotomy lesions interrupt these feelings. The distinct role of lamina I STT neurons in sensation is convincingly demonstrated by the selectively histamine-responsive cells that correspond uniquely with the sensation of itch. Whereas lamina I is usually related to pain and temperature, its role in homeostasis is underscored by the neurons that respond selectively to small-diameter muscle afferents, which subserve ongoing cardiorespiratory adjustments to muscular work but when strongly activated signal muscle burn and pain.

In particular, single-unit recordings from monkey STT neurons reveal that the responses of nociceptive-specific lamina I STT cells correspond precisely with the human psychophysiological profile of stinging (first) pain, whereas the polymodal nociceptive lamina I STT neurons correspond with the profile of burning (second) pain. Definitive evidence for these associations is provided by use of a graded series of mechanical probes and use of the repeated brief contact heat test, which selectively elicit sensations of stinging and burning pain (Craig, 2003).

In stark contrast, the large lamina V wide-dynamic-range (WDR) neurons studied by many investigators show response patterns to these tests that are significantly different from the profile of human pain. This is an important distinction because the proponents of the widely held pattern/intensity concept of pain sensation (so-called gate control theory) profess that WDR lamina V STT neurons are pain transmission cells that are necessary and sufficient for all types of pain sensation (Willis and Westlund, 1998; Price *et al.*, 2003). However, the inherent modality ambiguity of lamina V WDR cells, their high ongoing discharge rate, their musculotopic receptive field organization, their interpolation in the flexor reflex motor pathway, and their distinct responsiveness to proprioceptive input are all features consistent with a role in sensorimotor integration, rather than pain sensation. They provide input to sensorimotor cortex. Contrary to the common presumption that the topographic maps of somatosensory cortices are necessary for pain localization, the somatotopically organized and modality-selective representation of different types of pain in the primate lamina I spinothalamocortical pathway to dpIns can explain the haptic characteristics of pain sensation quite well. Furthermore, this association emphasizes the fundamentally important distinction that pain is an evolutionarily emergent aspect of homeostasis, not an aspect of sensorimotor integration.

3.18.3 Comparative Neurobiology of the Pain Pathway

The lamina I projection system provides a homeostatic afferent pathway that conveys modality-selective sensory information on the physiological condition of all tissues of the body. Lamina I neurons project at the spinal level directly to the intermediolateral and intermediomedial cell columns that contain autonomic motor neurons, and

they project at the brainstem level selectively to homeostatic integration and control regions that contain autonomic premotor neurons. A parallel projection system conveys vagal and glossopharyngeal afferent input to such sites by way of the projections of the nucleus of the solitary tract.

Comparative analyses (mainly in rat and cat; for detailed references, see Craig, 2004a) suggest a possible archetypical mammalian pattern in the telencephalic projections of this system and highlight the phylogenetic novelty of the direct lamina I projections to VMpo and MDvc in primates. These analyses indicate that, in subprimates, the telencephalic extension of the spinal and brainstem lamina I projections appears to provide a medial thalamic pathway for autonomic control and a lateral thalamic pathway for viscerosomatic control. In primates, however, phylogenetically novel direct projections to both medial and lateral thalamus are present that are enormously expanded in humans (Craig, 2002, 2004a).

3.18.3.1 An Overview

In cat, trigemino- and spinothalamic projections occur mainly in the ventral aspect of the ventral tier nuclei, the motor thalamus, the intralaminar nuclei, and the submedial nucleus. Within this distribution, lamina I projections in cat are concentrated in three main sites: the submedial nucleus, the ventral aspect of the ventral tier (including VMb and VPI), and the dorsomedial aspect of VPM.

In rat, trigemino- and spinothalamic projections occur mainly in VP, the posterior nucleus, the overlying VP, and the intralaminar nuclei, as well as a variety of extrathalamic regions (hypothalamus, amygdala, septal and pallidal nuclei, and limbic cortices). Within this distribution, lamina I projections in rat are concentrated in VP, the posterior nucleus, and the posterior triangular nucleus, with weak terminations in the parvicellular part of VPM (VPMpc; equivalent to VMb in the present terminology) and the submedial nucleus.

A parsimonious interpretation of these data is that all mammals have a dual lamina I projection to medial (limbic) thalamic circuits and to lateral (sensorimotor) thalamic circuits, albeit with differing routes in different species. A dual projection is consonant with the global view that small-diameter homeostatic afferents from the entire body activate pathways (by way of lamina I and the nucleus of the solitary tract) that are important, first, for homeostatic and autonomic processing (i.e., control of smooth muscle) and, second, for viscerosomatic sensorimotor processing (i.e., control of striate muscle). Evidence in other mammals is consistent with this interpretation (e.g., hedgehog, opossum, and bush baby; Hazlett *et al.*, 1972; Pearson and Haines, 1980; Kunzle, 1998).

3.18.3.2 The Medial Lamina I Pathway

The data suggest that the direct lamina I projection to MDvc in the primate can be viewed as a phylogenetically novel route to ACC that has a potential evolutionary basis in an ancient mammalian homeostatic medial thalamic pathway that was directed in primordial species to more primitive telencephalic autonomic control regions.

The medial lamina I pathway in both rat and cat effectively targets MD-related, autonomic control regions in orbital and infralimbic cortices, either directly or by way of the submedial nucleus or the parabrachial complex. In cat, in contrast to rat, there are few extrathalamic projections and no direct cortical projections, yet the medial lamina I pathway similarly targets orbital cortex by way of the submedial nucleus.

In both cat and rat, the developmental origin of the submedial nucleus from the ventral caudal portion of the medial pronucleus suggests an evolutionary kinship with the primate MDvc (in fact, there is an identical posterior-to-anterior topography in both sites), albeit with a fundamentally different cortical target. This highlights the shift in primates to anterior cingulate. Notably, the medial (limbic) homeostatic afferent pathway in rat seems to reach the ACC (i.e., limbic behavioral motor cortex) only by way of parabrachial input to medial thalamus. In cat, homeostatic afferent lamina I activity similarly attains anterior cingulate mainly by way of the parabrachial input to medial thalamus, although curiously, in cat there is also lamina I input to anterior cingulate by way of the ventral aspect of the ventral tier nuclei of lateral thalamus.

3.18.3.3 The Lateral Lamina I Pathway

The data suggest that the direct lamina I projection to VMpo in the primate can be viewed as a phylogenetically novel route to insular cortex that has a potential evolutionary basis in an ancient mammalian homeostatic lateral thalamic pathway that was directed in primordial species to sensorimotor regions, but that in primates became redirected to limbic sensory cortex.

The lateral pathway in subprimates is directed mainly to sensorimotor cortex. In rat this projection ascends by way of VP and in cat by way of the ventral margin of the ventral tier nuclei (especially VPI).

In rat there is little differentiation in this pathway (VP supplies input to the overlapping sensory and motor cortices), but in cat both the lamina I and the vagal afferent projections seem to target area 3a, the transitional region between the primary motor and somatosensory cortices that also receives proprioceptive and vestibular inputs. Similarly, in primates a corollary lamina I projection by way of VMpo and a parallel vagal afferent projection by way of VMb also target area 3a. These observations support the interpretation that the archetypical ascending homeostatic afferent pathway by way of lateral thalamus provides a substantive basis for a generalized role in viscerosomatic sensorimotor integration.

The predominant lateral thalamic homeostatic afferent pathway in primates is the lamina I projection to VMpo and the parallel direct pathway to VMb from the nucleus of the solitary tract. This provides a novel modality-selective, topographic interoceptive sensory representation in insular cortex that is distinct from the primordial sensorimotor pathway. A rudimentary homologue of the lamina I projection to VMpo appears to exist in cat, but in rat this pathway is only tenuously present, if at all. Thus, in cat there is a moderately dense, weakly topographic lamina I projection to the ventral aspect of VMb. This appears to be homologous to VMpo, because it also projects to insular cortex adjacent to gustatory cortex and it is significant (though not critical, in sharp contrast to primates) for thermosensory behavior. Similarly, in rat a few trigeminal and spinal lamina I fibers terminate in the ventral and medial aspects of VPMpc, the parabrachio-recipient gustatory nucleus that is equivalent to VMb, but apparently no solitary nucleus fibers do so. A few neurons in this locus reportedly project to a small portion of rostral agranular insular cortex adjacent to gustatory cortex that is important for nociceptive behavior, and this area may respond to somatic C-fiber, vagal, and thermosensory inputs.

The primordial role of the insular cortex seems to be modulation and telencephalic control of brainstem homeostatic integration sites, such as the parabrachial nucleus, where its descending projections terminate. It is associated functionally with the autonomic nervous system, because stimulation of insular cortex affects cardiorespiratory, gastrointestinal, and thermoregulatory activity, and because it is closely interconnected with hypothalamus, amygdala, and ventral striatum. It can be regarded as limbic sensory cortex, whereas anterior cingulate can be regarded as limbic motor cortex. Thus, the encephalized representation of the condition of the body in primates and humans appears to have emerged evolutionarily from the afferent limb of the hierarchical homeostatic system. Activity that produces pain in humans ascends in the telencephalic extension of a pathway whose primary role has been homeostasis for hundreds of millions of years.

3.18.3.4 Summary

The comparative analyses indicate that the homeostatic afferent lamina I projections to MDvc and VMpo in the primate can be described as phylogenetically novel pathways that produce direct activation of ACC and the interoceptive representations in insular cortex. These pathways appear to signify fundamental primate innovations on the archetypical mammalian pattern of dual telencephalic pathways for autonomic and sensorimotor control. The most significant innovation appears to be the emergence of a primary limbic sensory representation of the physiological condition of the body as an extension of a primordial pathway for telencephalic control of mesencephalic and hypothalamic homeostatic integration.

3.18.4 Evolution of the Human Pain Pathway

The findings summarized in the above text indicate that pain in humans is a homeostatic emotion that consists of a feeling (a discriminative sensation engendered in dpIns) and a motivation (a behavioral drive engendered in ACC) that reflects an adverse condition in the body requiring a behavioral response. It is subserved by phylogenetically novel ascending pathways to the thalamus (to VMpo and MDvc) that are not present or, at best, are primordial in subprimates. It is associated in particular with progressive re-representations of the interoceptive afferent inflow in the anterior insula that are present only in humanoid primates. These functional anatomical findings have several implications.

First, these observations imply that subprimates cannot experience feelings of pain in the same manner as humans, because they do not have the same neuroanatomical substrates. Assumptions that animals can feel pain without the direct interoceptive sensory representation in dpIns and the progressive re-representations in the anterior insula that humans use are simply examples of anthropomorphism. On the contrary, survival behaviors elicited by homeostatically challenging stimuli (including thermal and noxious stimuli) are naturally emitted by invertebrates as well as vertebrates.

Second, these observations imply that the neural separation of skeletal and smooth muscle afferent

control systems, which is embedded in the development of the spinal cord, is evolutionarily ancient. The archetypical mammalian homeostatic afferent spinothalamic projections seem to reflect this basic functional division, with a pathway to the medial thalamus for limbic integration and a pathway to the lateral thalamus for viscerosomatic sensorimotor integration. The appearance of the high-resolution interoceptive representation in primate limbic sensory cortex, distinct from sensorimotor cortices, conforms with this fundamental functional distinction as well.

Third, these observations imply that the primate innovation of direct ascending homeostatic afferent input to limbic sensory and limbic motor cortices provided a profound evolutionary advantage. This is consistent with the view that encephalization in primates enabled more refined telencephalic control of hierarchical processing and enhanced postprocessing in all neural systems. The encephalized representation of the condition of the body in primates enabled modality-selective and topographically organized descending control of homeostatic integration in the brainstem, hypothalamus, and ventral striatum. This certainly had profound effects on adaptability. For instance, it seems reasonable to infer that the incredibly complex homeostatic adjustments (i.e., thermoregulatory, vestibuloautonomic, cardiorespiratory, etc.) that were necessary for the development of upright, bipedal locomotion must have required refined control capability. It is also noteworthy that the facial (branchiomeric) expression of emotion, an essential feature of mammalian reproductive and social interaction, became graded in primates (De Waal, 2003), and this evolutionary advancement in conspecific communication must have been greatly enhanced by the high-resolution homeostatic afferent representation in primates.

A clear example of the survival value of the direct, high-resolution interoceptive sensory representation in primates is provided by the owl monkey (Craig *et al.*, 1999). This New World primate is the only nocturnal monkey known, and accordingly it has several beneficial evolutionary adaptations. For example, its visual system is highly adapted for movement detection in the dark over a broad field of view, appropriate for its appetite for moths and other insects that fly at night. The owl monkey is also highly osmotic and has a greatly enhanced ability to use olfactory cues for orientation and food localization. In particular, these animals use scent markings for navigation and trail marking in the rain forest at night, when the humid air is cold and still – a significant aid to its nocturnal foraging. Notably, the cold night air also provides the appropriate condition for enhanced thermal discrimination of warm scent markings, and owl monkeys have a specialized thermosensitivity focused at the nose that inherently provides a valuable accessory signal when sampling olfactory cues during nocturnal foraging (or perhaps when another animal is near in the dark). This capability is provided by a highly developed lamina I trigeminothalamic thermosensory pathway to dpIns that is not present in other monkeys. An enhanced discriminative thermosensory capability for detecting the warmth or freshness of a scent trail must have considerable survival value for a small nuclear family of owl monkeys trying to follow one another, in pitch darkness, along an arboreal foraging trail through the rain forest (where separation can mean fatal exposure to felid predators). One can easily infer that this pathway evolved as an adaptive adjunct to nocturnal olfactory behavior by virtue of the refined thermosensory capability provided by the direct interoceptive cortical substrate in this primate.

Finally, these observations imply that humanoid primates benefited from an enormous evolutionary advantage due to the alignment of the homeostatic afferent representation in the insular cortex with the asymmetric autonomic efferent representation that had been present there in all mammals. In other words, the lateralized re-representations of parasympathetic feelings (e.g., taste) in the left anterior insula and of sympathetic feelings (e.g., pain) in the right anterior insula aligned these homeostatic afferent systems with the parasympathetic cardiac drives in the left insula and sympathetic drives in the right insula that are apparent even in rodents, which probably emanate anatomically from the inherent asymmetry in peripheral cardiac innervation. I infer that this adaptation in humanoid primates enabled numerous qualitatively significant adaptive refinements in conspecific emotional communication, including subjective self-awareness across time, empathy, and music (Craig, 2004b, 2005). The concomitant evolutionary development of the ability to feel pain from psychic (emotional) as well as physical causes, of course, has its disadvantages as well.

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3.19 Visceral Afferents

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Glossary

nociceptor(s) — A sensory receptor for which the adequate stimulus is one that damages or threatens damage (to skin; Sherrington, 1906). The adequate stimuli for activation of visceral nociceptors include distension of hollow organs, ischemia, inflammation, and traction on the mesentery.

nocifensor (nocifensive behavior) — Lewis (1936; see Lewis, 1942; LaMotte, 1992 for discussion) termed as nocifensor a system of 'nerves' associated with local defense against injury. The term has since expanded to describe behaviors associated with protection against insult and injury. Nocifensive behaviors are more complex than simple withdrawal reflexes initiated by activation of a nociceptor(s). Nocifensive behaviors produced by visceral stimulation are also considered pseudaffective (Sherrington, 1906; pseudoaffective) because responses to visceral stimulation are organized supraspinally.

polymodal nociceptor — A nociceptor that responds to multiple modalities of stimulus energies (i.e., thermal, chemical, and mechanical). Most (and perhaps all) visceral nociceptors are polymodal in character.

3.19.1 Introduction

Responses to wide-ranging stimuli characterize living organisms as simple as a single cell, which reacts to environmental cues and is attracted by chemical signals (chemotaxis). The molecular mechanisms linking an environmental stimulus or chemical signal to a cellular response (e.g., movement) may be similar across a wide range of organisms. Ion channels, such as the degenerins or members of the TRP (transient receptor potential) channel family, exhibit a high degree of structural homology between invertebrates and vertebrates, revealing evolutionary conservation of successful strategies. These highly conserved mechanisms for stimulus transduction are found in many cells in addition to neurons, a specific group of which are the focus of this article. Complex organisms have developed specialized organs serving diverse functions from gas exchange to nutrient absorption and excretion of toxins or waste products. Despite differences in structure and function, all contribute to maintaining an internal milieu (homeostasis) that is essential for survival of the organism.

As the complexity of organisms increases, the nervous system plays a greater role in the integration of information and maintenance of homeostasis. For example, feedback circuits evolved for controlling and adjusting homeostatic functions, including the regulation of visceral function. The innervation of the viscera ensures rapid and targeted transfer of

information, including flexible processing of this information, and triggers responses in and by effector organs. Interestingly, overlapping nervous systems developed in the viscera, not only in organs such as the heart, but importantly in groups (systems) of organs such as the gastrointestinal tract. In the gastrointestinal tract, an intrinsic (enteric) nervous system contains all the elements necessary to regulate basic functions such as absorption, secretion, motility, and blood flow. The enteric nervous system is modulated by various levels of extrinsic nervous control, characterized by neurons located outside of the gut wall in prevertebral ganglia, the spinal cord or brainstem and higher centers within the central nervous system. Figure 1 schematically illustrates several characteristics of visceral innervation and interaction, employing the gastrointestinal tract as representative of the viscera. The characteristics and functions of the extrinsic visceral innervation (visceral afferents) are considered below.

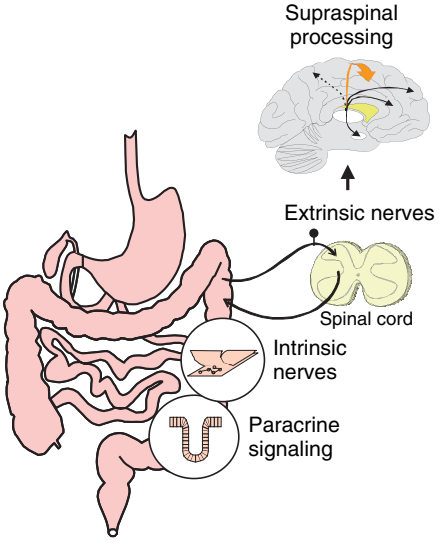

Figure 1 Diagrammatic illustration of visceral innervations and signaling, employing the gastrointestinal tract as the example. Extrinsic nerves convey information to the spinal cord (spinal afferents) and brainstem (vagal afferents, not shown; see Figure 2), which forwards visceral sensory information to supraspinal sites. As detailed in the text, most visceral input to supraspinal sites does not lead to conscious appreciation of visceral events, but is important to maintaining homeostasis by virtue of interacting with regulatory systems important to many visceral functions. The viscera are also innervated by an intrinsic nervous system, which interacts with nerve terminals of the extrinsic innervation in ways not fully understood and contribute to local events. Finally, the epithelium of all hollow viscera have the ability to secrete bioactive substances that can influence activity of either or both the intrinsic and extrinsic innervations of the organ, indicated here as a paracrine mechanism of signaling.

3.19.1.1 Activation-Sensation

When discussing the function of visceral afferents, it is essential to distinguish between activation of primary sensory neurons (or higher order visceroceptive sensory neurons) from activation of cortical structures. Cortical activation is necessary, but not sufficient for cognitive processes such as conscious perception of visceral stimulation. In humans and likely many other mammals, sensory input from skin, eyes, ears, or the olfactory system generally leads to activation of cortical structures and conscious perception of the event. Similarly, visceral input reliably activates central nervous system neurons, but only infrequently leads to awareness of the input in higher mammals. The most commonly perceived visceral events are either uncomfortable (e.g., bloating, over filling, gas, nausea) or painful, and visceral pain is relatively rare in health. By far, most visceral afferent activity conveyed to the central nervous system goes unnoticed. For example, the regularly beating heart and resulting pulsatile flow in arteries activates stretch receptors in cardiac and vessel walls, but despite this almost constant barrage of visceral afferent input, humans do not sense their heart as beating or register cyclical blood pressure changes. The exceptions confirm the rule; laypersons and physicians talk about palpitations as an unusual perception of the heartbeat.

The above emphasizes a unique aspect of the extrinsic (afferent) visceral innervation. The regulation of vital functions, such as respiration and blood flow, requires constant feedback from visceral afferent activity that is not consciously perceived. The afferent activity is critical to informing regulatory systems that are independent from intentional control mechanisms (hence the term 'autonomic nervous system'). As mentioned above, when visceral input is consciously perceived, it is often given special weight by the organism as a threat to vital functions, and therefore potentially to survival. However, there are notable and important exceptions. The proximal gastrointestinal tract is linked to digestion (and indigestion), and signals about its filling state are essential for the regulation of feeding behavior and the feeling of satiety. Conversely, the distal gastrointestinal tract and urinary bladder are important in storage and evacuation, which are linked to complex social functions (e.g., marking, appropriateness for defection) and thus require afferent input into higher brain centers.

Obviously, striking differences exist between the gills of fish and the lungs of mammals. Such differences multiply further if one includes invertebrates

in comparisons of organs serving similar vital functions (see Evolution of Visceral Control in Invertebrates). However, some similarities emerge when examining visceral structures responsible for gas exchange and their afferent innervation (Adriaensen *et al.*, 1998; Taylor *et al.*, 1999). It becomes more difficult to identify similarities or parallels between different species if one considers sensation as a feeling or perception, which requires some form of consciousness and relies on cortical structures. Using the musculocutaneous sensory system (commonly referred to as somatic, which is a misnomer given that viscera are part of the body) and the senses of vision, smell, and hearing as reference points, what are the unique aspects of visceral sensation? How do those unique aspects compare between different species? This article attempts to address these questions, primarily focusing on peripheral sensory mechanisms in the viscera. Because much of the research in neurobiology is driven by questions related to human disease, most studies have been performed in mammalian species, primarily mice and rats, thereby limiting our ability to compare detailed information about structure and function across the different branches of the phylogenetic tree.

3.19.2 Innervation of the Viscera

The innervation of the internal organs is uniquely associated anatomically with the autonomic nervous system (e.g., see Langley, 1921). Efferent fibers comprising sympathetic and parasympathetic divisions of the autonomic nervous system are contained in the same nerve bundles as are the afferent fibers that convey sensory information from the viscera to the central nervous system. Visceral afferents reach the central nervous system by two routes: cranial nerves directly enter the brainstem, primarily through the paired vagus nerves, the largest visceral sensory nerves in the body; a second group of afferents projects to thoracic, lumbar, and sacral dorsal root ganglia, conveying information to second-order neurons in corresponding segments of the spinal cord (Figure 2). Essentially all organs within the chest cavity and most of the gastrointestinal organs are innervated by both vagal and spinal afferents. Similarly, anatomically distinct nerves innervate the pelvic organs, conveyed in lumbar splanchnic (to thoracolumbar dorsal root ganglia) and pelvic (to lumbosacral dorsal root ganglia) nerves. Based on the anatomical organization and presence of efferent fibers in these nerves, an older nomenclature labeled these visceral afferents sympathetic ('afferent sympathetic fibers', Langley, 1921) and parasympathetic, respectively, analogous

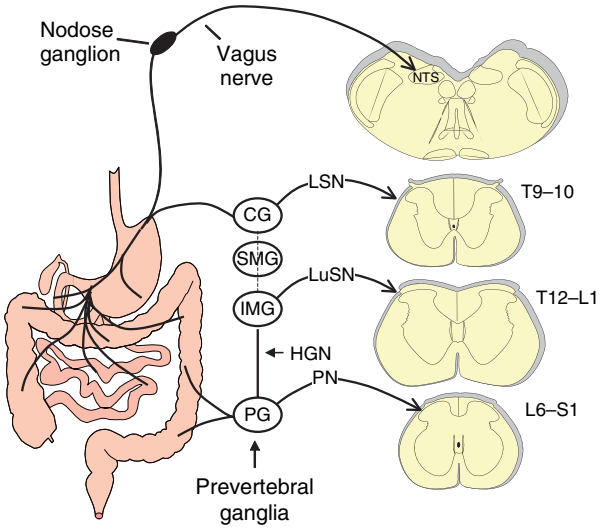

Figure 2 Diagrammatic representation of the extrinsic spinal and vagal innervation of the viscera. The diagram illustrates that each organ (e.g., distal colon, stomach, etc.) is innervated by two sets of nerves that terminate in different areas of the central nervous system. For example, the distal colon is innervated by the pelvic nerve (terminating principally in the sixth lumbar (L6) and first sacral (S1) segments of the rodent spinal cord) and hypogastric nerve (HGN)–lumbar splanchnic nerve (LuSN) (terminating in the 12th thoracic (T12) and L1 segments of the rodent spinal cord). Note that spinal visceral nerves pass through prevertebral ganglia, where they give off axon collaterals and interact with secretory and motor neurons in the ganglia, en route to the spinal cord. Note also the extensive innervation of the abdominal viscera by the vagus nerve, which terminates centrally in the nucleus of the solitary tract (NTS). The cell bodies of vagal afferents are contained in the nodose ganglia and those of spinal afferents in dorsal root ganglia (not shown).

to the dual efferent innervation of abdominal and thoracic viscera. Although Langley (1921) considered the presence of visceral afferents in autonomic nervous system nerves an important component of autonomic regulation, his and related terminology is confusing and the taxonomy has been largely abandoned.

There is no doubt that an important function of visceral afferents is to provide critical input to autonomic centers that modulate efferent outflow to the internal organs. Several lines of evidence suggest that these distinct pathways serve different functions. Interruption of spinal, but not vagal, afferents abolishes aversive responses to noxious mechanical stimulation of stomach, suggesting that spinal visceral afferents encode gastric nociceptive information (Ozaki *et al.*, 2002). This is consistent with the long-held notion that "visceral pain is transmitted to the spinal cord by afferent fibers traveling in sympathetic nerves" (Cervero and Foreman, 1990). However, growing evidence suggests that vagal afferents are also involved in

nociception, as they carry information about threatening conditions in heart and lungs, and may play a role in chemonociception within the gastrointestinal tract (Undem and Weinreich, 1993; Foreman, 1998; Schuligoi *et al.*, 1998; Benson and Sutherland, 2001; Lamb *et al.*, 2003; Kollarik and Undem, 2004). Taken together with the convergence of both pathways at the level of the brainstem and the significant overlap in functional characteristics, it is likely that vagal and spinal afferents both regulate visceral function in addition to their essential contributions to conscious perception of visceral events.

By far, most information about the function of visceral afferents has been derived from studies in mammalian vertebrates, and thus the information that follows is limited in an evolutionary context. Considerable attention, however, has been directed at the evolution and function (excitation, inhibition) of the autonomic nervous system (e.g., see Burnstock, 1969 for review), revealing that even though some features of the mammalian autonomic nervous system are represented in primitive vertebrates, there are striking variations in the function and autonomic innervation of organs in related species. Similarly, Walters (1994) has extensively studied and reviewed comparative and evolutionary features of nociceptor function, which although studied in leech, snail, and mollusk, in addition to vertebrates, addresses only surface (cutaneous) nociceptors. Given the importance of detecting injury and tissue damage from the viewpoint of survival, it is not surprising that there is considerable conservation of nociceptor function throughout animal evolution. A subset of visceral afferents are nociceptors, but stimuli adequate for activation of surface/cutaneous nociceptors (e.g., cutting, crushing, burning) are typically inadequate for activation of visceral nociceptors, which in mammalian vertebrates are activated most effectively by distension of hollow organs, ischemia, and traction on the mesentery (see Ness and Gebhart, 1990 for review). Although not the principal focus of the limited studies of which we are aware, work on frog (Tower, 1933) and toad (Niijima, 1960, 1961, 1967) support differences in adequate stimuli for surface nociceptors versus visceral nociceptors established in mammals. Thus, while extrapolation of information below to the general evolutionary trend may not be inappropriate, such extrapolation needs to be undertaken cautiously.

3.19.2.1 Sensory Endings

We have limited information about the structure of afferent nerve terminals within the viscera. The low innervation density of the viscera and the small size of these mostly unmyelinated fibers pose a significant challenge to direct examination. Much of our current understanding is derived from studies using tracer dyes, which stain both peripheral and central terminations after being transported distally and/or proximally from the injected site in the primary sensory ganglion. Current estimates suggest that less than 10% of neurons in spinal dorsal root ganglia project to a visceral target (McMahon, 1997). Even the most sophisticated tracing techniques will label only a fraction of these neurons, making direct studies of peripheral endings of spinal visceral afferents very difficult. While it has been generally assumed that most visceral afferents are unencapsulated, free nerve endings within their target tissues, recent reports have identified structural specializations on the terminals of some visceral afferent fibers that allow inference about their function. Current information remains largely restricted to vagal afferent nerve terminals in visceral organs, which have been described in some detail for the cardiovascular, respiratory, and gastrointestinal tract. However, a recent report suggests that pelvic afferents innervating the rectum form terminal specializations similar to those seen in some vagal afferent fibers projecting to the proximal gastrointestinal tract (see below).

In the gills or airways and in the gut, nerve endings can be found in close proximity to epithelial cells containing secretory granules at the basolateral site (Adriaensen *et al.*, 1998; Gershon, 1999; Taylor *et al.*, 1999; Brouns *et al.*, 2003). The close apposition between these specialized cells and nerve endings suggests that release of mediators from epithelial cells activates visceral afferent fiber terminals (i.e., paracrine signaling, Figure 1). Indeed, enteroendocrine cells isolated from the gut respond to appropriate stimuli and release mediators, such as serotonin or the neuropeptide cholecystokinin (CCK) (Kim *et al.*, 2001a, 2001b). Serotonin or CCK receptor antagonists block vagal responses to mucosal stimuli, such as flow of luminal contents, high luminal glucose or fat concentration, providing functional evidence for the indirect activation of neurons through epithelial chemical signaling (Davison and Clarke, 1988; Blackshaw and Grundy, 1990; Ladabaum *et al.*, 2001; Uneyama *et al.*, 2002; Li *et al.*, 2004).

Using anterograde tracers, more complex structural specializations of nerve endings have been described in the heart and the gastrointestinal tract. Within the tunica muscularis of the mammalian gut, some vagal afferents form two morphologically distinct endings in organ tissue: intraganglionic laminar

endings (IGLEs) and intramuscular arrays (IMAs) (Holst *et al.*, 1997). IGLEs are found in myenteric ganglia, a component of the enteric (or intrinsic) nervous system, at the interface between the circular and longitudinal muscle of hollow organs. Nerve fibers branch within these ganglia, surround one or many of the enteric neurons, and intercalate with the glial cells that form the scaffold of these ganglia. This structure prompted Phillips and Powley (2000) to speculate that IGLEs are activated by organ distensions, which passively distort (stretch) these structures. By combining physiologic studies with anterograde tracers, Zagorodnyuk *et al.* (2001) were able to show a close spatial relationship between the punctate receptive fields of mechanosensitive vagal afferent endings and IGLEs in the guinea pig stomach. Such a role in mechanosensation is further supported by experiments in genetically engineered mice lacking the gene for neurotrophin 4 (NT4). These knockout mice show a decrease in IGLE density within the proximal intestinal tract, and display an atypical eating behavior characterized by spurts of feedings, rapidly filling the stomach, interspersed with breaks (Fox *et al.*, 2001). These findings suggest that a satiety signal triggered by distension of the gastrointestinal tract is altered, possibly due to the change (reduction or absence) in peripheral mechanosensory elements. The close proximity between enteric neurons and vagal afferent fiber terminals certainly raises the question of whether and how the intrinsic and extrinsic afferent innervations of the gut interact. Inhibition of calcium-dependent transmitter release or the use of antagonists to neurotransmitters of enteric nervous system neurons, such as acetylcholine, did not affect vagal responses to mechanical stimulation, arguing against a sequential activation of enteric and – secondarily through synaptic transmission – vagal afferent fibers (Zagorodnyuk *et al.*, 2003).

Less information is available about IMAs, which are defined by branching fibers that run parallel to the smooth muscle cells within the circular or longitudinal muscle layers of the gastrointestinal tract (Wang and Powley, 2000). Interestingly, they are mostly found close to sphincteric regions, suggesting that they may provide important information about active tension generated by these muscles. While structure and distribution along the axis of the gut are consistent with IMAs functioning as tension receptors, no experimental evidence has directly shown the importance of IMAs in responses to muscle tension or stretch (Phillips and Powley, 2000).

Using anterograde tracers, several investigators have demonstrated that peripheral nerve terminals branch, at times forming hybrid endings in distinct areas, such as the myocardium and endocardium, or having endings with two distinct morphologies (Berthoud and Powley, 1992; Cheng *et al.*, 1997; Phillips and Powley, 2000). Consistent with such branching patterns, injection of different retrograde labels into distant areas of the same organ, such as the gastric fundus and pylorus, leads to double-labeling of a significant fraction of vagal and spinal neuron cell soma in nodose and dorsal root ganglia, respectively, suggesting that one neuron can innervate different areas of the same viscus (i.e., has multiple receptive endings or receptive fields in an organ; an example is given in Figure 3). That one visceral sensory neuron and its peripheral ending may have more than one receptive field is supported by nonanatomic, electrophysiological reports showing several distinct receptive fields, separated by as much as 3 cm, in about 10% of gastric or colorectal afferents (Berthoud *et al.*, 2001).

It is not unprecedented that a sensory neuron may have a widely branching terminal tree and multiple receptive fields in a tissue or organ. One defining characteristic of visceral pain is that it is referred to muscle and skin, and it has long been argued that a single visceral sensory neuron in the nodose or a dorsal root ganglion may give rise to axons that innervate different tissues (see Ness and Gebhart, 1990 for review). Although anatomical evidence in support of such dichotomizing afferents is limited and has been criticized methodologically in the past, several recent reports suggest that a single dorsal root ganglion neuron may give rise to axons that terminate in two different organs, such as urinary bladder and distal colon (Malykhina *et al.*, 2004).

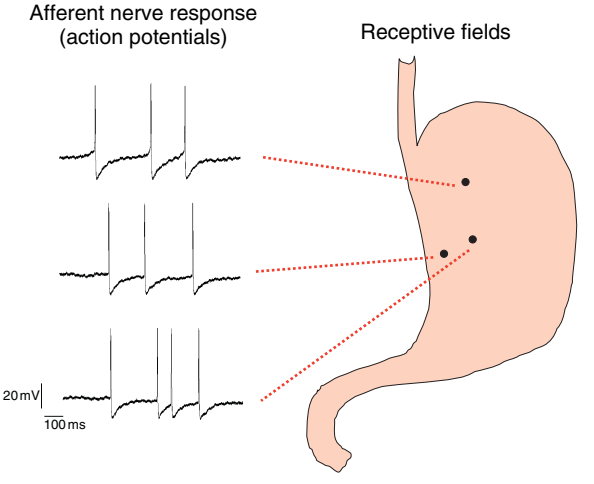

Figure 3 Example of a single visceral afferent with multiple receptive endings, or receptive fields, in an organ. In this example of a gastric vagal afferent, action potentials are shown to be evoked by probing at three different sites on the glandular portion of the rat stomach.

This observation raises the question of whether such promiscuous targeting of different organs is restricted to the pelvic organs and might be a phylogentic imprint of the cloaca. While physiologic studies have demonstrated the presence of two or more receptive fields within a single organ (Janig and Koltzenburg, 1991; Berthoud *et al.*, 2001), there is no complementary evidence showing that visceral afferents respond to stimulation of two anatomically distinct organs (Sengupta and Gebhart, 1994a, 1994b).

3.19.2.2 Sensory Axons

Electron microscopic and physiologic studies have demonstrated that virtually all visceral sensory neurons have unmyelinated or thinly myelinated axons (C and Aδ fibers), with a predominance of C fibers (Sengupta *et al.*, 1989, 1990; Berkley *et al.*, 1990; Undem and Weinreich, 1993; Sengupta and Gebhart, 1994a, 1994b; Navaratnam *et al.*, 1998). As metabolic demands increased in homeothermic animals, or visceral functions became linked to complex social function, more rapid sensory feedback became increasingly important. Myelination of neurons thus granted an advantage for survival. Changes in myelination of visceral axons also reflect phylogentic differences in vital organs, such as those for respiration, and their associated social function, such as vocalization. For example, the need for rapid adjustments of gas exchange during exercise requires monitoring of respiratory expansions of the lung. Consistent with these theoretical assumptions, most stretch receptors in the airways and lungs of mammals are associated with myelinated axons (Taylor *et al.*, 1999). Vocalization, in turn, relies on rapid changes in airflow, associated with movements of laryngeal, pharyngeal, and/or oral structures (Canning *et al.*, 2004). This evolution of afferent pathways with distinct conduction velocities fits with a phylogenetic tree described by Porges, who proposed that increasingly complex metabolic and social demands led to the evolution of more rapidly adjusting control mechanisms for autonomic function (Porges, 1998, 2003).

3.19.2.3 Central Terminations

As mentioned above, the central terminations of visceral afferent neurons project into the brainstem or spinal cord. Vagal sensory neurons send their central endings to the ipsilateral nucleus of the solitary tract and – for some gastrointestinal afferents – the area postrema. The terminals branch along the rostrocaudal axis of the brainstem and may even cross the midline, making connections with several second-order neurons (Altschuler *et al.*, 1989). Some visceral input from the cardiovascular system and upper respiratory tract, most notably the larynx, projects to the nucleus ambiguus, the main sensory ganglion of the glossopharyngeal nerve, allowing the integration of afferent information from pharynx and larynx, which is important for the regulation of deglutition and vocalization (Taylor *et al.*, 1999; Saper, 2002). Despite the tremendous differences in brain structures between fish and mammals, this basic organizational structure can be found in all vertebrates.

Within the mammalian spinal cord, most visceral sensory neurons project via the dorsal roots to superficial laminae I and II of the dorsal horn, with some extending to laminae V and X. Unlike spinal patterns of termination of cutaneous afferents, which are circumscribed in area and limited largely to a single spinal segment on the side ipsilateral to the input, the spinal terminations of visceral afferents spread rostrocaudally above and below the spinal segment of entry into the spinal cord and also cross to the contralateral spinal dorsal horn (Sugiura and Tonosaki, 1995). Visceral afferent terminals in the spinal dorsal horn are further distinguishable from cutaneous afferent terminals ultrastructurally in that they possess a greater number and density of terminal swellings, suggestive of a greater number of synapses and thus potentially greater and more diffuse input (Sugiura and Tonosaki, 1995).

In the sacral spinal cord, many visceral afferents terminate in the intermediolateral region and provide important input to the sacral parasympathetic nucleus, a component of the autonomic nervous system that sends preganglionic efferents out of the ventral roots of the spinal cord (Vizzard *et al.*, 2000). As already mentioned for visceral afferents projecting to the brainstem, spinal afferents that terminate within the lumbosacral cord form synapses with many second-order neurons over several segments within the rostrocaudal direction. This spread of central terminations may be important in responses requiring coordination of different organ functions. For example, micturition generally inhibits defecation in mammals (Pezzone *et al.*, 2005). However, the interactions with several central neurons also contribute to the poor ability to precisely locate and/or discriminate visceral stimuli.

3.19.3 Neurochemical Properties of Visceral Sensory Neurons

The expression of surface markers, structural proteins, ion channels, or transmitters has enabled us to

identify subgroups of sensory neurons, which may serve distinct functions. While helpful in the study of cutaneous sensory neurons, the utility of such surrogate markers appears to be limited in visceral sensory neurons. For example, the heat- and acid-sensitive ion channel TRPV1 (capsaicin receptor) is primarily expressed by small-diameter dorsal root ganglion neurons, which cannot be stained with neurofilament antibodies, a surrogate marker for myelination (Michael and Priestley, 1999; Zwick *et al.*, 2002; Yoshimura *et al.*, 2003). Based on physiological studies, most of these cells likely function as polymodal nociceptors (Perry and Lawson, 1998; Fang *et al.*, 2005a). However, this classification does not hold for visceral sensory neurons. Cough receptors in the extrapulmonary airways by definition respond to irritants (i.e., potentially noxious substances). However, many of these neurons are capsaicin-insensitive (Carr *et al.*, 2003; Kollarik and Undem, 2002, 2004; Canning *et al.*, 2004; Undem *et al.*, 2004). Conversely, nearly all colon sensory neurons show immunoreactivity for TRPV1 or respond to capsaicin (Robinson *et al.*, 2004; Sugiura *et al.*, 2004). Finally, while virtually all gastrointestinal sensory neuron axons in mice conduct in the C fiber range, about 30% of nodose neuron axons exhibit neurofilament immunoreactivity commonly associated with myelination (Ruan *et al.*, 2004). These findings highlight our still limited understanding of visceral sensory innervation.

It is generally held that groups of neurons with distinct functions and, therefore, also distinct anatomical and neurochemical markers likely exist. This assumption is supported by two neurochemical markers, which do provide some important information. The presence of immunoreactivity for substance P or calcitonin gene-related peptide (CGRP) is restricted to a small number of vagal sensory neurons, most of which innervate the upper airways and have their cell bodies in the proximal (jugular) portion of the ganglion (Altschuler *et al.*, 1989; Ruan *et al.*, 2004; Undem *et al.*, 2004; Thai Dinh *et al.*, 2005). Both neuropeptides are also found in greater numbers of spinal visceral sensory neurons (Vizzard, 2001; Robinson *et al.*, 2004). Their afferent terminals in organs may also have an effector function in their target area, as these neurons can release substance P or CGRP from both central and peripheral terminals (Fischer *et al.*, 1996; Castagliuolo *et al.*, 1997; Grady *et al.*, 2000). Significant activation of the nerve terminal or invasion of action potentials past branch points with propagation into other terminals (axon reflex) may trigger such peripheral release of substance P or CGRP. Both neuropeptides affect smooth muscle function, attract and activate immune cells, most notably mast cells, thereby contributing significantly to the development of neurogenic inflammation (Heiman and Newton, 1995; Riegler *et al.*, 1999; Theodorou *et al.*, 1996; Renzi *et al.*, 2000; Willis, 2001).

3.19.4 Functional Properties of Visceral Afferents

The preceding section emphasizes that visceral sensory neurons within a single species do not constitute a homogeneous population, making it difficult to discuss the function of these neurons across different species. In vertebrates, most viscera receive a dual sensory innervation, with vagal and spinal afferents having distinct functional properties, further confounding this picture. Despite these limitations, many recent studies have shed significant light on the functional properties of visceral sensory neurons.

3.19.4.1 Molecular Mechanisms of Signal Transduction

Visceral sensory neurons in dorsal root ganglia express many of the known ion channels involved in the transduction of mechanical, thermal, and chemical stimuli. Different members of the TRP channel family provide a molecular basis for thermosensation (McCleskey and Gold, 1999; Zhang *et al.*, 2004). This includes the capsaicin receptor TRPV1, which can be found in about half of all nodose ganglion neurons and most spinal dorsal root ganglion neurons innervating the colon (Michael and Priestley, 1999; Robinson *et al.*, 2004; Sugiura *et al.*, 2004, 2005). The functional importance of these results is supported by single-fiber recording studies showing that gastrointestinal afferent endings respond to heat and/or cold stimuli (El Ouazzani and Mei, 1982; Kang *et al.*, 2004). TRPV1 is expressed widely, particularly in sensory neurons, where it is considered by some to designate a neuron as a polymodal nociceptor (McCleskey and Gold, 1999). Although TRPV1 has been extensively studied, knowledge about its role in visceral sensation and nociception is limited. Significantly, TRPV1 is expressed in the bladder, including the urothelium, and bladder function is significantly altered in TRPV1 knockout mice, leading to the suggestion that TRPV1 is important in detecting bladder stretch (Birder *et al.*, 2001, 2002; Dang *et al.*, 2005a).

Vagal and spinal visceral sensory neurons also express acid-sensitive ion channels (ASIC), which are related to the degenerin/ENaC super family of voltage-insensitive, amiloride-sensitive Na^+ channels, a family of mechanosensitive ion channels

identified in *Caenorhabditis elegans*. ASICs are encoded by four different genes and ASICs 1–3 are expressed, typically as heteromers, in sensory neurons (Kellenberger and Schild, 2002). The name ASIC was chosen based on proton sensitivity of these ion channels. However, these channels are also activated by stretch. Consistent with a potential contribution of these channels in visceral mechanosensation, experiments in ASIC3 knockout mice revealed differences in afferent fiber activation during visceral distension (Jones *et al.*, 2005). However, a complex picture is emerging with respect to the role(s) of ASICs in mechanosensation. Some knockouts (ASIC1) exhibit increased mechanosensitivity rather than decreased sensitivity to visceral distension, which has been attributed to different contributions of various subunits forming the multimeric ion channel pore (Page *et al.*, 2004).

As described above, mechanical or chemical stimuli can also trigger the release of various mediators from specialized epithelial cells, which in turn then interact with ionotropic and/or metabotropic receptors on the peripheral terminals of visceral afferents (Figure 4). A different interaction between epithelial cells (urothelium) and the urinary bladder has been demonstrated in mammals, where no specialized paracrine cells have been identified within the epithelium. Despite the absence of cells with special endocrine activity, bladder distension triggers ATP release from urothelial cells, which activates purinergic receptors (P2X receptors) on bladder afferent terminals, potentially triggering micturition and/or sensation (Cockayne *et al.*, 2000, 2005; Vlaskovska *et al.*, 2001). Seven P2X receptors have been cloned ($P2X_{1–7}$), all of which are permeable to monovalent cations and several of which, either as homo- or hetero-oligomers, have been implicated in visceral function and sensation (Burnstock, 2001). $P2X_2$, $P2X_3$, and $P2X_{2,3}$ receptors are expressed in small- to medium-diameter sensory neurons (Petruska *et al.*, 2000) and in the micturition reflex pathway (Zhong *et al.*, 2003). Mice with engineered deletions of $P2X_3$ receptors exhibit bladder hyporeflexia, thereby demonstrating the physiologic importance of urothelial ATP release in bladder function (Vlaskovska *et al.*, 2001). It is unclear whether epithelial ATP release similarly affects gastrointestinal or respiratory function. Vagal and spinal neurons innervating the gut also express P2X receptors and respond to purinergic receptor agonists (Robinson *et al.*, 2004; Dang *et al.*, 2005b). However, P2X receptor antagonists do not alter responses of vagal afferents to esophageal stretch (Zagorodnyuk *et al.*, 2003). Considering the distinct microscopic structure of the esophageal epithelium (squamous epithelium), it remains to be seen if mechanical or chemical stimuli trigger epithelial ATP release in other areas of the gastrointestinal tract or in the airways.

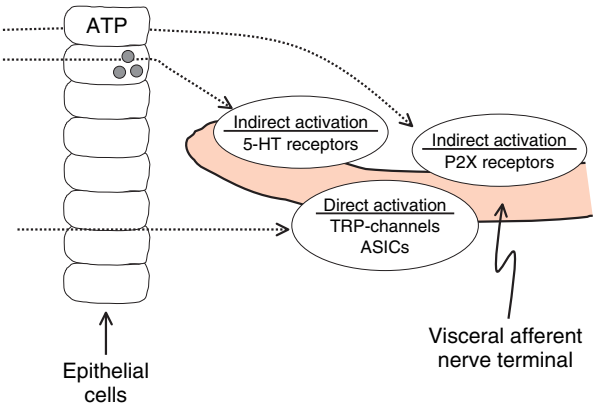

Figure 4 Diagrammatic illustration of direct and paracrine-like signaling in the viscera. Illustrated is a visceral afferent nerve terminal that is directly sensitive and activated by protons (e.g., ASIC and/or TRP channels) or thermal stimulation (TRP channels) applied to the organ. Other stimuli release bioactive substances (e.g., serotonin, 5-HT, or ATP) from epithelial cells which in turn activate visceral afferent nerves, an indirect action of a visceral stimulus.

3.19.4.2 Functional Characterization of Visceral Afferents

The function of visceral afferents has been studied extensively *in vivo* and *in vitro*, however almost exclusively in mammals. Most of the detailed information is derived from single-fiber recordings. The distal end of the cut nerve is split into small filaments, which are placed on a recording electrode to monitor action potentials in response to stimulation of a target organ (e.g., stomach). As most fibers in a given nerve filament will not innervate this target organ, recordings generally reflect the activation of a single nerve fiber. This approach requires a search stimulus to determine whether a fiber bundle contains processes projecting to the organ of interest. The choice of search stimulus, however, obviously biases results in favor of sensitivity to the search stimulus. Accordingly, claims of response specificity must be evaluated carefully.

Using the single-fiber recording method, early investigators functionally characterized mucosal, muscle, serosal, and mesenteric receptors based on responses to chemical and principally mechanical stimuli. Unlike the structural elements discussed previously (IGLEs and IMAs), the designations 'receptor' and further specifications, such as 'mucosal' or 'serosal', were based solely on

functional characterization in the absence of anatomical evidence.

Mucosal receptors were identified on the basis of rapid adaptation to mechanical stimulation and apparent selective chemosensitivity, both of which were absent after the mucosa was carefully scraped away, confirming a location in the mucosa (e.g., Iggo, 1957; Paintal, 1957; see Sengupta and Gebhart, 1994c for review). Whereas nutrient chemosensitivity of mucosal receptors was an early focus of study (e.g., glucose, lipid, and amino acid sensitivity), most recent studies used mechanical stimuli to functionally characterize muscle, serosal, and mesenteric receptors. Mechanical stimuli, such as blunt probing, tissue compression, traction on the mesentery, or hollow organ distension, have the experimental advantage that they can be easily and repeatedly applied. Single-fiber recordings from different species generally show punctate or narrowly circumscribed receptive fields for most mechanosensitive fibers. However, some visceral neurons may have two or more receptive fields, which can be located in anatomically distinct areas of an organ, such as the fundus and distal corpus of the stomach in mice (see Figure 3) (Berthoud *et al.*, 2001).

Most investigations have studied afferent fiber responses to distension, corresponding to different filling states and/or intraluminal pressures. That distension-sensitive visceral afferent fiber endings are located in organ muscle has been demonstrated by removal of mucosal, submucosal, and serous membranes of organs without compromising fiber responses to distension (see Sengupta and Gebhart, 1994c). All hollow organs contain muscular elements, and thus two distinct mechanisms are conceptually possible: stretch and tension (Phillips and Powley, 2000). Using the orientation of muscle cells as reference, stretch receptors are in parallel with muscle cells and will be activated by organ distension, such as propelled chyme or – under experimental conditions – inflation of a balloon. Contraction of the muscle running in parallel should cause a shortening in length and thus decrease firing of these receptors. In contrast, tension receptors are located in series with muscle cells. Therefore, muscle contractions will activate tension receptors even in the absence of changes in length. This model is borrowed from analogous structures involved in the regulation of skeletal muscle function (muscle spindle and Golgi tendon apparatus).

Sustained hollow organ distension typically triggers a slowly adapting response of visceral afferents. The response profiles of mechanosensitive visceral afferents to balloon distension have not yet convincingly demonstrated the existence of distinct receptors that distinguish between stretch or tension in hollow viscera. The best, but still controversial experimental evidence relates to mechanoreceptors in the atria (Taylor *et al.*, 1999). The type B receptor is activated by filling during the first part of diastole, associated with small pressure changes (V-wave of the pressure profile); the type A receptor is activated in response to atrial contraction at the end of diastole (A-wave of the pressure profile), associated with a second and higher pressure increase (Paintal, 1973). Most fibers identified based on their stretch sensitivity (i.e., volume change) also respond to muscle contraction (tension). This may in part be due to technical limitations, such as difficulty in achieving truly isometric (only tension changes) or isotonic (only stretch changes) conditions in viscera. In addition, the deformation associated with filling of a hollow organ may generate passive tension, which further blurs the distinction between stretch and tension (Lynn *et al.*, 2005; Zagorodnyuk *et al.*, 2005).

As already indicated, most investigators use graded distension of hollow organs with balloons or fluid when studying mechanosensitive visceral afferent fibers. Despite some variation between different organs and species, the results generally reveal that mechanosensitive visceral afferents dynamically encode stimulus intensity over a wide range. Under normal conditions, luminal pressures remain well below 20 mmHg in the urinary bladder and gastrointestinal tract. However, localized pressures can briefly exceed 100 mmHg during muscle contractions. Most healthy human volunteers perceive luminal pressures exceeding 30 mmHg as uncomfortable or painful (Corsetti *et al.*, 2004; Mayer, 1994). Consistent with these findings, distension pressures of 30 mmHg and higher trigger aversive, pseudaffective, and/or autonomic responses in experimental animals (Ness *et al.*, 1991; Ozaki *et al.*, 2002; Ness and Elhefni, 2004). Interestingly, a relatively small proportion of mechanosensitive fibers (~20–25%) require distending pressures of at least 25 mmHg for activation (Sengupta *et al.*, 1989, 1990; Sengupta and Gebhart, 1994a; Ozaki *et al.*, 1999; Ozaki and Gebhart, 2001). Such high-threshold fibers have only been found in spinal, and not vagal afferents, corresponding to the widely held importance of spinal sensory pathways in nocifensive reactions to noxious stimuli, discussed above (Cervero and Foreman, 1990; Cervero, 1994; Cervero and Laird, 1999). This prompted speculations that high-threshold mechanoreceptive fibers function as nociceptors in the viscera (specificity theory). Opponents of this interpretation point to the fact that low-threshold

mechanosensitive fibers also encode stimulus intensity well into the noxious range and thus contribute to nociception (intensity theory). Using the human percept as an indirect means to resolve this controversy, Tack *et al.* (2004) demonstrated that changes in descriptors for nonpainful sensations change over a wide range of stimulus intensities, described as a stimulus-response function, which shifts in parallel with that of painful sensations when visceral sensation is altered using pharmacological interventions. These findings support a model with high- and low-threshold visceral mechanosensitive pathways, both contributing to pain and the defensive responses triggered by the organism.

In addition to response threshold, temporal changes in response to a persistent stimulus are an important physiologic property, potentially allowing identification of distinct subgroups. Afferent fiber responses to organ distension or stretch generally show slow adaptation. Differences in time course and degree of adaptation have led, at times, to complex classification schemes, although the impact of these different firing patterns on visceral sensation or regulation of visceral function remains undefined. The very rapidly adapting stretch receptors described in stomach and colon may be an exception, as these afferents are activated by light mucosal stroking, suggesting that they have receptive endings in the mucosa and sense luminal flow (Page and Blackshaw, 1998; Lynn and Blackshaw, 1999; Page *et al.*, 2002). A similar response profile has also been described for irritant receptors of the airways (Paintal, 1973), which can also be activated by chemicals and are involved in triggering the cough reflex.

3.19.4.3 Functional Characterization of Chemosensitive Visceral Afferents

As mentioned previously, relatively little information exists about chemosensitive visceral afferents. A small number of afferents innervating the small bowel can be activated by luminal perfusion with different nutrients (Jeanningros, 1982; Mei, 1985). It is likely that most nutrients activate specialized epithelial cells, triggering the release of CCK and/or other mediators, which, in turn, depolarize afferent fiber terminals. Consistent with this interpretation, enteroendocrine cells of the small intestine express bitter receptors that have been identified in taste buds of the tongue. Exposure to cycloheximide increases the intracellular calcium concentration in these enteroendocrine cells, thereby initiating a cascade of events that ultimately leads to mediator release (Wu *et al.*, 2002). The activation of afferent neurons through such paracrine cells provides the body with dual and overlapping feedback regulatory mechanisms. The local release may affect neighboring structures and regulate or modulate their function, such as increasing blood flow or altering secretion or motility. The parallel activation of neuronal circuits, intrinsic and extrinsic, may further optimize digestion by rapidly and effectively modifying organ function locally and/or by affecting distant organs, such as activating pancreatic secretion or slowing the further delivery of luminal contents by inhibiting gastric motility. At the same time, extrinsic afferent fiber activity may alter the organism's behavior, decreasing food intake and/or even more complex actions required for procurement of food, such as foraging or hunting (Berthoud, 2004). At first glance, it seems counterintuitive that visceral, specifically vagal afferent input, which presumably goes largely unnoticed, may have such a profound effect. However, circumstantial evidence derived from clinical data and some experimental results support this interpretation. As already described above, a decrease in mechanosensory elements (IGLEs) within the gastrointestinal tract of NT4 knockout mice changes patterns of food intake (Fox *et al.*, 2001). Initial results suggest that electrical stimulation of the stomach nonselectively activates gastric vagal afferents, decreases food intake, and shows positive effects in the treatment of obesity (Xu *et al.*, 2005). Direct vagal nerve stimulation reportedly improves depression and is beneficial in the treatment of refractory epilepsy, presumably by inhibiting areas in the medial areas in the forebrain (see below) (Zagon, 2001).

While it is not known whether such specialized enteroendocrine cells can be found in close proximity to visceral sensory nerve terminals within the gut of species as different as fish and mammals, neuroepithelial signaling is an evolutionarily old signal. Glomus cells, which sense oxygenation and signal through the ninth and/or tenth cranial nerve, affect respiratory effort in organisms from elastomeres to humans (Taylor *et al.*, 1999). Despite this parallel, significant differences in sensory feedback that regulates gas exchange emerge, when comparing lung breathers with fish or tadpoles, which rely on gills for this function. With the development of lungs, the main signal driving respiration is hypercapnia and the associated drop in pH, sensed by central chemoreceptors located mainly on the floor of the fourth ventricle in the brainstem (Paintal, 1973). Amphibians apparently recapitulate this switch during metamorphosis. In early larval stages, acidification does not alter the rhythmic discharge of cranial nerves involved in respiratory efforts. With the onset of lung breathing, a slight

decrease in pH significantly enhances neuron bursting patterns.

Not all chemical stimuli activate visceral afferents indirectly through specialized epithelial or other non-neuronal cells. As discussed earlier, neurons express proton-sensitive ion channels (ASIC and TRPV1). Acid exposure can directly activate cardiac, gastrointestinal, bladder, and pulmonary neurons *in vitro* and *in vivo* (see Figure 4) (Benson *et al.*, 1999; Kollarik and Undem, 2002; Kang *et al.*, 2004; Sugiura *et al.*, 2004, 2005). Studies of cardiac afferent fibers suggest that such acid-sensitive receptors sense ischemia, as oxygen-deprived tissue relies on anaerobic metabolism, which generates lactate and rapidly decreases tissue pH (Benson and Sutherland, 2001). Similarly, irritant or cough receptors within the respiratory tract are likely activated by direct interaction between some irritants and the nerve terminal (Moore *et al.*, 2000; Kollarik and Undem, 2002; Canning *et al.*, 2004; Undem *et al.*, 2004). Ischemia-sensitive fibers and irritant receptors both trigger defensive responses to ensure the survival of the organism.

3.19.4.4 Polymodal Character of Visceral Sensory Neurons

Under normal conditions, sensory neurons respond to specific stimulus modalities, thereby allowing meaningful decoding of messages by higher-order sensory neurons in the central nervous system. Such modality specificity has also been described for afferents innervating different viscera. However, many visceral afferents are polymodal and respond to two or even three distinct stimulus modalities (Blackshaw and Grundy, 1989; Lynn and Blackshaw, 1999; Ozaki *et al.*, 1999; Ozaki and Gebhart, 2001; Berthoud *et al.*, 2001; Page *et al.*, 2002). Most chemosensitive visceral mucosal afferents and the majority of mechanosensitive visceral afferents conduct in the C-fiber range. Based on conduction velocity and lack of modality specificity, most visceral afferents resemble polymodal nociceptors that innervate the skin (Fang *et al.*, 2005b). However, it is unlikely that all or even most polymodal visceral afferents function as nociceptors in the viscera, thus leaving uncertain the physiological relevance of their polymodal character. Because the density of visceral innervation is low, and the rostrocaudal spread of visceral input within the spinal cord and brainstem is great, the polymodal character of visceral sensory neurons may further contribute to the poor ability of humans to discriminate and localize stimuli within the gut lumen. We also know that thermal or chemical stimuli can acutely alter excitability of mechanosensitive gastric vagal afferents (Kang *et al.*, 2004). Accordingly, sensitivity of visceral afferent ending to other modalities of stimuli may alternatively represent local modulatory influences on sensory feedback that can regulate food intake or satiety.

3.19.4.5 Sensitization

Sensitization is an increase in response magnitude of a sensory afferent fiber to an applied stimulus, frequently associated with a decrease in response threshold. Although initially introduced to describe the increase in excitability of cutaneous nociceptors following tissue injury (see Perl, 1996 for review), sensitization is now recognized as a representation of plasticity of neural function, and a property that defines most mechanosensitive visceral afferent fibers. Studies investigating sensitization of visceral afferents typically induce acute or chronic inflammation in hollow organs. These experimental forms of gastritis, colitis, or cystitis are associated with behavioral changes, such as increased micturition frequency or nocifensive reactions, as well as increased responses of afferent fibers innervating the organ. Detailed physiological studies show that both low- and high-threshold mechanosensitive visceral afferents sensitize, suggesting that both can contribute to visceral pain (because sensitization is a property unique to nociceptors in skin; see above). However, sensitization of visceral afferents has only been studied for mechanical stimuli. Considering the polymodal character of most visceral afferents, it is highly likely that responses to other stimuli are similarly sensitized, which may have implications beyond the development of visceral discomfort and/or pain. Accordingly, functions such as secretion and motility may be enhanced and play an important role in infections or parasitic infestations, where more rapid clearance of luminal contents may increase the chance for recovery and survival (e.g., intestinal infection).

3.19.5 Central Projections of Visceral Afferents

In vertebrates, the cranial (or oral) end of the organism is also the business end for key vital functions: gas exchange (gills and lungs), food intake, and – in fish – also electrolyte and fluid exchange (gills). Sensory input relevant for regulation of these functions is processed in the brainstem, where input from the vagus and

glossopharyngeal nerves to the nucleus of the solitary tract and the nucleus ambiguus is integrated with afferent information from other cranial nerves, triggering appropriate adjustments in visceral function and/or behavior (Paintal, 1973; Taylor *et al.*, 1999). Vagovagal reflexes execute relatively simple responses, such as gastric accommodation (Zhang *et al.*, 1998; Chang *et al.*, 2003; Travagli *et al.*, 2003). More complex responses rely on central pattern generators in the brainstem, which can also be activated by corticobulbar projections. The swallowing reflex is a good example of such a patterned response; it is triggered by pharyngeal stimulation, but can certainly also be initiated volitionally without such a peripheral stimulus (Goyal *et al.*, 2001).

Transneuronal tracing studies using viral vectors reveal projections of visceral afferent neurons to many structures in the brainstem, midbrain, diencephalon, and forebrain, including the rostral ventrolateral medulla, reticular formation, locus coeruleus, mesencephalic tegmentum, hypothalamus, thalamus, and amygdala (illustrated in Figure 1) (Marson, 1997; Valentino *et al.*, 2000; Vizzard *et al.*, 2000; Cano *et al.*, 2001; Rinaman and Schwartz, 2004). Many of these structures play a role in the regulation of autonomic function or are important relay stations for sensory input. Interestingly, several of these areas are involved in arousal and alertness. Vagal stimulation can trigger lasting inhibition of locus coeruleus neurons, a potentially important adaptive response that promotes rest and decreases energy expenditure when food intake reaches satiety levels (Zagon, 2001).

With the development and increasing size of forebrain structures, the processing of information that relates to interaction between the organism and its surroundings (external environment) – touch, sound, light – occurs primarily in the enlarging cortical mantle structures. Afferents providing information about volitionally controlled aspects of visceral function such as deglutition, defecation, or vocalization, similarly project to these mantle structures. However, most visceral input (interoception) activates deeper brain structures closer to the midline, including regions often referred to as the limbic system (evolved from the entorhinal cortex), which processes olfactory signals and plays an important role in emotional responses (Vogt, 2005). The integration of visceral input with information about smell and taste and centers controlling emotion has several potential implications. A bitter taste and/or foul smell trigger disgust, an aversive reaction, which will stop the organism from ingesting possibly poisonous or spoiled material. The opposite will be true for tastes or smells that are perceived as pleasant, which will promote food intake (Berthoud, 2004). Another implication relates to consciously perceived visceral input. Psychophysical research has demonstrated that visceral stimuli are generally more strongly linked to affective dimensions than is cutaneous sensory input (Strigo *et al.*, 2002, 2003). Considering that affect does not simply define states, such as happiness, but constitutes a strong drive (emotive function of sensory input or pain), the more significant emotional impact of visceral stimuli may represent a protective mechanism for vital functions, increasing the likelihood of survival.

3.19.6 Conclusion

The study of visceral sensation across a wide range of species reveals specializations of structure and/or function that reflect gradual adaptation of sensory mechanisms during the evolution from aquatic to terrestrial animals, from poikilothermic to homeothermic organisms. The findings described in this article suggest a gradual development that increases speed and precision of information transfer, enabling more rapid and targeted regulation of visceral function and/or animal behavior. The associated decrease in metabolic costs, and more effective adjustments to changing environmental conditions, likely enhanced survival chances for the organism (see Table 1).

Table 1 Characteristics of visceral afferent (extrinsic) innervation

Anatomically associated with the autonomic nervous system
Unmyelinated (C) and thinly myelinated (Aδ) axons
Myelination of visceral afferents is largely restricted to areas requiring rapid sensory feedback and visceral functions that are part of social interactions
In mammals, organs are innervated by two sets of nerves, each with different function(s)
Medium-sized cell soma in vagal nodose or spinal dorsal root ganglia
Less than 10% of spinal afferent inflow arises from viscera
Intraspinal arborization of spinal afferent terminals is extensive
Most, if not all, are polymodal (thermo-, chemo-, and/or mechanosensitive)
Endings in all different layers of organs with some structural (and likely functional) specialization
Neuroepithelial signaling plays an important role in chemosensation and contributes, at least in the mammalian bladder and intestine, to mechanosensation
They sensitize (an increase in response magnitude, i.e., excitability)

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3.20 Evolution of Sound Localization in Mammals

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Glossary

binaural	With both ears.
contralateral	On the opposite side.
exteroceptive	Perception of external objects.
fossorial	Adapted to digging and underground life.
inferior colliculus	Midbrain nucleus of the ascending primary auditory pathway.
interaural	Between both ears.
isocortex	Top layer of the cerebral hemispheres in mammals (= neocortex).
lemniscal pathway	Pathway that involves the lateral lemniscus in the brainstem.
marsupials	Mammals in which the female typically has a pouch (marsupium), in which it rears its young through early infancy.
mesencephalic	Located in the midbrain.
monaural	With one ear.
monotremes	Egg-laying mammals.
Placentalia	Mammals that rear young through early infancy with a placenta.
sauropsids	Birds and reptiles.
taxon (plural taxa)	Taxonomic unit, a grouping of organisms.

3.20.1 Introduction

The ability to localize sound sources in a complex environment is of paramount importance for orientation in a biological habitat, and intraspecies as well as interspecies communication. The accurate localization of an approaching predator or competitor can be a matter of life or death. This topical essay discusses the major features of the evolution of sound localization in mammals in terms of the physical cues for sound localization, the major brain structures involved, and behavioral measures of localization acuity across taxa. Sound localization in mammals builds upon similar systems shared by lower vertebrates that utilize bilateral hearing, and adds unique abilities arising from the development of the external ear and from the development of the isocortex (neocortex). The evolution of sound localization in mammals is not characterized by a straightforward sequence of changes in the underlying brain structures concomitant with an improvement in ability but rather is more related to the ecological niche of each species. Different habitats and different behavioral strategies for survival require different solutions for sound localization. Many species from a variety of taxa have developed a remarkable acuity for localizing sounds, which comes close to or matches human localization acuity. However, while acuity may be similar, for example, across echolocating bats (microchiroptera), homologous brain structures involved in sound localization can be anatomically or functionally different.

3.20.2 Cues for Sound Localization

The cues for sound localization comprise interaural cues, which are based on the information derived from both ears, and monaural cues, which are caused by the filtering of each external ear, the pinna (for overview see Blauert, 1997). The interaural cues are used for lateralization, that is, the left/right discrimination of sound. The monaural cues give additional information on the elevation of a source and allow front/back discrimination. All vertebrates that have bilateral hearing use binaural cues to some extent (e.g., frogs, reptiles, and birds). For low-frequency sounds from the side, the time of arrival of the sound waves differs between the two ears. This is the interaural time difference (ITD). If the sound source direction is located along the mid-line axis, for example, front, rear, or directly above, the ITD would have the value zero, because the sound wave reaches both

ears at the same time. For lateral incidence angles, the sound travels around the head and the body of the animal on various paths to reach the far ear, that is, the ear opposed to the sound event. The physiologically relevant ITD range depends on head size, head geometry, and pinna shape. A common measure to estimate the physiological range of ITDs in a species has been to simply measure the distance between the ear canal openings. However, depending on the angle of incidence and the shape or body position of an animal, sound has to travel on complicated paths around the animal. The ITDs in the fine structure of a sound can be detected up to ~2.5 kHz in many mammalian species. For both low and high frequencies, the ITDs in the envelope of a sound, for example, the onset slope or amplitude modulations, can also be utilized (Joris, 2003). For high-frequency sounds the head shadow attenuates the sounds at the far ear. This interaural cue is called the interaural intensity difference (IID). The IID for low frequencies is relatively small (a few dB), but for high frequencies it can be as large as 20–30 dB for certain directions, depending on the head size.

In contrast to amphibians, reptiles, and birds, most nonaquatic and nonsubterranean mammals possess a pinna of varying complexity in shape and mobility. The resulting pinna cue is what distinguishes the localization ability of the mammals. The angle of sound incidence upon the ridges and folds of the pinna causes certain frequencies to be amplified while others are attenuated. Therefore, the pinna cues provide information about the location of sound in space. Many mammals can move the pinna. Others, for example some primates, cannot. It is of paramount importance to emphasize that ITD, IID, and the monaural spectrum always vary together in a natural acoustic environment. Because of the influence of the pinna, the magnitudes of the IID and the ITD can vary nonmonotonically with frequency and direction, especially for high frequencies. For natural broadband sounds delivered in an ecologically valid environment, the left and right ear will have a spectral transfer function at each azimuth and elevation, and the differences between the ears lead to an interaural difference spectrum (for review see Blauert, 1997). The implication is that the advanced localization system of mammals can make use of the combined cues to achieve the highest possible acuity in all spatial dimensions. However, many fossorial species, for example the pocket gopher, show little ability to localize sounds, which is explainable by the lack of spatial cues in their subterranean habitat (Heffner and Heffner, 1990).

3.20.3 Brain Structures for Sound Localization

The brain receives acoustic information via only one row of inner hair cells located in the cochlea of the inner ear. Unlike the retina, which is a two-dimensional structure, no spatial information is inherent in the pattern of activation of the receptors. Hence, the spatial position of a sound source has to be computed by the auditory structures of the brain. Solving this complex computation has resulted in a variety of evolutionary adaptations. I will restrict the discussion of the role of brain structures for the evolution of sound localization to a couple of nuclei in the superior olivary complex, the auditory midbrain (inferior colliculus), and the auditory cortex, although other brain structures are also involved in sound localization. To describe them all would exceed the scope of this topical essay (see Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals, The Effects of Domestication on Brain Size, Cetacean Brain Evolution).

3.20.3.1 Brainstem

The superior olivary complex (SOC) is a group of auditory nuclei in the brainstem of amphibians, reptiles, and mammals. One major function of the SOC is to encode the cues that contribute to sound lateralization on the basis of convergent binaural ascending inputs arising from both ventral cochlear nuclei. The SOC consists of several nuclei. Of major interest for sound localization are the lateral superior olivary nucleus (LSO) and the medial superior olivary nucleus (MSO). The LSO is involved in the coding of IIDs (Boudreau and Tsuchitani, 1968) and envelope ITDs (Joris and Yin, 1995). The MSO processes ITDs in the fine structure of predominantly low-frequency sounds (Yin and Chan, 1990). The range of the interaural disparity cues increases with head size. A large head is especially advantageous for the use of fine structure ITDs. Hence, it is not surprising that mammalian species with large heads have a pronounced MSO and often a smaller LSO, while species with small heads have a relatively smaller MSO and a large LSO. However, a large-sized LSO does not necessarily indicate superior IID coding. For example, some hoofed species like the horse, are apparently not able to use IIDs to localize high-frequency tones, although they possess a well-developed LSO (Heffner and Heffner, 1986). The basic neuronal properties of the LSO appear to be similar across taxa (Tollin, 2003, review): most LSO neurons are excited by the ipsilateral ear and inhibited by the contralateral ear. In contrast, MSO neurons are excited by both ears in

the cat and many other mammals. Individual MSO neurons are tuned to a certain range of ITDs (Yin and Chan, 1990). There is some weak evidence that the neurons of the mammalian MSO are spatially arranged in a manner that is consistent with a 'delay line', the anatomical basis of a coincidence detector (cross-correlation) model of ITD processing (Yin and Chan, 1990) as proposed by Jeffress (1948). Although, in the barn owl, the delay line in the nucleus that corresponds to the mammalian MSO (nucleus laminaris, NL) appears to be more clearly developed than in mammals (Kubke *et al*., 2002; Carr and Konishi, 1988), the function shows strong similarities. Neurons of both the NL and the MSO time-lock to monaural and binaural stimuli, and respond maximally when time-locked spikes from each side arrive simultaneously, that is, when the difference in the spike conduction delays compensates for the ITD (Goldberg and Brown, 1969; Carr and Konishi, 1988; Yin and Chan, 1990). The homology between the mammalian MSO and the nucleus laminaris was accepted for decades but has recently been questioned (Carr and Soares, 2002, review). However, it is clear that the mammalian MSO appears to be a less ideal cross-correlator than the owl's NL (Batra and Yin, 2004), and additional mechanisms of ITD processing via inhibitory processes within the MSO have been proposed (Brand *et al*., 2002). These inhibitory mechanisms could actually improve the cross-correlator, and might therefore be an evolutionary step.

Until recently, the fossil finds of early, mesozoic mammaliformes and mammals included only small, shrew to rat-size species, for example, *Morganucodon*, whose head was only about 20 mm long. The average small size of these fossils suggested that those early mammals were nocturnal insectivores, and possibly specialized to hear predominantly high frequencies like extant small insectivores and many rodents. The finding that some insectivores, for example some bat species, have a predominantly monaural MSO (Covey *et al*., 1991) prompted the theory that the mammalian MSO was initially a monaural structure which became binaural when the early nocturnal insectivorous mammals explored more ecological niches and evolved into larger species after the decline of the dinosaurs (Grothe, 2000, review). However, other bat species possess a binaural or mixed binaural/monaural MSO (Harnischfeger *et al*., 1985), or a binaural MSO that resembles the MSO of nonecholocating mammals (Grothe and Park, 1998). This diverse functional organization, and especially monaural characteristic might reflect an adaptation for certain echolocation strategies in the different bat species rather than a primitive feature. More research in primitive nonecholocating insectivores is needed to evaluate this theory. The first true mammals were probably represented by *Hadrocodium*, *Repenomamus*, and *Gobiconodon* species, which would fill an intermediate position between the reptile-like *Morganucodon* and the earliest therian (therians = marsupial and placental mammals), *Triconodon*, a species whose cranial endocast morphology places it at the time of origin of the mammalian isocortex (Rowe, 1996). *Triconodon* was quite large, and was about the size of a Virginia opossum. However, recent fossil finds indicate that some of the early mammalian *Repenomamus* species were considerably larger, for example *Repenomamus giganticus*, which had estimated bodyweight of 12–14 kg, a skull length of 160 mm, and overall body length of more than 1 m. It has been proposed that *R. giganticus* was a carnivore (Hu *et al*., 2005). The apparently larger diversity in body size of those early mammals provokes the question whether some bigger species were sensitive to lower sound frequencies, and used the ITDs provided by their larger heads, and whether they possibly had a binaural MSO. A definite answer to this question cannot be provided by the fossils, because soft tissues are not preserved. In many primitive mammals, like monotremes and some insectivores the middle ear cavities are not bony, but in part covered by cartilage and connective tissue. What these species have in common is that their low-frequency hearing is poor independent of head size (Masterton *et al*., 1969; Aitkin and Johnstone, 1972; Gates *et al*., 1974). Hence, the evolution of the MSO and ITD coding in the brainstem remains an open question.

3.20.3.2 Midbrain

The central nucleus of the inferior colliculus (IC) is an obligatory station of the primary ascending auditory pathway. The neuronal sensitivity to ITDs was first described by Rose *et al*. (1966) for the ICC of the cat, and further examined by Goldberg and Brown (1969) for the beagle. In the mammal IC the processing of combined interaural and spectral cues requires convergence of monaural and binaural inputs and across-frequency integration. The neuroanatomical aspects of this convergence in the IC have been studied using simultaneous tracer injections in different monaural and binaural lower brainstem nuclei (Oliver, 1987; Oliver and Shneiderman, 1991; Oliver *et al*., 1997; Loftus *et al*., 2004). Further anatomical evidence for across-frequency interaction within the ICC was provided by

Malmierca *et al*., (1995). They found that distant isofrequency layers are interconnected in the ICC of the guinea pig. Some neurophysiological aspects of this convergence have been revealed for the ICC of the rabbit by combining the ITD with monaural amplitude modulation (D'Angelo *et al*., 2003). It became evident that most neurons integrate ITD and monaural information to a varying degree. Chase and Young, (2005) as well as Sterbing *et al*. (2003) found evidence for the integration of different localization cues in the ICC of the cat and the guinea pig, respectively. Therefore, the mammalian ICC is a potential source of substantial monaural and binaural integration in the lemniscal pathway of a variety of mammals, which exceeds the function of the auditory midbrain in lower vertebrates.

3.20.3.3 Isocortex

The origin of the mammalian isocortex, in which the auditory cortex is located, has been subject of contradicting studies (for review, see Aboitiz *et al*., 2002). In the past it was regarded as a homologue to a structure named the 'dorsal ventricular ridge' in reptiles, which receives thalamic auditory and visual inputs. However, recent studies suggest that the mammalian isocortex arose from the dorsal pallium, which is predominantly visual and somatosensory in reptiles. This suggestion is supported by molecular and developmental evidence (for a review on the evolution of the isocortex, see Aboitiz *et al*., 2002). As a result, the mammalian isocortex would be a new evolutionary target for auditory thalamic projections. The development of the isocortex is accompanied by a gradual expansion of the brain, which is evident from fossil skull endocasts of early mammals, for example, *Hadrocodium* and *Morganucodon*. While the mesencephalic sensory pathways are the main processors of topographically organized exteroceptive information in sauropsids, and the lemniscal and collicular pathways are largely separated in sauropsids, this is not the case in mammals. In the mammalian isocortex both pathways are integrated, and might therefore implement more ascending information. The enlargement of the isocortex has been associated with the development of a 'modern' middle ear, which makes the transmission of sound more effective. However, while most early mammals with an enlarged isocortex have a fully evolved middle ear, the reverse is not true. Not every mammal with a modern middle ear has an enlarged isocortex. This makes a theory that the enlargement of the isocortex occurred to process sounds inconclusive.

While there is consensus from lesion studies that at least parts of the auditory cortex (AC) are necessary for sound localization, it is unclear whether different fields of the AC contribute differently to or are specialized exclusively for sound localization. Unilateral lesions of the AC in carnivores and monkeys result in deficits of localization of exclusively contralateral sounds (Jenkins and Masterton, 1982; Jenkins and Merzenich, 1984). The only exception from this finding is the human, which might be caused by our hemispheric specialization (Zatorre and Penhune, 2001). If the inactivation is confined to certain fields of the auditory cortex of the cat, either a contralateral deficit or no deficit occurs (Malhotra *et al*., 2004). A deficit occurs for the tonotopically organized fields A1 (primary auditory cortex), PAF (posterior auditory field), as well as, for the nontonotopic multisensory field AES (anterior ectosylvian sulcus), but no deficit was found following inactivation of the tonotopical field AAF (anterior auditory field), nontonotopic A2 and other multisensory fields. These lesion studies prompted the question whether different fields of the auditory cortex contribute differently to sound localization in different species. As described above, monaural and binaural information converge in the ICC, where the majority of neurons are spatially tuned. One could assume that the actual processing of auditory space is complete at this level, and that the role of AC is to distribute this preprocessed information to higher-order areas (Schnupp *et al*., 2001; Kowalski *et al*., 1995). However, the auditory cortex offers new substrates for further processing. The first would be the connection between the cortical hemispheres (corpus callosum) in higher mammals (Placentalia) (for review, see Aboitiz and Montiel, 2003). Since monotremes and marsupials lack the corpus callosum, the placental mammals have the evolutionary advantage of an additional layer for the processing of spatial information. Unfortunately, the possible role of transcallosal projections for sound localization especially under evolutionary aspects is not understood yet.

The second new substrate would be additional thalamic inputs that bypass the primary auditory cortex, but target certain secondary areas. In primates, for example, the secondary caudo-medial area (CM), which is known to be in part serially activated by A1 (Rauschecker *et al*., 1997), receives – in contrast to A1– additional lemniscal and extralemniscal projections from other parts of the auditory thalamus than the ventral medial geniculate body (MGv). These derive, for example, from the dorsal and medial part of the MG (for review, see Jones, 2003). These additional inputs might indicate a specialization of this area for the processing of

certain sound attributes. Based on anatomical studies on the topography in the connections of the lateral belt and parabelt (Romanski *et al.*, 1999; Hackett *et al.*, 1998a, 1998b, 1999a, 1999b), and preliminary electrophysiological studies, it has been proposed that area CM is specialized to code for sound location (Tian *et al.*, 2001; Recanzone *et al.*, 2000). From these findings, a model for two, separate 'what' and 'where' streams of cortical auditory processing was introduced by Romanski *et al.* (1999) in analogy to the processing streams in the visual system (Mishkin and Ungerleider, 1982).

However, despite the fact that the architecture and connections of the different auditory cortical fields have been well described in the macaque (Hackett *et al.*, 1998a, 1998b, 1999a, 1999b), their functional specialization for sound localization remains largely unknown. Even if many areas were coding for sound location, there might be differences in the processing of spatial information. For the cat, the work of Middlebrooks and co-workers on field PAF suggests that in this area, in contrast to A1, the acoustic space is coded in the form of response latency and patterns of trains of action potentials, rather than the rate of action potentials (Furukawa *et al.*, 2000; Furukawa and Middlebrooks, 2002; Stecker *et al.*, 2003).

To summarize, the relative contribution of different fields of the AC has not been studied in enough species to clearly reveal an evolutionary trend of specialization of certain fields/areas for sound localization. However, all mammalian species studied so far (carnivores, rodents, bats, and primates) have in common that the AC can be divided in a core of 2–3 and a belt of additional areas. This seems to be the basic organization retained from a common ancestor. Because of the remarkable differences in the number of fields, their relative location, their connections, and different physiological properties in different species, it is likely that the complex cortical processing system has developed in a quite independent fashion across mammalian taxa, and is therefore an example for niche evolution.

3.20.4 Behavioral Measures of Sound Localization

The variations of the organization of the auditory pathway involved in sound localization between mammalian species raises the question whether this variation is correlated with sound localization acuity. One might further assume that a bigger head, that is, a larger interaural distance, might improve the performance in localizing sounds. The acuity of sound localization has been examined behaviorally in a wide variety of mammalian species. Most information is available on the 'minimum audible azimuth angle' (MAAA) which is defined as the minimum angle for which the animal (or a human listener) perceives two separate sound sources instead of only one fused sound source. However, this measure does not represent a sound localization task, for which an animal would have to point to a perceived invisible/unknown sound source, but a sound source discrimination task. The methods used to assess the MAAA varied, but in most cases some kind of operant conditioning was used. To allow comparison, only studies that used broadband signals in an operant conditioning setup in air (not under water) were included in the following analysis. The head width was plotted against the MAAA for 18 mammalian species (Figure 1). Surprisingly, the minimum audible angle does not clearly depend on the interaural distance of the animal ($R = -0.39$). Very small species, for example, echolocating bats, can have quite small MAAAs despite their specialization for high frequencies, suggesting that ITDs do not play a major role for this sound-source discrimination task in these species. On the other hand, rodents with comparable headsize can have MAAAs ranging from 12° to 27°.

For comparative behavioral measures of sound localization acuity Heffner and Heffner (1992) pointed out an important problem: parameters unrelated to the auditory system might affect sound source localization behavior. Mammals that have a visual streak, that is, a horizontally enlarged area of highest visual acuity, might only orient their head up to a position where the acoustic target reached the boundary of the visual streak, while animals with a small visual fovea, for example primates, would have to direct their gaze toward an acoustic target within a much smaller area of vision. The disparity between the 'localized' and physical position of the sound source would naturally be expected to be greater for mammals with a visual streak than for mammals with a small fovea. The actual sound localization acuity on the basis of the physical sound localization cues, however, might very well be comparable, but hard to test in an experimental setup.

In summary, it is compelling how many factors affect sound localization. It appears to be difficult if not impossible to correlate certain parameters, like head size, pinna shape, or middle ear volume with localization acuity. The evolution of sound localization in mammals is a multifaceted aspect that is driven by ecological needs independent of taxon and common ancestry.

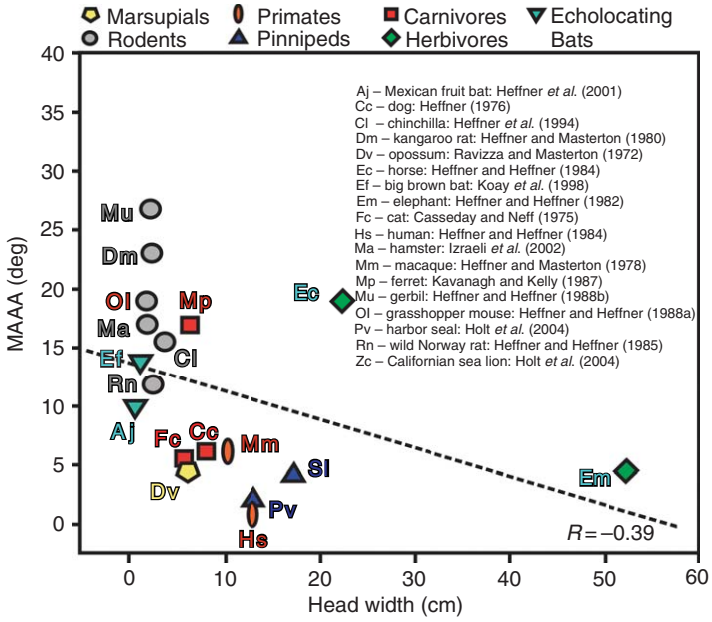

Figure 1 MAAA plotted against head width for 18 mammalian species. Only studies that used broadband signals in an operant conditioning setup in air (not under water) were included.

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3.21 Cetacean Brain Evolution

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Glossary

encephalization	An evolutionary increase in brain size relative to body size.
Mysticeti	The modern suborder of baleen and rorqual whales.
Odontoceti	The modern suborder of cetaceans consisting of dolphins, toothed whales, and porpoises.

3.21.1 Cetacean Evolution, Phylogeny, and Ecology

The origin and evolutionary history of the Cetacea represents one of the most dramatic physiological and behavioral transformations in the biological record. The first suborder, Archaeoceti, contained approximately 25 (known) genera (Thewissen, 1998) and derived from near-shore Indo-Pakistani locales (Thewissen *et al.*, 1996). Archaeocetes shared a common ancestry with modern Artiodactyla (the even-toed ungulates) over 60 Mya (Thewissen *et al.*, 2001) and survived until the late Eocene, around 37 Mya (Barnes *et al.*, 1985) when the modern suborders, Mysticeti (comprising 13 species of baleen and rorqual whales) and Odontoceti (comprising 67 species of toothed whales, dolphins, and porpoises) appeared (Barnes *et al.*, 1985).

Today, modern cetaceans have large brains and are streamlined predators that are widely diversified, inhabiting all oceans and many rivers. Their dietary strategies range from straining of krill to predation on other marine mammals. Their social groups span from two or three individuals to herds of thousands. They are long-lived with relatively long juvenile periods, are wide-ranging, and have complex social structures. Furthermore, in odontocetes a sophisticated echolocation system has evolved.

3.21.2 The Cetacean Brain as an Alternative Route to Complexity

Cetaceans are a highly divergent order that has not shared a common ancestry with our own order, Primates, for at least 92 My (Kumar and Blair Hedges, 1998). Modern cetacean brain structure is extremely different from that of other large-brained mammals, including primates. Yet, despite these neuroanatomical differences, there is growing evidence for a striking degree of cognitive and behavioral convergence between cetaceans and primates in such domains as social complexity, cognition, and self-awareness (Marino *et al.*, 2002, for a review of this literature). Therefore, the study of cetacean brains offers a unique opportunity to address questions about how complex behavior based on very different neuroanatomical substrates can evolve independently. Cetacean brains and primate brains are arguably most meaningfully conceived as alternative evolutionary routes to neurobiological and cognitive complexity.

3.21.3 Increases in Absolute Brain Size and Encephalization

Archaeocete brains changed surprisingly little over the 15 My it took for these ancient whales to became fully aquatic. Archaeocete brain mass varied from about 290 to 2240 g (Marino *et al.*, 2004). At first glance, the higher values in the upper range might imply that archaeocete brains were elaborated relative to other mammals. But brain size

scales allometrically with body size, and when the enormous body sizes of the archaeocetes are taken into account, the brain weight/body weight ratios plunge into the range from 0.0003 to 0.001 (compared to a mean ratio of 0.02 in humans). But ratios of brain weight and body weight overestimate relative brain size in very small animals and underestimate relative brain size in very large animals. Although not a flawless measure, encephalization quotient or EQ corrects for this nonlinearity by using a regression line that represents the overall parameters of the whole sample of brain weight and body weight data. (Throughout this article, reported EQ values will be based on the equation: EQ = brain weight/0.12 (body weight)$^{0.67}$, from Jerison, 1973.) When EQ is applied, the below-average range for archaeocetes, from 0.3 to 0.6, reiterates the conclusion from brain-weight/body-weight ratios that this early suborder did not possess a highly elaborated mammalian brain (Marino *et al.*, 2004). Moreover, the last group of archaeocetes from the late Eocene possessed EQs no higher than those of the earliest archaeocetes (Marino, 2004), disproving the notion that the process of adapting to the aquatic habitat *per se* involved selection for brain enlargement in cetaceans.

Recent studies of the pattern of EQ in cetacean evolution over the past 47 My shows that there were two significant increases in mean EQ among odontocetes (there are insufficient data for mysticetes at this time) (Figure 1). A major increase occurred about 37 Mya with the emergence of the first odontocetes, who were significantly more highly encephalized than the Eocene archaeocetes from which they are derived. The mean EQ grew from 0.5 in archaeocetes, i.e., well below average, to 2.0, in early odontocetes, i.e., significantly above average, with no overlap in the range of values (Marino *et al.*, 2004). By the mid-Miocene period, approximately 15 Mya, almost the full range of modern

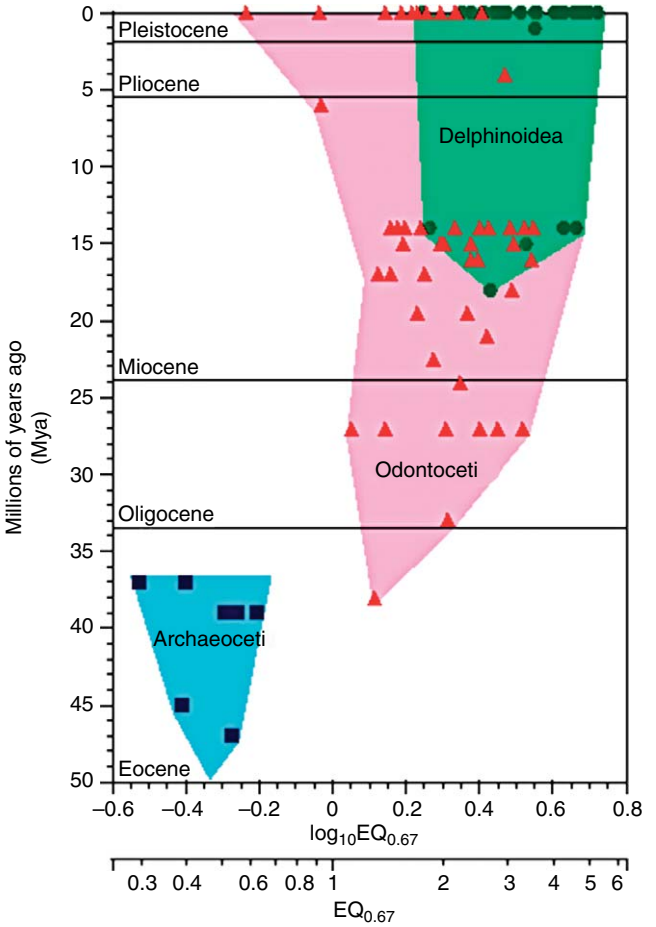

Figure 1 Mean encephalization quotients ($EQ_{0.67}$) of archaeocete and odontocete cetacean species over time. Scales for both raw $EQ_{0.67}$ and $\log_{10} EQ_{0.67}$ are shown across the bottom. Archaeocetes are shown in blue squares; delphinoid odontocetes are shown in green circles; nondelphinoid odontocetes are shown in red triangles. Timescale is in millions of years (My). Note the large shift in EQ at the origin of Odontoceti, and that the Delphinoidea form the upper range of odontocete EQ values from the middle Miocene to Recent. From Marino, L., McShea, D., and Uhen, M. D. 2004. The origin and evolution of large brains in toothed whales. *Anat. Rec.* 281A, 1247–1255.

odontocete EQ values had been achieved. The upper boundary in this period is occupied exclusively by members of the Delphinoidea superfamily (Delphinidae + Phocoenidae + Monodontidae). These mid-Miocene delphinoids enjoyed a second significant increase in EQ over their Oligocene ancestors but no significant change since then. Today, all odontocetes possess above-average EQs. Many modern species, with EQs ranging up to 4 and 5, possess encephalization levels second only to modern humans and significantly higher than any other animals (Marino, 1998; Marino *et al.*, 2004).

3.21.4 Changes in Major Neuroanatomical Structures

Archaeocete brains were characterized by small tubular cerebral hemispheres ending rostrally in relatively large olfactory peduncles and bulbs (Edinger, 1955). These features are further evidence that although some archaeocete brains were large in absolute size, they did not possess morphological characteristics of a highly elaborated mammalian brain such as an enlarged neocortex. But the brains of the earliest representatives of the odontocete and mysticetes that appeared in the late Eocene/early Oligocene did manifest the early stages of several neuroanatomical trends that culminate in the characteristics observed in modern cetaceans. Therefore, modern cetacean brains are not only different from those of other 'mammals' but are vastly divergent from the brains of their Eocene ancestors. These major morphological trends include the expansion of the cerebral hemispheres, the enlargement of auditory structures, the reduction of olfactory structures, the reproportioning of the limbic system, and the emergence of an unusual neocortical architecture.

3.21.4.1 Expansion of Cerebral Hemispheres

The cetacean telencephalon is arranged into three concentric tiers of limbic, paralimbic, and supralimbic tissue. The high degree of cortical gyrification and resulting expansive surface area of approximately 3745 cm^2 is unsurpassed among mammals, including humans (Ridgway and Brownson, 1984). Yet the cetacean neocortex is relatively thin, with a width between 1.3 and 1.8 mm, as compared with 3.0 mm in humans (Ridgway and Brownson, 1984). Cerebral enlargement in cetaceans occurred most exuberantly in the parietal and temporal regions. The frontal lobes, in particular, remain relatively hypoplastic. Whereas primate brains feature large frontal lobes, no homologous frontal lobe region in the cetacean brain has been identified, leading many investigators to substitute the term 'orbital lobe' for 'frontal lobe' when referring to these modest, ventrally oriented hemispheric regions (Morgane *et al.*, 1980).

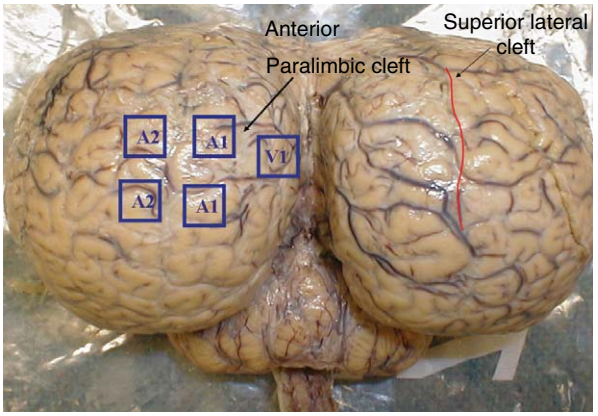

Figure 2 A dorsal view of a brain from an adult Blainville's beaked whale (*Mesoplodon densirostris*) showing the locations of the primary visual cortex (V1), primary auditory cortex (A1), and secondary auditory cortex (A2).

The idiosyncratic pattern of elaboration of the neocortex in cetaceans has resulted in a highly unusual configuration of sensory–motor projection zones. Electrophysiological mapping of cetacean cortex places primary visual cortex on the vertex of the hemisphere in the lateral gyrus and the primary auditory cortex lateral and directly adjacent to it in the suprasylvian gyrus. Secondary auditory cortex lies lateral to the primary auditory field in the medial ectosylvian gyrus (Supin *et al.*, 1978; Figure 2). Somatosensory and motor cortices lie immediately adjacent and rostral to the visual and auditory regions (Ladygina *et al.*, 1978). Therefore, all of the projection zones of the cetacean brain are confined to one region of the overall surface with a vast expanse of remaining nonprojection cortical tissue that can be tentatively described as integrative or associative. This is a highly unusual arrangement for large mammals in general.

3.21.4.2 Enlargement of Auditory Structures

Cetaceans are highly reliant on audition and use this special sense in an extremely complex and multifaceted manner. (Elaboration of auditory structures in cetacean brains has not been accompanied by reduction in visual structures or function, with the exception of the almost-blind river dolphins.) Generally, auditory structures are relatively larger in odontocetes than mysticetes because of the odontocete ability to echolocate. The vestibulocochlear nerve in cetaceans is immense in diameter and is

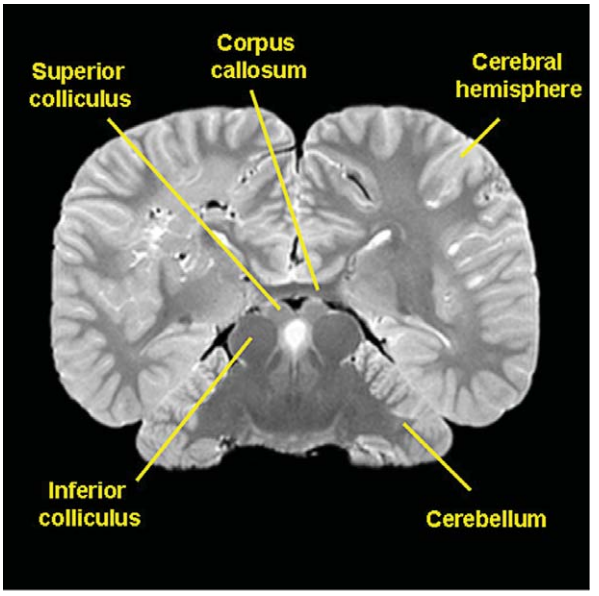

Figure 3 Labeled coronal magnetic resonance image of adult bottlenose dolphin (*Tursiops truncatus*) brain showing that the inferior colliculus is much larger than the superior colliculus. This reflects the emphasis in dolphins on processing auditory information.

composed of relatively more auditory than vestibular fibers (Oelschlager and Oelschlager, 2002). The ventral cochlear nucleus, trapezoid bodies, lateral lemniscus, and inferior colliculi (auditory tectum) are all greatly enlarged in comparison with terrestrial mammals. Whereas in mysticetes the inferior colliculus can be larger or smaller than the superior colliculus (visual tectum), in odontocetes the inferior colliculus is always larger than the visual tectum and can be at least four times the size of the superior colliculus (Marino *et al.*, 2003; Figure 3). The auditory tectum projects to a large medial geniculate nucleus in the massive thalamus.

Likewise, the primary and secondary auditory projection zones on the cerebral surface are extensive (Ladygina *et al.*, 1978; Supin *et al.*, 1978) but are not apparently the entire reason for the great expansiveness of the total neocortical volume in cetaceans. Auditory information is undoubtedly processed at higher integrative levels in other hemispheric regions, as is the case in all mammals. The fact that cetaceans possess such a large expanse of integrative neocortex is consistent with the experimental literature showing very sophisticated general cognitive processing capacities (Herman, 2002).

3.21.4.3 Reduction of Olfaction

Olfaction has been completely lost in adult odontocetes. Fetal odontocetes possess small olfactory structures (Buhl and Oelschlager, 1988; Marino *et al.*, 2001) that regress completely by birth. Infrequently, a short olfactory peduncle remains in adult sperm whales (*Physeter macrocephalus*) and northern bottlenosed whales (*Hyperoodon ampullatus*) (Oelschlager and Oelschlager, 2002). Adult mysticetes, while possessing a vastly reduced olfactory system, have maintained small olfactory bulbs, a thin olfactory peduncle, and an olfactory tubercle (Oelschlager and Oelschlager, 2002). Interestingly, both cetaceans and primates (albeit less extensively) underwent a reduction of olfaction during their evolution. But whereas this has led to a reduction in the hippocampal formation in cetaceans (see below), in primates the hippocampus remains well developed.

3.21.4.4 Reproportioning of the Limbic System

The reduction of olfactory input has resulted in a reproportioning of the limbic system in cetaceans. Notably, the cetacean hippocampus (archicortex), fornix, and mammillary bodies are all highly reduced (Jacobs *et al.*, 1979; Morgane *et al.*, 1980). On the other hand, the amygdala is large and well-developed in cetaceans (Schwerdtfeger *et al.*, 1984), reflecting the maintenance of substantial nonolfactory sources of input to this structure. An interesting corollary feature to the small limbic system is the extremely well-developed cortical limbic lobe (the periarchicortical field above the corpus callosum and the entorhinal cortex) in cetaceans (Oelschlager and Oelschlager, 2002; Marino *et al.*, 2003). This juxtaposition of a vastly reduced archicortex and a highly elaborated periarchicortical/entorhinal zone leads to intriguing questions about whether there was a transfer of learning and memory functions from the olfactory-based hippocampal domain to other cortical, including periarchicortical and entorhinal, regions.

3.21.5 Cortical Architecture

The neocortex of primates (and many other mammals) consists of six well-defined layers. Main input from the thalamus is to granular layer 4 with feedback from layer 6. Layers 2 and 3 output to other parts of the cortex and layer 5 to subcortical structures. Pyramidal cells, with apical dendrites oriented perpendicularly to the neocortical surface, span much of the cortex and end in layer 1 (Allman, 1999). Although stellate and pyramidal cells dominate, other kinds of cellular morphotypes are clearly distinguishable and there is a considerable

amount of heterogeneity (Swanson, 2003). In contrast, cetacean neocortex possesses mainly five layers. It is characterized by a very thick layer 1 that contains apical dendrites of extraverted pyramidal cells from a highly accentuated layer 2 (Glezer *et al.*, 1988; Morgane *et al.*, 1988). Glezer *et al.* (1988) and Morgane *et al.* (1990) suggested that in cetaceans the entirety of thalamocortical afferents feed into the thick layer 1 and through the extraverted neurons of layer 2 to deeper levels.

One of the most salient features of cetacean neocortex is the general lack of granularity in layer 4. Morgane *et al.* (1988) identified two types of visual cortex in the bottlenose dolphin. Heterolaminar cortex contains a very meager layer 4, which is entirely absent in homolaminar cortex. The general lack of layer 4 in cetaceans has important implications for afferentation patterns. In primates and other mammals, some afferent connections come through layer 1 to dendritic connections from layer 2 neurons, whereas other specialized thalamocortical afferents synapse directly on neurons in layer 4. In cetaceans, the majority of afferents appear to go through the very thick layer 1 to synapse en passage on extraverted neurons of layer 2 (Glezer *et al.*, 1988). A small portion of afferents go to layers 3 and 5 as well (Revishchin and Garey, 1990). Some investigators view the segregation of afferents to layer 4 and 1 to be a later evolutionary development than the pattern evinced in cetacean neocortex (Morgane *et al.*, 1986; Glezer *et al.*, 1988; Morgane *et al.*, 1990), further adding to the notion that the cetacean neocortex has expanded on a highly conserved theme that essentially bypasses an entire stage of neocortical granularization found in many other mammals.

Despite the general lack of granularity, there is evidence for considerable cytoarchitectural heterogeneity in many cetacean cortical regions. Morgane *et al.* (1988, 1990) reported the presence of highly conserved cytoarchitectonic vertically oriented columns in both homolaminar and heterolaminar visual cortex of the striped dolphin, *Stenella coeruleoalba*. Also, in a study of insular cortex in the bottlenose dolphin, Manger *et al.* (1998) reported cellular clumps or modular subdivisions that are distinct from the vertical columns noted by Morgane and his colleagues. Furthermore, Glezer *et al.* (1999) reported the prevalence of calretinin and calbindin-immunoreactive neurons over parvalbumin-immunoreactive neurons in dolphin neocortex and noted that, given the role of calretinin and calbindin neurons in inhibiting intracolumnar signals, the preponderance of vertical flow of inhibition along the columnar axis over laminar flow is a chemoarchitectural indicator of strong verticality in cetacean neocortex. The expansive surface area may indicate an extreme multiplication of vertical functional units that provides an intriguing dimension of processing complexity in cetacean brains.

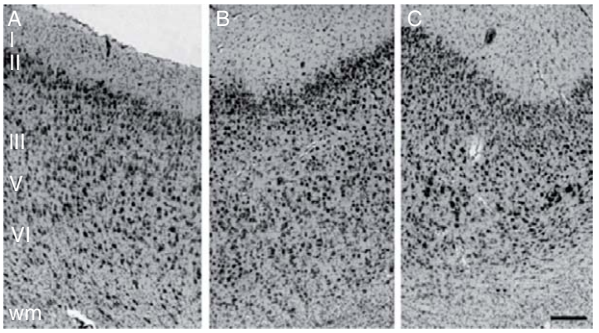

Figure 4 Examples of cytoarchitecture in the frontal cortex of the bottlenose dolphin. A, Lateral orbital gyrys; B, posterior level of the gyrus proreus; C, cortex on the lateral bank of the cruciate sulcus possibly corresponding to a motor field. Layers are indicated by Roman numbers. wm, white matter. Scale bar (on C): 100 μm. Reproduced from Hof, P., Chanis, R., and Marino, L. 2005. Cortical complexity in cetacean brains. *Anat. Rec. (Special Issue: Nature's experiments in brain diversity)* 287A, 1142–1152, with permission from John Wiley & Sons.

Very recent work has added to the view that cetacean neocortex is more complexly organized and diverse than previously thought. For instance, the frontal region of the cetacean brain is very different from that of other mammals, but it is characterized by a well-defined laminar pattern with considerable heterogeneity across regions (Hof *et al.*, 2005; Figure 4). Other areas of the cetacean brain, such as the insular cortex, the sensory cortices, and the posterior polar region, possess well-differentiated groupings of cells and vertically oriented modules. These characteristics provide the neurobiological substrate for the considerable behavioral complexity in many cetaceans (Marino, 2002).

3.21.6 Summary

The study of cetacean brains provides a unique opportunity to examine how large complex brains evolve outside of the primate lineage. Our present knowledge of cetacean brain evolution and modern neurobiology indicates that cetacean brains began a process of substantial elaboration in size and organization approximately 35 Mya with the emergence of the first odontocetes and mysticetes. Today many cetaceans possess encephalization levels exceeded only by modern humans. The highly expanded cetacean brain, however, is characterized by very different neuroanatomical trends and cytoarchitectural organizational themes than other

large mammal brains, i.e., primates. This juxtaposition of similar encephalization levels in cetaceans and primates and highly divergent neuroanatomical trends presents an intriguing picture of alternative neurobiological routes to cognitive and behavioral complexity and a unique opportunity to examine convergent behavioral and cognitive processes.

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3.22 The Evolution of Visual Cortex and Visual Systems

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Glossary

architecture The internal structure of a cortical area may contain unique histological features relative to adjacent cortical areas. There are several features that might distinguish an area. Two widely used histological markers result from (1) differences in metabolic activity of neurons driven by inputs from the thalamus or (2) by the degree of myelination of intrinsic axons.

cortical layers Neocortex is commonly portrayed as having six layers, although these layers can be divided into additional sublayers depending on the cortical area. Layer 1 sits at the pial surface of cortex, whereas layer 6 is the deepest layer.

extrastriate Refers to all of visual cortex outside of V1, as V1 is commonly referred to as striate cortex.

neuroanatomical tracing Because they are available in several colors, fluorescent tracers are particularly useful at retrogradely labeling multiple populations of neurons. Injected extracellularly in small volumes (0.2–1.0 μl) the tracer is taken in at synaptic sites of axon terminals over a relatively small region of cortex, spanning a half of a millimeter or less. Over a period of several days the fluorescent tracers are transported back along the axon to the cell body. In this way one is able to trace the origins of inputs to a particular cortical region.

3.22.1 Differences in Visual Cortex Complexity

Confronting the evolution of visual cortex is appealing in that it is perhaps the most dominant sensory system in humans, as evidenced by the large expanse of cortex devoted to visual processing. We have relatively big brains, so perhaps it is not surprising that we have a lot of visual cortex. Yet, compared to other sensory modalities, vision has a far greater representation in our cortex. Presumably our expanded visual system affords us a richer view of reflected light than animals with less cortex devoted to vision. Though our visual processing capabilities are due in part to a more elaborately constructed

retina, the visual thalamic relay stations and cortical areas in species with complex visual systems provide much more than a one-to-one correspondence of retinal ganglion cell output. The expanded size of our visual cortex can be used to infer an increased capacity for visual processing as size of mammalian cortex is often attributed to greater cognitive abilities. In line with such reasoning, comparisons of cortical sizes between several species of mammals are useful for determining a first approximation of the variability of cortically driven features. This is particularly informative when studying extinct species where sizes of brain endocasts can provide insights into cortical evolution (see What Fossils Tell Us about the Evolution of the Neocortex). But, the size of visual cortex in different mammals does not allow us to infer much about visual system complexity.

In addition to overall size, we know that mammalian cortex is subdivided into areas, or 'organs' of the brain (Brodmann, 1909). In the shrew, there is only one discernable visual area in cortex (Catania *et al.*, 1999). It is difficult to know what the shrew sees with that one area, but based on models of neuronal interconnectivity, the processing of visual features would be limited (Kaas, 2000; Mitchison, 1991; Ringo *et al.*, 1994). In mammals with greatly expanded visual cortex, such as carnivores and primates, as many as 19–40 visual areas have been proposed, based on anatomical and functional investigations (Felleman and Van Essen, 1991; Kaas, 1989b, 1997a; Sereno and Allman, 1991; Van Essen, 2004). The utility of having multiple visual areas is that each can be specialized for certain aspects of visual processing. By compartmentalizing there is a reduced need for intercommunication between all neurons, and thus fewer connections are required to process the information (Changizi and Shimojo, 2005; Kaas, 2000, 2002; Koulakov and Chklovskii, 2001; Mitchison, 1991; Ringo *et al.*, 1994). Areas can be further compartmentalized into processing modules, creating another level of complexity. Modular organization is more commonly reported in mammals that are considered to have moderate to high numbers of visual areas. Interestingly, while squirrels and tree shrews appear to have a similar, moderate numbers of visual areas, at least six (Kaas, 2002), they do not share the same level of modular organization (Bosking *et al.*, 1997; Van Hooser *et al.*, 2005a). Thus, one level of complexity, number of areas, does not necessarily lead to higher complexity of other levels, modularity.

We know that the numbers of cortical areas and degree of modularity within these areas varies across studied species (Kaas, 1989a, 2002). In the framework of cladistics, we can compare similarities in cortical organization across sister groups, and contrast with more distantly related species to determine what structures within the visual system represent the basic mammalian plan and from where features of more complex brains may have evolved (Kaas, 1995, 2002, 2004b; Striedter, 2005). However, sweeping comparisons are made difficult by the small number of examined species. Particularly lacking is a more detailed description of the number of visual areas, their functions, and modular organization. We have some evidence as to the number of cortical areas, their architecture and connection patterns in species from a handful of orders, but functional modular organization has been studied in detail in only a few species, namely monkeys, prosimians, cats, ferrets, tree shrews, and squirrels. Modular organization can and has been studied at the anatomical level in more species and this information, though less descriptive, is useful for making comparisons across several sister groups (Kaas, 2002). Another hindrance to species comparisons is that researchers who do study the organization of visual cortex in various mammals do not always agree with each other's interpretation of the evidence. Even in macaque monkeys, probably the most commonly studied animal for visual cortex organization, it has taken over 30 years to agree on the existence of the third visual area, V3 (Lyon and Kaas, 2002b; Van Essen, 2004; Zeki, 2003). Yet issues with V3 and the 20 or so other poorly defined areas remain, limiting our ability to make comparisons to other primates such as New World monkeys and even humans (Felleman and Van Essen, 1991; Kaas and Lyon, 2001; Rosa and Tweedale, 2005; Sereno and Tootell, 2005).

The goal of this article is to provide a reasonable interpretation of the available evidence on the organization of visual cortex and underlying structures in species from several mammalian orders (see Figure 1). Comparisons of the organizational schemes between species are made, highlighting several issues. How many visual areas were present in the earliest mammals? Have rodents diverged from the common mammalian plan? What are the organizational similarities and differences in species with moderate visual systems? How similar are the complex visual systems of cats and primates? Are any higher-order areas homologous? To address these questions, a focus is placed on a cladistic approach to species comparisons, but an emphasis is also made on similarities between distantly related species. The consolidation within this article of a wide body of comparative evidence on visual cortex organization should serve as a useful template for investigators probing cortical evolution.

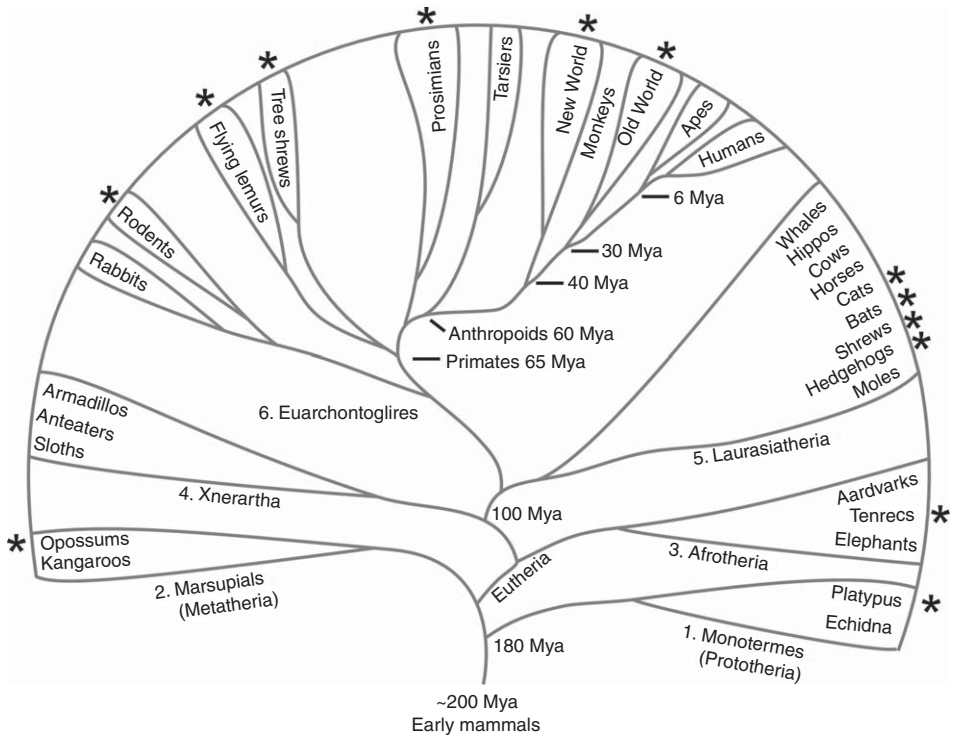

Figure 1 Phylogenetic relationships of the six mammalian superorders based on recent molecular studies (Murphy *et al.*, 2001). Early mammals diverged into prototherian monotremes, metatherian marsupials, and four superorders of eutherians. The organization of visual cortex in 13 taxa from five of the six superorders is covered in this article. * Species covered in text. Adapted from Kaas, J. H. 2005a. From mice to men: The evolution of the large, complex human brain. *J. Biosci.* 30, 155–165.

3.22.2 The Basic Mammalian Plan

3.22.2.1 Identification of V1 and V2

With perhaps only a couple of exceptions, mammals have at least two visual areas (Kaas, 1987; Kaas and Krubitzer, 1991; Krubitzer, 1998; Rosa and Krubitzer, 1999). An obvious exception would be mammals with vestigial eyes, such as subterranean species – the common mole (Catania and Kaas, 1995) and the naked mole rat (Catania and Remple, 2002) – where perhaps no visual cortex is present. Another exception can be found in the very small brains of the shrew where only a single visual area is present (Figure 2b; Catania *et al.*, 1999). Nevertheless, most mammals studied have at least two distinct visual areas. And, observed similarities in myeloarchitecture, subcortical inputs, and retinotopic organization across species have led to the generally accepted conclusion that the primary visual area (V1) and the secondary visual area (V2) are homologous across mammals (Kaas, 1987; Krubitzer, 1995; Krubitzer and Kahn, 2003).

V1 is typically found at the caudomedial extreme of neocortex and has several hallmarks of a primary sensory area that have been revealed through three basic techniques used for the study of cortical organization, namely architecture, connections, and retinotopic mapping (Kaas, 1987; Van Essen, 1979). The most conspicuous architectonic feature of V1 is the dense myelination of axons that can be revealed through a silver staining procedure (Gallyas, 1979). V1 also stains darkly for the metabolic enzyme cytochrome oxidase (CO; Wong-Riley and Carroll, 1984). Particularly when applied to flattened cortical preparations, these staining methods have proved very effective in identifying V1 in many species, from marsupials to monkeys (Kaas, 1987; Krubitzer, 1995), and have served as a useful landmark for further examination of the characteristics of V1.

Beyond the architecture, much of what we know about V1 in the majority of studied mammals comes through neuroanatomical tracing techniques and electrophysiological mapping of the representation of the contralateral visual hemifield. Through neuroanatomical tracing, we know that the primary retinal ascending pathway to V1 is relayed by the lateral geniculate nucleus (LGN) to the middle cortical layer, layer 4 (Jones, 1985; Steriade *et al.*, 1997). In most mammals, the LGN projects heavily to V1, and less so, if at all, to the remainder of visual cortex. Superficial layers 2 and 3 in V1 in turn project to layer 4 of V2. This basic cortical

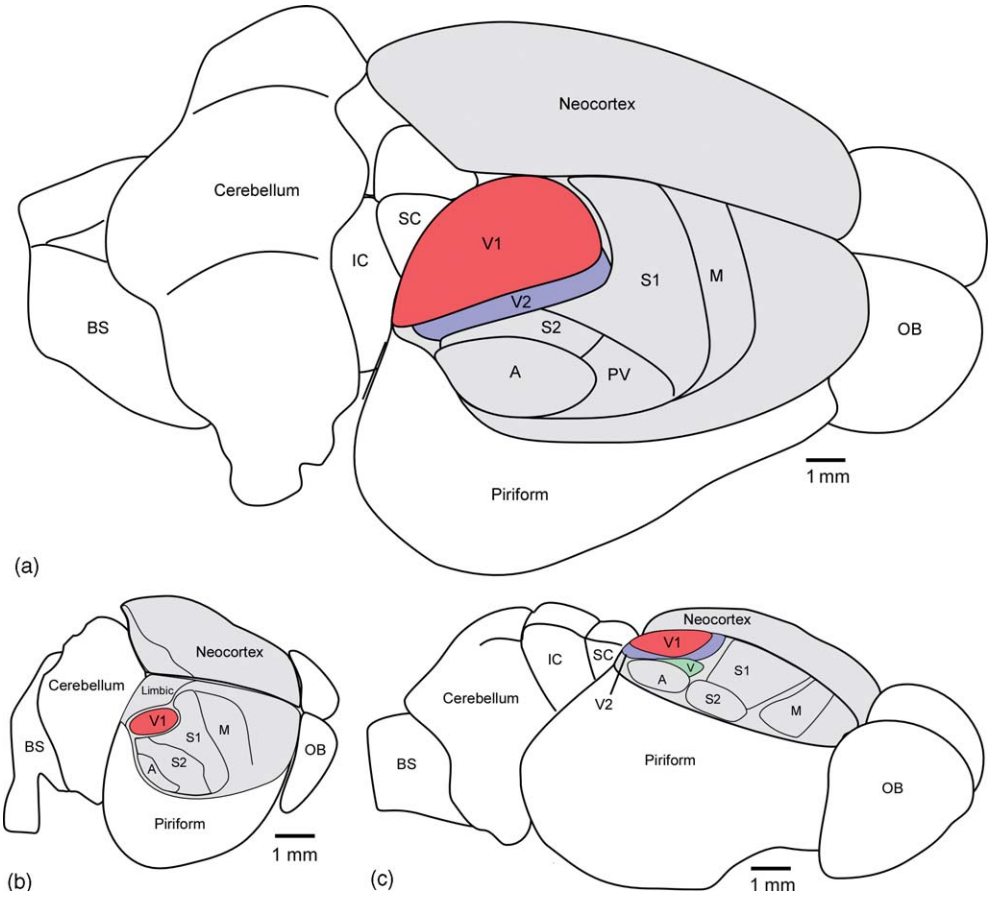

Figure 2 Visual cortex organization in small nonvisual mammals shown on whole-brain drawings. Based on similarities of several features, including brain sizes, insectivores (hedgehogs and shrews; (a and b) and tenrecs (member of the Afrotheria superorder; (c)) may have a cortical organization similar to the earliest mammals. Neocortex is relatively small and dominated by somatosensory areas 1 (S1) and 2 (S2), with a third area, parietal ventral (PV), found in the larger insectivore, the hedgehog. In insectivores, at most, only two visual areas are present, the primary (V1) and secondary (V2), with only a single area present in the smallest insectivores, the shrews. A third, visually responsive region (V) has been reported in the tenrec. Auditory (A) and motor cortex (M) are also present. Phylogenetically older piriform cortex is quite large. Likewise, the superior and inferior colliculi (SC and IC), the cerebellum, olfactory bulbs (OB), and brainstems (BS) are also relatively large. a, Data from Catania, K. C., Collins, C. E., and Kaas, J. H. 2000. Organization of sensory cortex in the East African hedgehog (*Atelerix albiventris*). *J. Comp. Neurol.* 421, 256–274. b, Data from Catania, K. C., Lyon, D. C., Mock, O. B., and Kaas, J. H. 1999. Cortical organization in shrews: Evidence from five species. *J. Comp. Neurol.* 410, 55–72. c, Data from Krubitzer, L., Kunzle, H., and Kass, J. 1997. Organization of sensory cortex in a Madagascan insectivore, the tenrec (*Echinops telfairi*). *J. Comp. Neurol.* 379, 399–414; and Kaas, J. H. 2002. Convergences in the modular and areal organization of the forebrain of mammals: Implications for the reconstruction of forebrain evolution. *Brain Behav. Evol.* 59, 262–272.

projection pattern is the early part of a general 'hierarchical' progression of areas within the visual system (Rockland, 1997). We also know that a secondary pathway provides less direct retinal input to cortex, by transmitting through the superficial layers of the superior colliculus (SC) to the pulvinar (also referred to as the lateral posterior nucleus, LP) and then to superficial layers of cortex. In contrast to the LGN, the pulvinar not only projects heavily to V1, but also provides large numbers of inputs to all areas of visual cortex (Casanova, 2004; Kaas and Huerta, 1988; Stepniewska, 2003).

Another basic tool for the examination of visual cortex is mapping of the visual topography (Allman and Kaas, 1971b; Gattass and Gross, 1981; Hubel and Wiesel, 1965; Kaas, 1997b; Kaas *et al.*, 1970; Rosa, 1997; Tusa *et al.*, 1979). By presenting isolated spots of light to different regions of the retina, the so-called retinotopic mapping has revealed a first-order map of the contralateral visual hemifield that is coextensive with architectonically defined V1 (Rosa and Krubitzer, 1999). Though the progressive placement of a microelectrode at sites traversing across the surface of V1 to determine the receptive fields of local neurons is painstaking, what emerges is an inverted map with the upper visual quadrant represented ventrally and the lower quadrant dorsally (for examples, see Figures 3–5; also, see

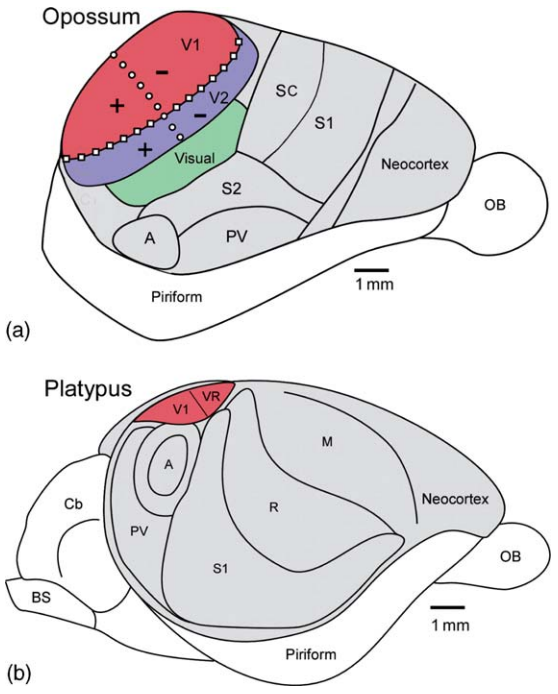

Figure 3 Visual cortex organization in nonplacental mammals, marsupials (a) and monotremes (b), shown on whole-brain drawings. a, The cortical organization of the opossum is typical of most small marsupials. Like the hedgehog, there are three large somatosensory areas, S1, S2, and PV, and a small auditory region as well as an additional caudal somatosensory area (SC) (A). However, unlike insectivores and tenrecs, visual cortex in opossum has expanded with comparatively large areas V1 and V2, and a large third region that contains visually responsive neurons. Areas V1 and V2 are both retinotopically organized (see schematic of visual hemifield in Figure 4a), with the representations of the upper (+) and lower (−) visual fields located ventrally and dorsally, respectively. The + and − visual field representations are bisected by the representation of the horizontal meridian (line of circles), while the representation of the vertical meridian (line of squares) separates areas V1 and V2, resulting in mirror-image representations of the visual field. b, In contrast to the marsupials, cortical organization in monotremes is dominated by three large somatosensory areas, S1, PV, and the rostral area (R), as shown in platypus. Motor cortex (M) is also quite large, and auditory cortex is comprised of at least two areas, a primary (A) and surrounding belt. Visual cortex is relatively small in monotremes. Though the evidence is limited, the caudal region of visual cortex may be homologous to V1 of other mammals, while the rostral visual area (VR) may represent a second visual area. a, Data from Beck *et al.* (1996), Rosa *et al.* (1999), and Kahn *et al.* (2000). b, Data from Krubitzer *et al.* (1995) and Krubitzer and Kahn (2003).

schematic in Figure 4a). The two visual quadrants are separated by the representation of the horizontal meridian (shown as a line of circles in several figures) which bisects V1. The representation of the vertical meridian (shown as a line of squares in several figures) is found at the rostral or anterior border of V1. Many more details of cortical organization are known from retinotopic mapping, such as variability in receptive field sizes, and in most species the magnified representation of central vision (see Allman and Kaas, 1971b; Rosa, 1997; Van Essen *et al.*, 1984). In addition to microelectrode mapping, the more recently developed techniques of intrinsic signal optical imaging and functional magnetic resonance imaging (fMRI) have been used to obtain retinotopic maps of large regions of cortex, making for easier comparisons across cortical areas (Sereno and Tootell, 2005). While retinotopic maps provide basic organizational information, neurophysiological studies describing the functional properties of V1 neurons have been reported for relatively few species, but they have provided for useful comparisons.

In most cases, the mammalian cortex also contains V2, located along the anterior border of V1. Characteristic of a nonprimary cortical area, V2 stains less darkly than V1 for CO and myelin (Krubitzer, 1995), receives feed-forward projections from V1 (Rockland, 1997), and receives its main subcortical retinal relays through the pulvinar, rather than the LGN (Jones, 1985; Steriade *et al.*, 1997). In addition, because V2 is substantially smaller than V1, it contains a compressed representation of the contralateral visual hemifield (see Rosa, 1997; Rosa and Krubitzer, 1999). The V2 retinotopic map can be distinguished from V1 by matching with CO and myeloarchitecture, but also because as the receptive fields for recording sites cross from V1 to V2 over the vertical meridian, the receptive field positions flip to form a rough mirror image of the representation in V1. The retinotopic organization of V2 has been revealed through microelectrode mapping and through connections with retinotopically defined regions of V1. Most evidence revealed through these techniques shows a similar organizational scheme for V2 in all mammals (see Rosa and Krubitzer, 1999).

3.22.2.2 Visual Cortex of Insectivores and Nonplacental Mammals

While most mammals have ample cortical space for more than two visual areas (Kaas, 1989b; Kaas and Krubitzer, 1991), certain constraints in the smallest mammals limit cortical representations to two or even only one visual area. Insectivores are notable for their small brain-to-body size ratio (Striedter, 2005), and in some species for small body size (Catania *et al.*, 1999). While the brain of a common laboratory rat may weigh as much as a few grams, the entire body weight of the smallest insectivore, the least shrew, is only 5 g. The extremely small size of the shrew brain is compounded further by the

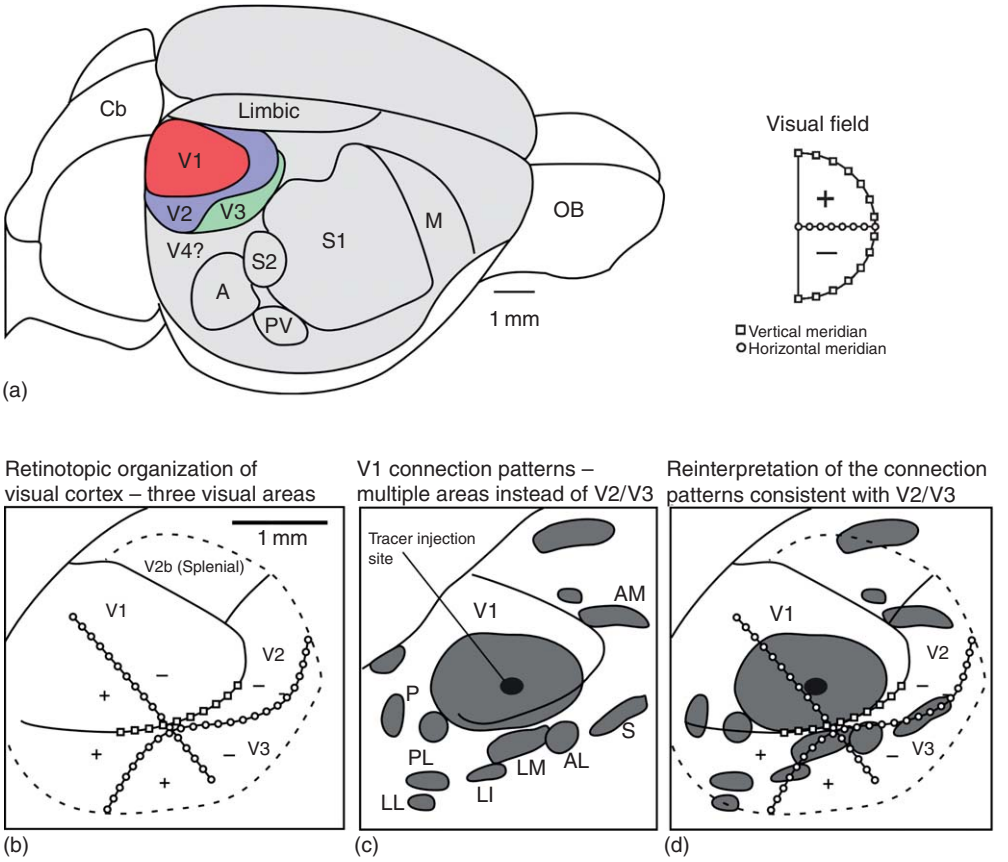

Figure 4 Visual cortex organization in a nonvisual rodent, the common mouse, shown on a drawing of the whole brain. a, Despite the somatosensory (S1, S2, and PV) dominance of cortical space, the mouse has at least three distinct cortical visual areas (V1, V2, and V3) and a fourth region (V4) that contains visually responsive neurons (Wagor *et al.*, 1980; Kaas and Krubitzer, 1991). b, While the visual areas are relatively small, V1, V2, and V3 are retinotopically organized (Wagor *et al.*, 1980; see schematic of the visual hemifield in panel a). c, In contrast, alternative schemes of the organization of visual cortex in nonvisual rodents propose as many as eight extrastriate areas in place of V2 and V3 (Olavarria and Montero, 1989). These results are based primarily on the multiple patches of neurons labeled from tracer injections in V1. In this example, a V1 injection was placed near the representation of the horizontal meridian. d, While several distinct clusters of labeled neurons are found in extrastriate cortex, these clusters are not inconsistent with the retinotopic organization of V2 and V3, and do not unequivocally support the proposal of eight extrastriate areas. For example, the majority of patches of labeled neurons can be found along the proposed V2/V3 border, which represents the horizontal meridian, and matches the retinotopic location of the injection site in V1. See Section 3.22.3 for abbreviations.

small size of neocortex relative to the rest of the brain, and further still by the majority of cortex representing somatosensory receptors, leaving little cortex representing vision (see Figure 2b; Catania *et al.*, 1999). With the larger shrews weighing upwards of 50g, these insectivores provide a close approximation to the size constraints presented to our earliest mammalian ancestors, which are estimated to have weighed ~30g (Allman, 1999). In addition to body and cortical size constraints, insectivores in general are considered to have retained many other structural features of the first mammals and to occupy similar habitats (see Kaas *et al.*, 1970). Because of these similarities to early mammals, the organization of visual cortex in shrews and other insectivores is likely to have diverged little from the original mammalian plan.

In five species of shrew, only a single visual area is present (Figure 2b; Catania *et al.*, 1999). It has the characteristic dark CO staining of mammalian V1, its neurons respond robustly to flashes of light presented to the eyes, and it receives projections from the LGN. Other insectivores, such as the European hedgehog (Figure 2a) and the tenrec of Madagascar (Figure 2c), do not fair much better than the shrew in terms of the amount of visual representation in cortex. However, there is a V2, present in these species. The hedgehog is much larger than the shrew, weighing about 1000g. Both V1 and V2 have been demonstrated through microelectrode mapping, with V2 forming a mirror representation of V1 (Kaas *et al.*, 1970). Indicative of a V1, hedgehog V1 stains very darkly for myelin and CO (Catania *et al.*, 2000; Kaas *et al.*, 1970; Krubitzer,

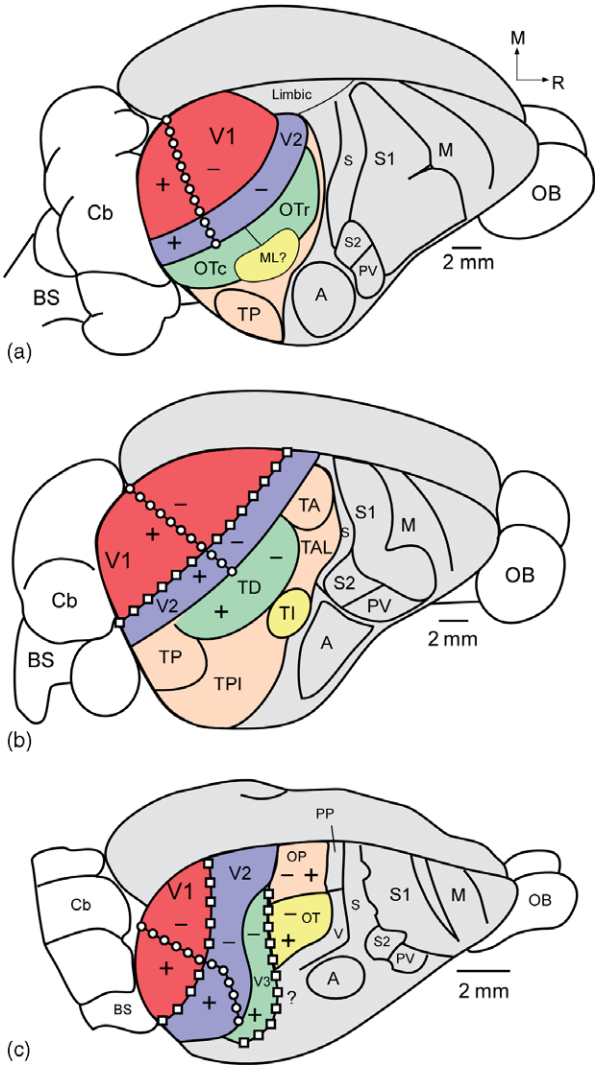

Figure 5 Visual cortex organization in species with moderately complex visual systems: squirrels (a), tree shrews (b), and flying foxes (c). At least half of cortex in these three highly visual species is devoted to vision (colored shading). In all three species, V1 and V2 are retinotopically organized and quite large, comprising nearly half of visual cortex. A V3-like region is found in all three species as well (green shading), with a fourth region (yellow shading) that in limited ways resembles area MT in primates (see Figures 12–14). Overall, several visual areas comprise the cortex. At least six visual areas have been defined in squirrels (Kaas *et al.*, 1989; Paolini and Sereno, 1998) and flying foxes (Rosa, 1999), and eight in tree shrews (Lyon *et al.*, 1998). Additional visual areas may be present in rostral cortex which has been shown to be visually responsive. See Section 3.22.4 for explanation of visual areas and abbreviations.

1995) and receives projections from the LGN (Hall and Ebner, 1970). Because many characteristics of the hedgehog resemble that of some of our earliest mammalian ancestors, it is postulated that V1 and V2 likely represent the prototypical mammalian plan for visual cortex (Kaas, 2005a; Kaas *et al.*, 1970), and this is supported by the evidence that most mammals, including marsupials, also have a V1 and a V2 (Rosa and Krubitzer, 1999). While the organization of the tenrec and hedgehog visual cortex is very similar, this similarity may be more of a reflection of the limitations imposed by small cortical size, as recent evidence shows that tenrecs should not be considered insectivores (Murphy *et al.*, 2001). In fact, tenrecs are now considered part of the African superorder of mammals, Afrotheria (see Figure 1), which includes golden moles, elephant shrews, aardvarks, and even elephants and sea cows.

Metatherian mammals, or marsupials, diverged from an older common ancestor than insectivores, but nevertheless, by most accounts, all studied marsupials have a V1 and V2 (Figure 3a). In several small marsupial species V1 is clearly delimited through CO or myeloarchitecture (Beck *et al.*, 1996; Kahn *et al.*, 2000; Martinich *et al.*, 2000; Rosa *et al.*, 1999). In addition, an orderly retinotopic map is evident (Rosa *et al.*, 1999; Kahn *et al.*, 2000). The case for a marsupial V2 initially was less certain. For example, in an earlier study on the mouse opossum (*Marmosa elegans*) tracer injections in V1 resulted in several patches of labeled neurons in cortex adjacent to the anterior V1 border (Bravo *et al.*, 1990). In a manner similar to conclusions made in some rodent studies (see Section 3.22.3.1) it was proposed that each patch represented a separate visual area, rather than a single V2. However, subsequent experiments on V1 connectivity in other small marsupials showed connectivity with V2 that is more consistent with the concept of a single visual area, V2 (Beck *et al.*, 1996; Kahn *et al.*, 2000). The existence of V2 was confirmed through detailed retinotopic mapping (Rosa *et al.*, 1999).

Prototherian mammals, or monotremes, were the first to diverge from the mammalian line, having split perhaps as many as 180 Mya (Figure 1; Murphy *et al.*, 2001). In the cortex of monotremes, at least two visual areas have been described (Figure 3b; Krubitzer, 1998; Manger, 2005). However, there is some question as to whether they can be considered homologous to V1 and V2 of metatherian and eutherian mammals. As a result, rather than V1 and V2, they have been tentatively termed the caudal and rostral visual areas, or Vc and Vr. Krubitzer *et al.* (1995) described a region of dark CO and myeloarchitecture that is coextensive with both visual areas. While Vr is somewhat lighter in appearance, there is no sharp architectonic division between the two areas as between V1 and V2 in other mammals. Both regions contain fairly complete retinotopic maps, but Vc can be distinguished from Vr in that it responds more vigorously to visual

stimulation (Krubitzer, 1998). Because of the slightly darker architectonic features, more vigorous response properties, and the fact that the caudalmost area occupies cortical territory similar to that of mammalian V1, it is possible that Vc is homologous to V1. However, there is some evidence that visual cortex in monotremes receives ascending inputs from a secondary visual thalamic nucleus, rather than the LGN. Thus, while monotreme cortex contains at least two visual areas, they only partly resemble visual areas in other mammals. Largely because so few studies have been done on the visual system in monotremes, homologies to visual cortex in marsupials and eutherian mammals remain in doubt, and it is plausible that the basic mammalian plan for the visual system underwent several modifications after having split from the monotremes (see Krubitzer, 1998).

3.22.2.3 The Basic Mammalian Plan: One Area, Two, or Even Three?

From the cortical organization evidence in marsupials and insectivores, as well as the implication that most other eutherian mammals have a V1 and a V2, there emerge two approaches for reconstructing the cortical organization of the earliest mammals. These approaches are somewhat in conflict and result in different conclusions. On the one hand, shrews and other insectivores retain several anatomical features of the earliest mammals and would be under similar constraints brought on by a small cortex. This overwhelming similarity has led to the conclusion that insectivore cortical organization reflects the basic mammalian plan. On the other hand, shrews have one less visual area than other, larger insectivores (see Figure 2), and the argument that all other mammalian sister groups, including marsupials, have a V1 and a V2 is used to support the view that the common ancestor to marsupials and eutherians was likely to have a V1 and V2 as well. So, do we devise the basic mammalian plan based on similarities between shrews and early mammals, or common features across all mammalian sister groups? Or can we use both criteria?

While the proposal that similarities in body and brain sizes, other morphological characteristics, and habitat make insectivores an ideal candidate for the species most resembling early mammals is fairly supportable, it clashes with earlier conclusions derived from comparisons across all mammalian sister groups. In particular, though all studied mammals (other than shrews, and possibly monotremes) possess at least a V1 and V2, many insectivores tend to have fewer visual areas than most other mammalian orders, including marsupials, which have at least three (Figure 3a). The issue now becomes whether any of the three or more visual areas are homologous or evolved independently in separate orders. While some have made the argument for homologies of at least three visual areas – V1, V2, and V3 (Rosa, 1999; Rosa and Manger, 2005) – others conclude that V3 is not homologous (Kaas, 2002).

Let us assume that only two areas are homologous across eutherian mammals. Even so, is the hedgehog, with V1 and V2, more representative of the basic mammalian plan than the shrew, which only has a V1? The shrew having only a V1 can be explained through evolutionary digression to adapt to an extremely small body and brain size, where there just is not enough room for a second visual area. However, it is important to consider here that all species studied that do have a V2 are significantly larger than shrews, and these species are also significantly larger than the estimated size of the first mammals. Shrews, on the other hand, are about the same size as the earliest known mammals. Thus, it is not implausible that shrew cortical organization is most representative of the first eutherian mammals.

If we postulate that only a V1 was present in the earliest mammals, then this has implications for the evolution of subsequent visual areas – V2 and every other extrastriate area could have evolved independently in some or several mammalian orders. Whether these areas are homologous would depend on whether a pre-existing mechanism allowing for the emergence of multiple visual areas in a similar fashion was in place in the earliest mammals (see Striedter, 2005). However, such a viewpoint would represent an extreme. What we can say for certain is that shrews and larger insectivores possess a visual cortex that is minimal in design, possessing only one or two visual areas and occupying a relatively small portion of cortex compared to the somatosensory system. As the visual system has expanded in other orders that place a greater emphasis on visual processing, it is generally agreed that V1 and V2 have retained enough of their basic features to remain homologous across species.

3.22.3 Rodent Visual Systems: Simple or Complex?

3.22.3.1 Have Small Rodents Diverged from the Common Mammalian Plan?

If we conclude that the basic mammalian plan for visual cortex calls for, at most, two areas, V1 and

V2, and we know that most mammals have at least three visual areas and probably more, then it follows that cortical evolution has resulted in the addition of visual areas. There are several theories as to how cortical areas have increased in number (see Captured in the Net of Space and Time: Understanding Cortical Field Evolution; Allman, 1999; Allman and Kaas, 1971a; Kaas, 1989a; Krubitzer, 1995; Krubitzer and Kaas, 2005; Krubitzer and Kahn, 2003; Northcutt and Kaas, 1995; Rosa, 1999, 2002; Rosa and Krubitzer, 1999; Rosa and Tweedale, 2005; Striedter, 2005). Rodents provide a good example for both slightly and more moderately expanded cortex, as has been shown in mouse and squirrel, respectively (see Figures 4 and 5a). And, because rodents are the most abundant of the mammalian orders, insights into their organization are important for the study of cortical evolution. Despite using similar techniques, efforts over the past 30 years to determine the organization of visual cortex in rodents have led to two very different conclusions as to the organization of extrastriate cortex (see Sereno and Allman, 1991; Rosa and Krubitzer, 1999). Discrepancies in extrastriate organizational schemes begin as early as V2. As detailed by Rosa and Krubitzer (1999), opposing opinions relate to whether subsequent extrastriate areas have been added to a pre-existing V2 or whether pre-existing extrastriate cortex in an early rodent ancestor, instead of a V2, was already subdivided into several small areas. As we have seen in species that branched from earlier common ancestors – marsupials, tenrecs, and insectivores – only a single area V2 is present, if at all (Figures 2 and 3). Thus, multiple areas in place of V2 in rodents would represent a divergent path in mammalian visual cortex evolution.

The controversial conclusions stem from studies in mouse and rat. Building upon an earlier microelectrode mapping of rat cortex anterior to V1 (Montero *et al.*, 1973b), the patchy extrastriate connection patterns of V1 in mouse and rat were interpreted as support for several distinct visual areas (see Figure 2c; Olavarria and Montero, 1989), rather than a single V2 (Figures 2a and 2b), as postulated by others (Malach, 1989; Rumberger *et al.*, 2001; Wagor *et al.*, 1980). A subsequent study used two to three distinguishable tracer injections placed in different retinotopic locations of V1 in single animals (Montero, 1993) to further establish the retinotopic organizations of the multiple regions. The emergence of multiple distinguishable tracers has proved to be a useful tool for estimates of extrastriate cortex, as will be demonstrated more fully in subsequent sections. Retinotopic maps by others reported similar organizational schemes in rat and hamster (Espinoza *et al.*, 1992; Espinoza and Thomas, 1983). In addition, supporting evidence was derived from callosal connections used to approximate the vertical meridian borders of several of the proposed areas (Olavarria and Montero, 1981, 1989; Thomas and Espinoza, 1987). For example, Thomas and Espinoza (1987) proposed seven extrastriate visual areas, four of which border V1. The largest of these proposed areas, LM, contains a retinotopic map similar to that of V2. Other schemes have postulated as many as nine areas along the V1 borders (see Sereno and Allman, 1991). While it is not unreasonable to consider LM a V2 homologue (Rosa and Krubitzer, 1999), its truncated size leaves several areas adjacent to V1, a pattern not typically reported in other mammalian orders (Kaas and Krubitzer, 1991).

Despite several corroborating experiments, other results contradict the interpretation of multiple areas in place of V2. For example, an early anatomical study using lesions in V1 to look for degenerated neurons in extrastriate cortex, revealed only a single patch of connected neurons in extrastriate cortex nearest V1 (Montero *et al.*, 1973a). In addition, microelectrode mapping studies in the hamster and mouse supported a retinotopic organization more consistent with a single V2 along the lateral border of V1 (see Figures 4b and 4d; Tiao and Blakemore, 1976; Wagor *et al.*, 1980). Interestingly, these results are similar to those reported for lagomorphs (see Sereno and Allman, 1991), the closest relatives to rodents (Figure 1; Murphy *et al.*, 2001). More recently, intrinsic signal optical imaging in mouse visual cortex also yielded results consistent with a single V2 lateral to the V1 border (Kalatsky and Stryker, 2003).

Likely contributing to the differences in data on the retinotopic organization in small rodents are inherent logistical problems in obtaining a clean, detailed retinotopic map in a cramped cortical space while trying to stabilize the very small eyes (Rosa and Krubitzer, 1999; however, see Wagor *et al.*, 1980). In addition, a heavy reliance on callosal connectivity patterns to reveal multiple areas could be misleading in that callosal connections in species with a well-defined V2 extending the entire lateral border of V1, as found in primates, reveals a similar pattern as that seen in rat (Cusick *et al.*, 1984). Thus, callosal patterns are often irregularly distributed within single areas and give the impression of multiple areas if one assumes their location marks the vertical meridian. For these reasons, the use of callosal input patterns as a primary means of

delimiting borders between visual areas is questionable. In addition to the evidence in small rodents, results from brains of more visually dependent squirrels (see the discussion below), argue against multiple areas immediately outside of V1 in rodents, and point to a single V2 (Figure 5a), consistent with the common mammalian plan (Kaas and Krubitzer, 1991).

Before moving on, it should be noted that many rodent studies designate a separate V2, V2b, sometimes referred to as area 18b (Wagor *et al.*, 1980), that lies medial to V1. While the lateral area 18a (Wagor *et al.*, 1980), or LM (Espinoza and Thomas, 1983), is considered to be the homologue to V2 in other mammals, Rosa *et al.* (1999) contend that the medial region is similar to the splenial visual area of limbic cortex found in other mammals, including primates (Rosa *et al.*, 1997), and is not part of mammalian V2.

3.22.3.2 How Many Visual Areas in Rodent Cortex?

Most of the upwards of 12 extrastriate areas described in rodents are all located along the V1 border (Sereno and Allman, 1991), three along the medial border and six along the lateral border. But, as discussed above, alternative evidence concludes that the region immediately lateral to V1 comprises a single V2 (Wagor *et al.*, 1980; Kaas and Krubitzer, 1991), and the medial region may be more consistent with limbic visual cortex found in other mammals (Rosa and Krubitzer, 1999). Moving beyond the V2 controversy, we know that the rodent has additional cortex devoted to vision, and has presumably expanded from the basic visual plan found in insectivores. So, how many visual areas do rodents have?

If we jump ahead to results from more modern techniques, we find two retinotopically organized extrastriate areas outside of V2 in a mouse. Kalatsky and Stryker (2003) optimized visual display signals to avoid confounding biorhythms. In this manner, they were able to maximize intrinsic neuronal activity measured through optical imaging (movies of the activation of visual cortex as stimuli sweep across the visual field are available in their online supplemental material – see 'Relevant Website'). In all, four extrastriate areas were described – V2, V3, V4, and V5. V2 represents a condensed mirror image of the representation found in V1, while V3 mirrors V2, and likewise V4 mirrors V3. Area V5 is found medially in a similar location to V2b described above, and is probably the visual region of the limbic cortex (see Rosa and Krubitzer, 1999). The optical imaging measurements are consistent with earlier microelectrode maps in the mouse, where V1, V2, a V3 and an additional lateral region were reported (Figures 4a and 4b; Wagor *et al.*, 1980).

3.22.3.3 The Complexity of Squirrel Visual Cortex

Although mice have an expanded visual cortex compared to insectivores, they rely largely on somatosensation through vibrissa on the snout, and through their sense of smell. The importance of the somatosensory system is reflected by a cortex dominated by the barrel fields representing the vibrissa. This leaves little cortical space for visual areas, and subsequently as few as two extrastriate cortical areas outside of V2 have been reported (Figure 4a; Wagor *et al.*, 1980; Kalatsky and Stryker, 2003), as described in the previous section. In contrast, squirrels, which are diurnal, have retinas comprised primarily of cones, 90–95% (Jacobs *et al.*, 1980), and have an extremely high density of ganglion cells projecting to the LGN and SC (Johnson *et al.*, 1998; Major *et al.*, 2003) allowing for higher visual acuity. This increase in visual input is reflected in the greater expanse of cortex devoted to vision (Kaas, 2002), and an increased number of extrastriate visual areas (Figure 5a; Kaas *et al.*, 1989; Paolini and Sereno, 1998). Thus, the somatosensory subfield for the barrel field is one-third the size in squirrels than in rats, whereas striate cortex and extrastriate cortex are four and eight times larger, respectively, in squirrels (Paolini and Sereno, 1998).

An early study identified three retinotopically organized visual areas 17, 18, and 19 (Hall *et al.*, 1971), similar to V1, V2, and V3 found in other mammals. Like other rodents, V1 is easily distinguished through myelin staining (Kaas *et al.*, 1989; Paolini and Sereno, 1998). However, an advantage of studying squirrels over smaller, less visual rodents is that V2 is also distinguishable as a uniform darkly stained myelin region along the lateral border of V1. Consistent with the common mammalian plan, squirrel V1 receives topographic projections from the LGN (Kaas *et al.*, 1972a), whereas the main visual thalamic input to extrastriate cortex comes through the relatively large pulvinar (see Figure 6a; Robson and Hall, 1977). V2 and V3 in squirrels are also relatively large compared to the descriptions for mice (Figures 4a and 5b). Connection patterns from tracer injections placed in V1 and V2 of squirrels revealed several features of these large regions (Figure 7a; Kaas *et al.*, 1989). Similar to small rodent studies described above, patchy

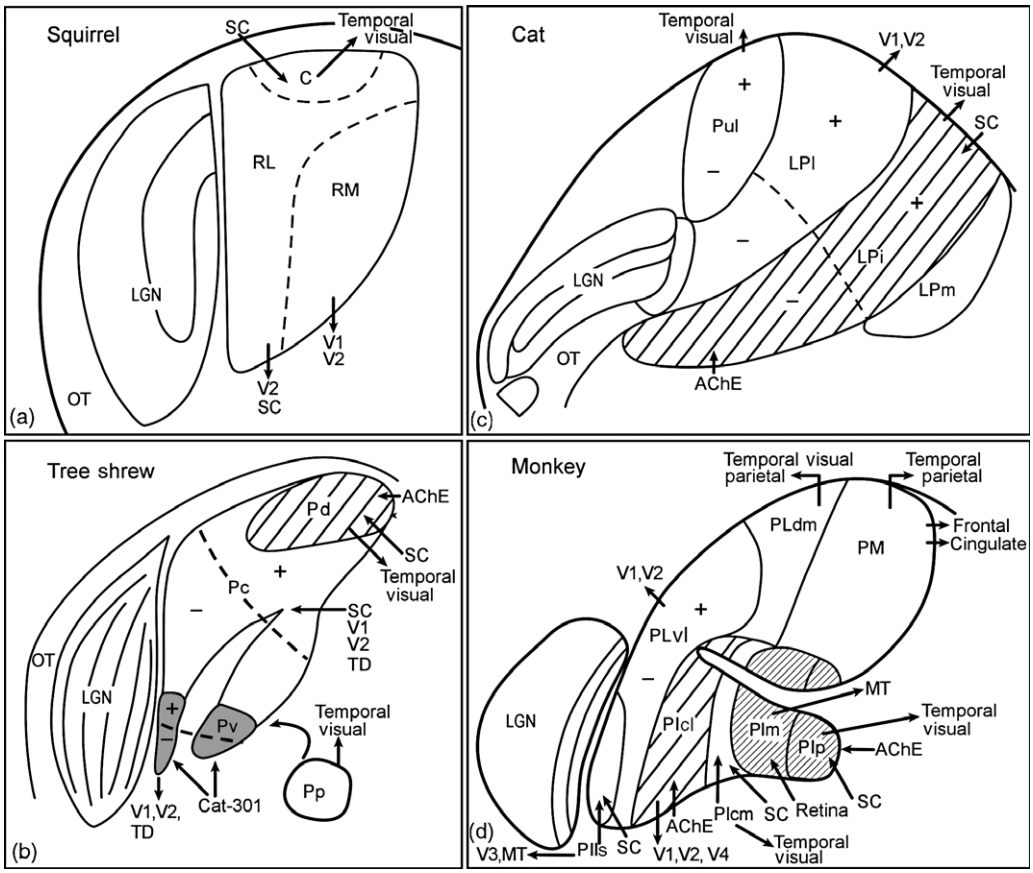

Figure 6 Organization and cortical connections of the pulvinar nucleus in four highly visual species. A thalamic nucleus relaying inputs from the superior colliculus (SC), the pulvinar receives feedback from and provides inputs to all of visual cortex. In highly visual species the pulvinar can be subdivided into distinct nuclei, each with a distinct pattern of connections to extrastriate visual cortex. a, There is evidence for three subdivisions in the pulvinar of squirrels, the rostrolateral (RL), rostromedial (RM), and caudal (C) divisions (Robson and Hall, 1977). RL receives projections from SC and projects to V2, whereas RM does not get SC projections but projects to both V1 and V2. The C subdivision is only found in the caudal extent of pulvinar, and is distinguished by SC inputs and connections with temporal visual cortex. b, Tree shrew pulvinar has four subdivisions distinguished through differences in architecture (Lyon *et al.*, 2003a), input from SC (Luppino *et al.*, 1988), and projections to visual cortex (Lyon *et al.*, 2003b). The central (Pc) is the largest subdivision. It receives inputs from SC and has retinotopic projections to V1, V2, and TD. The ventral subdivision (Pv) also provides retinotopic inputs to V1, V2, and TD, and can be distinguished through immunoreactivity to Cat-301 (gray shading). A dashed line in both Pc and Pv separates portions representing the upper (+) and lower (−) visual field representations in these subdivisions. The dorsal subdivision (Pd) with high levels of acetylcholinesterase (AChE; thatching) receives projections from SC and projects in turn to temporal visual areas. A posterior subdivision (Pp) is located in the posterior-most extent of the pulvinar and projects exclusively to temporal visual areas. c, The cat pulvinar is formed by the 'pulvinar' (Pul) and three subdivisions of the lateral posterior nucleus including lateral (LPl), intermediate (LPi) and medial (LPm; Casanova, 2004; Lyon *et al.*, 2003b). Inputs from SC and projections to distinct cortical visual areas are shown (based on Lyon *et al.*, 2003b; Casanova, 2004). For more details see Section 3.22.5.1. d, Monkey pulvinar has been split into at least eight subdivisions (Cusick *et al.*, 1993; Stepniewska, 2003). The inferior pulvinar is comprised of five subdivisions, the posterior (PIp), medial (PIm), central medial (PIcm), central lateral (PIcl) and lateral shell (PIls). The lateral pulvinar has been split into at least two subdivisions, the ventral lateral (PLvl) and dorsal medial (PLdm), while conservative estimates treat the medial subdivision as a single region (PM). Inputs from SC and projections to distinct sets of cortical areas are shown (Lyon *et al.*, 2005; Shipp, 2001; Stepniewska *et al.*, 2000; Stepniewska, 2003). In all panels, the dorsal lateral geniculate nucleus (LGN) and the optic tract (OT) are shown for reference. Modified from Lyon, D. C., Jain, N., and Kaas, J. H. 2003b. The visual pulvinar in tree shrews. II: Projections of four nuclei to areas of visual cortex. *J. Comp. Neurol.* 467, 607–627.

connections in V2 resulted from V1 injections. Furthermore, injections in V2 resulted in intrinsic long-range connections of up to 6 mm. In addition, callosal connections revealed a patchy pattern, much like that reported for rats (Gould, 1984). In conjunction with the myelin pattern and retinotopic maps, these patterns are taken to reflect modular organization within V2, rather

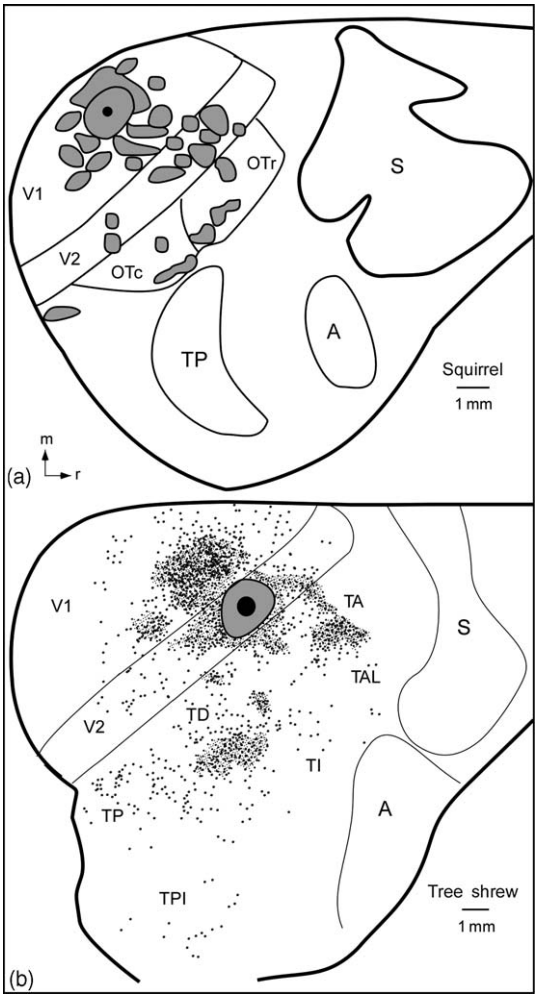

Figure 7 Connection patterns of early visual cortex are useful in identifying multiple extrastriate areas. a, An injection in V1 of squirrels results in a patchy pattern of label both intrinsically within V1 and in extrastriate cortex. In extrastriate regions, a wider displacement of these patches is attributed to separate cortical areas, OTc and OTr. b, In tree shrews, following a tracer injection in V2 the displacement of patches of labeled neurons in extrastriate cortex is more distinct, revealing several visual areas, TA, TD, TP, TPI. In both instances, these connection patterns can be used as guides for further exploration of extrastriate visual cortex, such as, targeting extrastriate areas for microelectrode recordings and further tracer injections. See Section 3.22.4 for abbreviations and more details. a, Based on Kaas, J. H., Krubitzer, L. A., and Johanson, K. L. 1989. Cortical connections of areas 17 (V-I) and 18 (V-II) of squirrels. *J. Comp. Neurol.* 281, 426–446. b, Modified from Lyon, D. C., Jain, N., and Kaas, J. H. 1998. Cortical connections of striate and extrastriate visual areas in tree shrews. *J. Comp. Neurol.* 401, 109–128, with permission from John Wiley & Sons.

than an indication of several distinct visual areas bordering V2.

Following V1 and V2 injections, patchy connections were also found in V3, termed the occipital–temporal zone (OT; Kaas *et al.*, 1989). However, because of widely spaced patchy connectivity it was proposed that V3 could be broken up into 2–3 areas labeled as rostral, middle, and caudal divisions of the occipital temporal cortex (OTr, OTm, and OTc; Kaas and Krubitzer, 1991; Kaas, 2002). Still, the connection patterns are somewhat consistent with a single area V3 (OT), if one considers multiple patches as a sign of modular organization as for V2, but in some cases the distance between patches in V3 is much larger than that found in V2 (Kaas *et al.*, 1989). In addition, earlier retinotopic maps of V3 were very limited (Hall *et al.*, 1971), leaving this region open to other interpretations. More conservative estimates split OT into two regions, OTr and OTc (Figures 5a and 7a; Kaas *et al.*, 1989; Kaas, 2002). Additional studies exploring the retinotopic organization with multiple tracer injections and retinotopic mapping would help determine whether OT is a single retinotopically organized region or actually comprised of 2–3 separate areas. As we shall see in the next section, the squirrel pattern of connectivity is similar to multiple divisions of V3 described for the highly visual tree shrew (Figures 5 and 7; Kaas and Krubitzer, 1991; Kaas, 2002).

The expanded temporal lobe of the squirrel provides additional space for two more large visual regions between OT and anterior cortex devoted to audition and somatosensation. These regions were identified as visual in nature through their connection patterns with V2. It is worth noting that tracing techniques, such as this, have proved to be a valuable technique for providing a first approximation of the number of extrastriate areas (see Kaas, 2004c). These visual areas have been termed the temporal posterior (TP) and temporal intermediate (TI). TP extends laterally from OTc, along the base of sensory cortex. TP stains very darkly for myelin and is sparsely interconnected with V2 (Kaas *et al.*, 1989). TI stains lightly for myelin and can be divided into at least two distinct regions based on interconnectivity with V2. Thus, outside of V2 and OT, the squirrel has at least three more extrastriate areas.

Based on response properties of neurons found in the OT visual region, Paolini and Sereno (1998) have designated two areas, the middle lateral (ML) and lateral (L). ML and L occupy territories similar to OTr and OTc. Further investigation revealed that ML and L are comprised of neurons selective for speed and the direction of moving stimuli (Paolini and Sereno, 1998).

The functional characteristics of neurons have been sparingly discussed up to this point. So, what

does it mean to find an area specialized for a visual feature? V1 neurons of rats, as well as squirrels, prefer particular aspects of features of a visual stimulus – orientation, direction, and length (Girman *et al.*, 1999; Ohki *et al.*, 2005; Van Hooser *et al.*, 2005b). However, V1 receptive field sizes are typically very small and thus will only respond to a stimulus presented in a very small portion of the visual field, but converging inputs to subsequent extrastriate areas enable neurons to respond to a larger region of the receptive field. In addition, particular features of a stimulus activate neurons in different extrastriate cortical areas to different degrees such that some areas may contain neurons that respond more vigorously to the motion of the object while being less influenced by the shape or color of the object, features which are important to neurons in other visual areas. While there are no reports of functionally specialized extrastriate areas in rats, a large body of evidence has detailed functionally selective areas in mammals with more complex visual systems, such as cats and primates (see Section 3.22.5).

With an extrastriate visual cortex eight times larger than the rat, and a conservative estimate of at least five extrastriate areas beyond V2, it seems clear that squirrel visual cortex is more complex than the rat or mouse, where likely only two more areas can be found beyond V2 (see previous section; Figure 4). This observation conflicts with a recent proposal that species within a particular order typically have the same level of cortical complexity (Manger, 2005). In support of similar complexity between rat and squirrel, one could argue that the multiple subdivisions proposed for V2 in rat and mouse increase the number of extrastriate cortex tremendously. Yet, this intricate pattern is not found in squirrels. Furthermore, if we give the rat nine visual areas along the V1 border, this would represent an organization more complex than visual cortex found adjacent to V1 in any other mammalian order, including primates.

3.22.4 The Moderate Visual Systems

In the species discussed thus far, we have seen as few as one visual area in the shrew, two in monotremes, hedgehogs, and tenrecs, two or three in some small marsupials, and perhaps as many as four in small rodents. While these species rely heavily on modalities other than vision, the number of areas jumps to at least seven in the more visually dependent squirrel. As we will see in this section, the number of visual areas is similar to that proposed for megachiropteran bats (flying foxes) and tree shrews, species that have been or still are considered close relatives of primates. These species, particularly tree shrews, are thought to have retained many of the features found in the common ancestor that led to primates, so an understanding of their cortical organization is essential for understanding the early evolution of the primate brain.

3.22.4.1 Visual Systems of the Tree Shrew and Flying Fox

Until recently, the superorder Archonta was used to group primates together with their three closest relatives – the gliding lemur, tree shrew, and flying fox. Two species of gliding lemur make up the order Dermoptera. While not actually a lemur, and despite having a rather large wing-like web of skin encircling its entire body, the gliding lemur resembles prosimian primates. In fact, recent DNA analysis indicates that gliding lemurs are indeed closely related to primates (Murphy *et al.*, 2001). The tree shrew, a squirrel look-alike, despite its name is more closely related to primates than shrews and other insectivores. Early classification based on brain similarities placed tree shrews within the primate order, though they have since been moved to their own order, Scandentia (see Martin, 1990). DNA evidence also supports a very close relationship between tree shrew and primates (Murphy *et al.*, 2001). The flying fox is the largest of the fruit-eating bats (Megachiroptera). While some unique anatomical similarities to primates led to the proposal that the flying fox should be classified separately from smaller, microchiropteran bats, and considered close relatives of primates (Pettigrew, 1986; Pettigrew *et al.*, 1989), more recent genetic evidence maintains bat monophyly (Van Den Bussche *et al.*, 1998) and has removed megabats from Archonta, claiming a closer relationship to cats than to either rodents or primates (Kaas, 2004b; Murphy *et al.*, 2001).

Despite the recent distancing of flying fox from tree shrew, neuroanatomical and physiological studies indicate some similarities in visual system organization (Figure 5). Both species are diurnal and boast retinas packed densely with ganglion output cells (Pettigrew, 1986; Kaas and Preuss, 1993) allowing for high visual acuity (Petry *et al.*, 1984). In thalamus there is a well-laminated LGN, which provides the main relay of retinal inputs to V1. Tree shrews have six architectonically distinct layers (Figure 6b), which segregate input from each eye and contain functionally distinct classes of neurons

(see Conley *et al.*, 1984; Jain *et al.*, 1994; Kretz *et al.*, 1986; Lyon *et al.*, 2003b; Wong-Riley and Norton, 1988). It has been suggested that the LGNs of the flying lemur and flying fox each have as many as six layers as well (see Kaas and Preuss, 1993), though only three layers are architectonically distinguishable in the flying fox (Ichida *et al.*, 2000; Manger and Rosa, 2005) and only Nissl stained sections, less than ideal for determining LGN layers, have been examined for the flying lemur (Kaas and Preuss, 1993). Thus, the similarity between the LGN of these species is uncertain. Furthermore, though these species show a relatively high number of geniculate layers compared to other species such as rodents, it has been argued that this feature is easy to evolve and has appeared several times through convergent evolution in distantly related species (Striedter, 2005).

As for most mammalian species, flying foxes and tree shrews have areas V1 and V2 that are clearly defined (Figure 5). Like the squirrel, these areas are fairly large, contain a retinotopic map of the contralateral hemifield (Kaas *et al.*, 1972b; Rosa *et al.*, 1993, 1994), and are easily distinguished through CO or myelin staining (Lyon *et al.*, 1998; Rosa *et al.*, 1994). While the LGN projects primarily to V1 in tree shrew, it provides a topographic but diffuse input to V2 (Lyon *et al.*, 2003b). A similar connection pattern appears to be present in the flying fox as well (Manger and Rosa, 2005). More detailed analysis of the tree shrew has also revealed several levels of modularity within V1 and V2 (Bosking *et al.*, 1997; Lund *et al.*, 1985; Lyon *et al.*, 1998), and these are described in Section 3.22.4.2.

Anterior to V2 in the flying fox, detailed microelectrode recordings revealed several fairly complete retinotopic maps (Figure 5c; Rosa, 1999). A V3 was identified adjacent to V2, as a narrower and shorter band of cortex representing a compressed mirror image of the retinotopic map of V2. Anterior to V3, two more retinotopically organized areas, the occipital temporal (OT) and occipital parietal (OP), were also identified. Anterior to OT was a narrow strip of cortex that contained visually responsive neurons. Because these neurons had large receptive field sizes, no retinotopic order was apparent. Anterior to OP, in the posterior parietal cortex (PP), just posterior to somatosensory areas S1 and S, neurons were responsive to both vision and touch. Uncharted cortex (?) ventral to OT and posterior to auditory cortex could also be part of visual cortex, as is the case for this region of cortex in squirrels (see previous section) and tree shrew (see below). In all, at least seven visual areas and visually responsive regions have been identified in the flying fox.

In tree shrews, injections of different, distinguishable tracers into different retinotopic locations of V1 and V2 (see Figure 7b) revealed several extrastriate visual areas lateral to V2 (Figure 5b; Kaas, 2002; Lyon *et al.*, 1998; Sesma *et al.*, 1984). In tree shrews, rather than a single V3 strip extending along the lateral border of V2, the existence of three separate areas has been proposed – the temporal anterior, dorsal, and posterior areas (TA, TD, and TP). The largest of the three areas, TD, is the most V3-like, in that it extends across much of the V2 border, and has retinotopic connections with areas V1 and V2. Areas TA and TP are found at the medial–anterior and lateral–posterior ends of TD. TA receives retinotopic inputs from V2, and interconnects with areas TD and TP. Likewise, TP receives crudely retinotopic projections from V2 and connects with TD and TA. The retinotopic pattern of connections with V2 and interconnections between areas TA, TD, and TP strongly support the proposal that a single, large V3 is not present in tree shrews (Lyon *et al.*, 1998). This conclusion may represent a divergence from the common mammalian plan wherein several species have an area resembling V3 (Rosa, 1999). Or, it may reflect the proposal that V3-like visual areas have evolved separately and are not homologous (Kaas, 2002). One suggestion is that a narrow strip more anterior in the temporal cortex, just adjacent to V2, could be homologous to a V3 (Rosa, 1999); however, retinotopic microelectrode maps of this region remain to be done. Alternatively, perhaps TD is homologous to V3, in that it is not unlike the proportions of V3 in the flying fox (Rosa, 1999) and compared to V2 it contains a condensed retinotopic map (Lyon *et al.*, 1998).

At least three more visual areas have been identified in cortex more anterior to TA, TD, and TP (Lyon *et al.*, 1998, 2003b). The temporal inferior area (TI) situated anterior–lateral to TD can be distinguished architectonically through staining for myelin and connections with TP. Interestingly, tree shrew TI is in a similar location to squirrel TP (see Figure 5) which also has some visual connections and stains darkly for myelin. Another extrastriate area in the tree shrew, TPI, lies lateral and inferior to TP with which, like TI, it is primarily connected. Lateral to TA, there is the temporal anterior lateral area (TAL) which receives input from both visual and somatosensory thalamus (Lyon *et al.*, 2003b) and is connected with visual and somatosensory cortex (Lyon *et al.*, 1998; Remple *et al.*, in press).

These bimodal anatomical inputs resemble the bimodal neuronal properties reported for OP of the flying fox (Rosa, 1999). In all, at least eight visual areas and regions have been identified in the tree shrew.

Both the tree shrew and flying fox also have an enlarged and subdivided pulvinar (Figure 6b; Luppino *et al.*, 1988; Lyon *et al.*, 2003a, 2003b; Manger and Rosa, 2005). This increase in pulvinar size is typically seen in species with a greater expanse of extrastriate visual cortex, including squirrels (Figure 6a; Robson and Hall, 1977). In addition, distinct subdivisions of the pulvinar project differently to regions of visual cortex, and evidence of these connection patterns is used as support for the existence of multiple extrastriate areas. In the flying fox, three architectonic subdivisions are discernible through CO staining, the lateral, intermediate, and medial (Pl, Pi, and Pm, respectively) (Manger and Rosa, 2005). Tracer injections across several areas in visual cortex, show that Pl, which is located adjacent to the LGN, projects strongest to V2, whereas subsequent subdivisions project more strongly to visual areas V3, OT, and OP, which are located progressively anterior in cortex (Manger and Rosa, 2005). In tree shrews, four pulvinar subdivisions have been identified through different connections to extrastriate cortex and through distinct architecture (Figure 6b; Lyon *et al.*, 2003a, 2003b). Adjacent to the LGN, is the largest, central subdivision, Pc, which projects topographically to V2 as well as to adjacent cortex in area TD. The ventral subdivision, Pv, stains darkly for the antibody to Cat-301, a marker for large diameter neurons, and projects topographically to V2 and TD, as well as to TA. The dorsal subdivision, Pd, stains darkly for acetylcholinesterase (AChe) and projects to posterior extrastriate areas TP and TPI. A posterior subdivision, Pp, projects exclusively to the most anterior extrastriate visual areas, TAL, TI, and also to TPI.

Despite the rather distant relationship between tree shrews and megachiropteran bats, these visually dependent species have a similar proportion of cortex devoted to vision, with a similar number of areas. Areas V1 and V2 are similar in size and retinotopic organization; there is a third visual area, V3 in the bat and TD in the tree shrew, that both have a compressed mirror image of the representation of the visual field in V2. Each species has two more visual areas that are retinotopically organized, OT and OP, in the bat, and TA and TP in the tree shrew. Additionally, a bimodal sensory zone, representing somatosensory and vision, is present in each species, OP in bat and TAL in tree shrew. While tree shrews are considered a good approximation of the primordial primate, the basic cortical organization seen in tree shrew has more in common with megabats, and even squirrels, than with extant primates. However, comparisons up to this point have only focused on the location, retinotopic organization, and connection patterns of visual areas. The modular organization within visual areas, both anatomical and functional, indicates that tree shrews are more primate-like than squirrel-like, as discussed below.

3.22.4.2 Building Levels of Complexity: Number of Areas and Functional Modularity in Tree Shrew and Squirrel Visual Cortex

While tree shrews are considered among the closest relatives to primates and interspecies comparisons can provide insights into the evolution of primate visual cortex, it is also useful to compare tree shrews to other species occupying a similar ecological niche (see Kaas, 2002). In the previous section, tree shrews were compared with the flying fox. These mammals shared many common organizational features of visual cortex. In addition, squirrels and tree shrews have many similarities in behavior, and in fact were considered squirrels by the local people in their native Southeast Asian habitat (see Martin, 1990). Both of these highly visual mammals have two types of cone photoreceptors in the retina allowing for some color vision (Jacobs *et al.*, 1980; Petry and Kelly, 1991), and have a high density of ganglion cell output (Kaas and Preuss, 1993). Furthermore, both species have a large and well-differentiated LGN (Figures 6a and 6b; Johnson *et al.*, 1998; Kaas, 2002), a large distinctive pulvinar (Figures 6a and 6b; Lyon *et al.*, 2003a), an unusually large SC (Kaas and Collins, 2001), and a moderate number of visual areas, seven or eight (Figures 5a and 5b). Finally, genetic studies have shown that tree shrews are more related to squirrels than bats (Figure 1; Murphy *et al.*, 2001).

An additional organizational component in mammals with expanded visual systems is that of anatomical and functional modules within early visual areas V1 and V2. At first glance, the organization of V1 and V2 between tree shrews and squirrels seems similar. In each species, the two areas can be identified through myelin staining and they have retinotopic maps that form mirror images. In addition, they show patchy intrinsic connection patterns. However, a closer examination reveals that the anatomical organization of visual cortex in tree shrews is much more elaborate than in squirrels (Bosking *et al.*, 1997; Fitzpatrick, 1996; Rockland and Lund, 1982; Rockland *et al.*, 1982;

Van Hooser *et al.*, 2005c). One distinguishing feature of tree shrews is the distinct ocular dominance layers of V1 (Hubel, 1975). These layers subdivide layer 4 and preserve the ocular segregation of the inputs from separate geniculate layers. While individual neurons in rodent visual cortex can receive relayed input primarily from one eye or the other, there is no evidence for structural preservation of ocular dominance. Ocular dominance is a prominent feature in ferrets, cats, and Old World monkeys, which take the shape of columns rather than layers. While ocular dominance is very distinctive in some highly visual species, it remains unclear whether a functional advantage can be attributed to these modules (Horton and Adams, 2005).

Anatomically, tree shrews and squirrels both exhibit long-range intrinsic connections (Figures 7a and 8a; Rockland and Lund, 1982; Kaas *et al.*, 1989). Yet, the pattern is patchy and more widespread in tree shrews (Figure 8a; Rockland and Lund, 1982; Rockland *et al.*, 1982; Lyon *et al.*, 1998; Van Hooser *et al.*, 2005c). Rockland and colleagues (1982) revealed several, fine 200μm wide bands of labeled cell bodies and axon terminations evenly distributed throughout a several millimeter wide region of V1 (up to 8mm) from a single tracer injection in V1. The size and periodicity of the bands resembled banding from 2-deoxyglucose (2-DG) uptake following full field visual stimulation of moving bars presented at a single orientation (Rockland *et al.*, 1982). In 2-DG experiments, radioactive glucose is introduced to the blood supply. Regions of the brain that are metabolically active will incorporate more of the 2-DG into the local neurons. Repetitively stimulating the visual field with bars of a single orientation will preferentially activate only those neurons that prefer that orientation. Thus, this experiment not only demonstrated that the tree shrew brain contains regularly distributed clusters of neurons preferring a similar orientation, it suggested that these regions are likely to be interconnected.

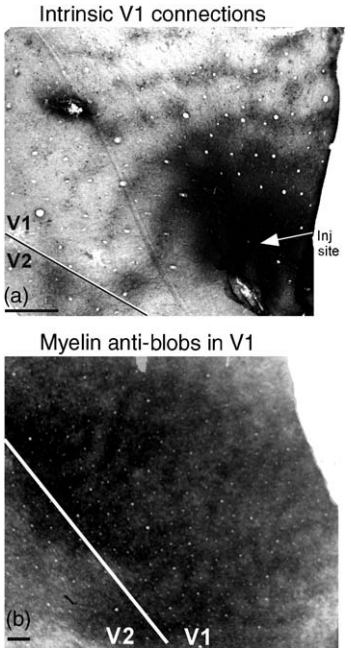

Figure 8 Anatomically defined modules in tree shrew V1. a, An injection of biotynilated dextran amine (BDA) reveals the intrinsic anterograde and retrograde connections of V1. Shown on a tangential section through cortical layer 3, a single injection resulted in a repeating pattern of regularly spaced clusters of labeled terminals and cell bodies within V1. b, Regularly spaced dense and light patches of myelin are also discernable across superficial cortical layers. Scale bar: 500μm. Reproduced from Lyon, D. C., Jain, N., and Kaas, J. H. 1998. Cortical connections of striate and extrastriate visual areas in tree shrews. *J. Comp. Neurol.* 401, 109–128, with permission from John Wiley & Sons.

Consistently with this prediction, recent studies using intrinsic signal optical imaging showed that the connections tend to link similar regions of cortex containing neurons that prefer similar orientations of a visual stimulus (Figure 9c; Bosking *et al.*, 1997). While presenting different oriented sinusoidal gratings to the tree shrew, intrinsic signals related to the neuronal activation were imaged optically and converted into a cortical map of orientation preference (Figures 9a and 9b). Similar maps are also a feature of primary visual cortex in ferret, cat, and all studied primates (see next section). When compared to patterns of connections from tracer injections placed into single orientation domains a high correlation between the location of axon terminals and similar preferred orientation was found (Bosking *et al.*, 1997). In contrast, recent work by Van Hooser *et al.* (2005a, 2005b) has shown that orientation domains are not present in the squirrel (Figure 10). In keeping with this observation, the intrinsic connectivity of squirrel V1 shows qualitatively only a limited patchy pattern (Figure 7a; Kaas *et al.*, 1989) and quantitatively this weak patchiness is not statistically significant (Van Hooser *et al.*, 2005c).

Other anatomical modules in tree shrew include the myelin dark 'anti-blobs' seen in V1 (Figure 8b; Lyon *et al.*, 1998) and the banding pattern of connections in V2 (Sesma *et al.*, 1984; Lyon *et al.*, 1998). While these features are not present in the squirrel, they are characteristics of primate V1 and V2. In tree shrew myelin patches are found in the superficial layers of V1. Such myelin patches have also been demonstrated in the superficial layers of primate V1, and they tend to occupy regions in-between CO blobs, hence the term 'anti-blob'. The functional significance of these myelin-dense patches remains to be determined, but axon

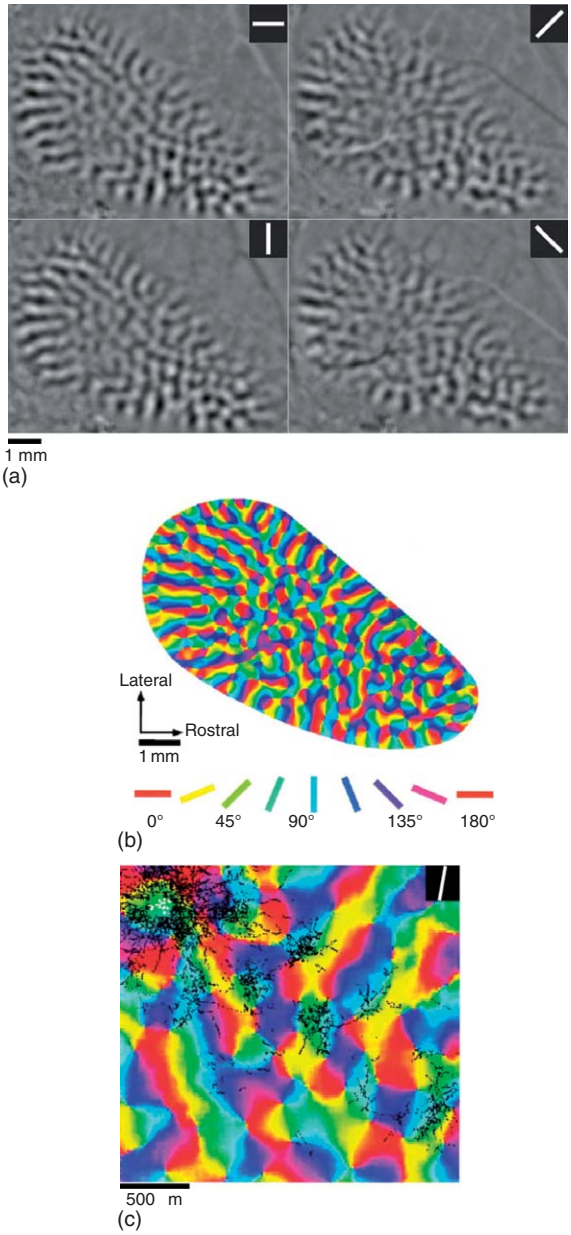

Figure 9 A regular pattern of functionally defined modules in tree shrew V1 can be revealed through intrinsic signal optical imaging. A major advantage of this technique is that it can simultaneously measure the responses of a large group of neurons over a wide area of cortex. a, Different populations of neurons are activated (black region) by a visual stimulus presented at one of four orientations (indicated in the upper right of each panel). Neurons preferring the same orientation are clustered together into modules, called orientation domains. b, A regular pattern of eight different orientation domains in V1 is apparent following stimulation with eight different orientated bars (below). The color-coding of the bars matches the color-coding of the domains. c, An anterograde neuronal tracer injected (white stipple) into a domain comprised of neurons preferring 45° (green) preferentially projects to like-domains. Reproduced from Bosking, W. H., Zhang, Y., Schofield, B., and Fitzpatrick, D. 1997. Orientation selectivity and the arrangement of horizontal connections in tree shrew striate cortex. *J. Neurosci.* 17, 2112–2127, copyright 1997 by the Society for Neuroscience, with permission.

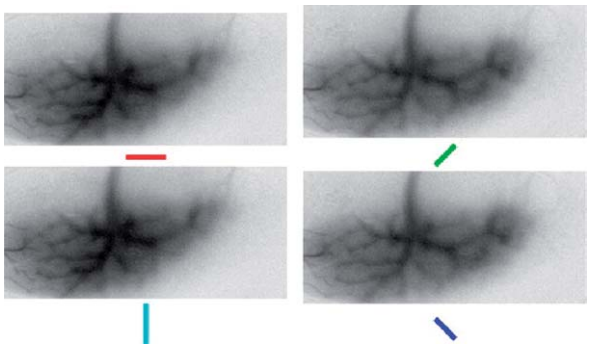

Figure 10 While intrinsic signal optical imaging of V1 in most highly visual mammals has revealed modular organization with respect to orientation processing (see Figure 9), orientation domains are not found in squirrels. These highly visual rodents, while having a comparable number of areas to tree shrews, exhibit less refinement of the internal structure of V1 and in this respect are similar to nonvisual rodents, such as mice and rats. Reproduced from Van Hooser, S. D., Heimel, J. A., Chung, S., Nelson, S. B., and Toth, L. J. 2005a. Orientation selectivity without orientation maps in visual cortex of a highly visual mammal. *J. Neurosci.* 25, 19-28, copyright 2005 by the Society for Neuroscience, with permission.

myelination is a key factor in increased conductivity speed so perhaps these clusters subserve some form of faster processing in V1.

The banding pattern in tree shrew V2 has been revealed through bidirectional tracers injected into V1 and V2. Terminals and cell bodies in V2 labeled by V1 tracer injections were arranged into several bands about 250–300 μm thick that extended from the V1/V2 border to the outer V2/TD border (Sesma *et al.*, 1984). Similar, but more constricted banding was observed following some individual V2 tracer injections, with the bands appearing up to 3 mm from the injection (Lyon *et al.*, 1998). These bands may be similar to bands found in primate V2 (see next section).

While tree shrews and squirrels have in common a moderate number of visual areas, the level of complexity in modular organization is very different. Functional and architectonic modular organization has not been demonstrated in V1 of squirrels, and the organization appears no different than V1 of mice and rats. In contrast, tree shrew V1 is highly modular and resembles many features found in V1 of carnivores and primates. Additionally, tree shrew V2 contains some of the modular anatomical features seen in primates. While the similarity in V1 and V2 between tree shrews and primates can be used to bolster their status as a close primate relative, the lack of modular organization in squirrels (Van Hooser *et al.*, 2005a, 2005b, 2005c) refutes the generally held belief that modularity and expanded visual systems come hand in hand. It may come as a

surprise that squirrels, with a relatively expansive visual system, lack modular organization, but, differences in modular organization in visual cortex between squirrels and tree shrews present an opportunity to explore the functional contributions of modular organization in otherwise similar complex systems.

3.22.5 Highly Complex Visual Systems in Carnivores and Primates

In contrast to the available data on the cortical organization of insectivores, rodents, bats, and tree shrews, the reported literature for cats and monkeys is vast, and includes detailed studies of functional characteristics of neurons while the animals are unanesthetized and performing visual tasks. The number of proposed visual areas in each taxa ranges from 15–19 in cats (Kaas and Krubitzer, 1991; Sereno and Allman, 1991; Payne, 1993) to 25 or more in monkeys (Kaas, 1997a; Van Essen, 2004; Rosa and Tweedale, 2005). While there is widespread agreement that monkeys and cats have a large number of visual areas, there has been less agreement as to each area's name, exact location, function, and even its very existence. In the first three sections here we will sample the available evidence to form a general overview of the organization of visual cortex in cats and ferrets, prosimian primates, and monkeys. In the subsequent sections, a more detailed description of certain cortical areas defined in primates – V1, V2, V3, and middle temporal (MT) – will provide the basis for comparisons with similar areas described in carnivores and other mammals.

3.22.5.1 Cat and Ferret Visual System

The cat as a model system for the study of visual cortex came into prominence through the early experiments of Hubel and Wiesel (1962, 1965, 1998). This seminal work was instrumental in developing an understanding of the receptive field properties of individual neurons in early cortical areas, identified as areas 17, 18, and 19 (but referred to here as areas V1, V2, and V3; see Figure 11). These three areas were considered to represent serial steps in a hierarchical progression where cells found early in the system were characterized as 'simple' and led to 'complex' and 'hypercomplex' cells at subsequent stages in visual cortex (see Payne and Peters, 2002). Though this description is oversimplified and there is a lot of mixing of simple and complex cells at early and later stages in the hierarchy, this organizational scheme set the stage for how mammalian visual cortex is viewed today, namely that visual processing becomes increasingly complex as it progresses across subsequent areas in visual cortex (see Bullier, 2004; Gross, 1997; Salin and Bullier, 1995).

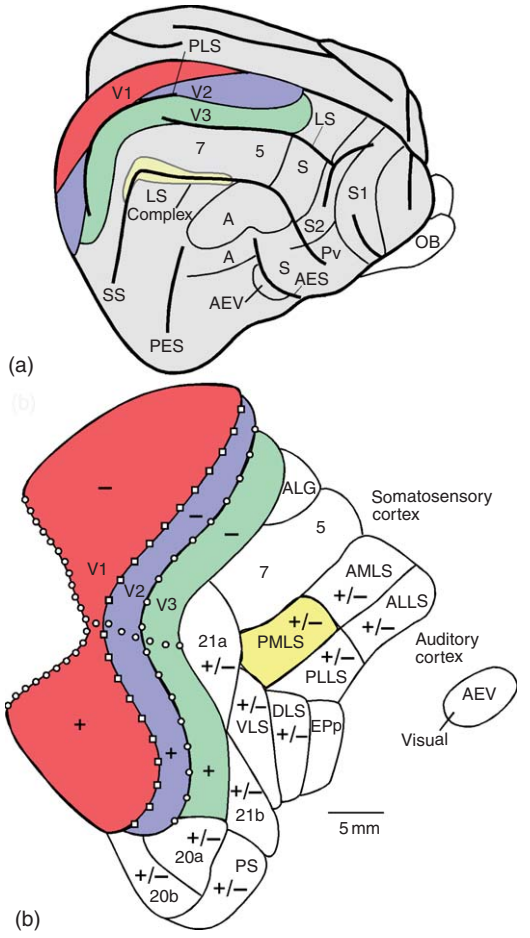

Figure 11 The organization of visual cortex in cats. a, Prominent visual areas, V1, V2, V3, and the LS complex (lateral suprasylvian) are shown relative to sulci, other visually responsive areas (5, 7, and anterior ectosylvian visual area (AEV)), and several nonvisual areas on the surface of an intact cortical hemisphere. Visually responsive areas occupy approximately half of the entire cortical surface. Anterior ectosylvian sulcus (AES); lateral sulcus (LS); posterior ectosylvian sulcus (PES); posterior lateral sulcus (PLS); suprasylvian sulcus (SS). b, A flattened cortical view of the relative sizes and locations of cat visual areas. Of the 19 visual areas shown, many are retinotopically organized (see schematic in Figure 4), though several of the higher-order areas contain incomplete representations of the contralateral visual field (see Kaas and Krubitzer, 1991). V1 (red) and V2 (blue) are considered homologous to early cortical areas found in most mammals. However, whether V3 (green) and PMLS (yellow) can be considered homologous to visual areas in other mammals, in particular areas V3 and MT in primates, remains a matter of debate (see Sections 3.22.5.5 and 3.22.5.6). See Section 3.22.5.1 for further description of cat visual areas and abbreviations. a, Based on Kaas and Krubitzer (1991) and Sereno and Allman (1991). b, Based on Sereno and Allman (1991) and Payne (1993).

As we have seen throughout this article, the earliest cortical stage in the visual system of mammals is V1, as it is the main recipient of the geniculate relay of retinal information. In addition to V1, cat V2 also receives direct projections from the LGN. While V2 in most mammals receives input from the LGN, the projections are less dense than those in cats, especially in other highly visual mammals, such as tree shrews (see Lyon *et al*., 2003b) and primates (Benevento and Yoshida, 1981; Bullier and Kennedy, 1983) – and somewhat less pronounced in the flying fox (Manger and Rosa, 2005). In cats, a large proportion of geniculate projections, stemming primarily from the Y-type ganglion cells (see Section 3.22.5.4.1), go directly to V2 (Stone *et al*., 1979). In this sense, V2 can be considered primary-like, an issue considered in great detail by Payne and Peters (2002). The specific projections of the Y cells directly to V2, provides a nonduplicative primary role for the region. As a result, cell properties in V2 reflect the fast conducting, transient response properties of the Y-cell inputs, whereas many of the V1 cells reflect the slower latency and sustained firing properties of the X-cell inputs. The lack of distinction between primary–secondary is also reflected in the myeloarchitecture of both cat and ferret cortex where, particularly in flattened preparations, there is no major differences between V1 and V2 at the border (Matsubara and Boyd, 2002; Olavarria and Van Sluyters, 1985; unpublished observations). Furthermore, CO blobs, a hallmark of primate V1 (Carroll and Wong-Riley, 1984; Horton and Hubel, 1981), are distributed evenly throughout V1 and V2 in the cat (Matsubara and Boyd, 2002).

Despite certain histological similarities between V1 and V2 in cats, differences in retinotopic organization can be used to reliably delineate the two areas. Through retinotopic microelectrode recordings pioneered by Hubel and Wiesel (1965), a stepwise progression from V1 to V2 revealed that these areas were split by the representation of the vertical meridian, whereas V2 and V3 were separated by the representation of the horizontal meridian. Subsequent studies exploring the retinotopic organization and connectional patterns of cat visual cortex revealed the size and extent of areas V1–V3 (see Figure 11; Tusa *et al*., 1978, 1979). As seen in tree shrew, bat, and squirrel visual cortex, nearly the entire laterorostral extent of V1 is bordered by V2. However, unlike these other species, cat V3 is substantially larger as it is nearly coextensive with the outer border of V2. As for other mammals, the lower visual field is represented dorsally, whereas the upper visual field is represented ventrally. One prominent feature of the retinotopy in these early areas in cats, which is even more pronounced in monkeys, is the greater representation through cortical magnification of the central visual field (see figure 1 in Payne, 1993).

In comparison to the mammals covered earlier in this article, cat cortex is quite expansive, and subsequently in this highly visual species there is room for a larger number of visual areas. Due to the overwhelming number of studies on the retinotopy of these areas and their interconnections, the focus here will be on presenting an overview of the relative locations of each area, while subsequent sections will highlight some of the characteristics of a few of these areas (for extensive reviews of extrastriate cortex in cat, see Payne, 1993; Payne and Peters, 2002; Rosenquist, 1985; Sereno and Allman, 1991; Tusa *et al*., 1981). Figure 11b, adapted and modified from Sereno and Allman (1991) and Payne (1993) shows 19 total visual areas on an unfolded, or flattened, cortical sheet. Beyond V3, as for the moderately complex visual systems in other species, the areas are substantially smaller, and contain complete or nearly complete representations of the contralateral visual hemifield. The relative locations of these areas to the two main sulci, the lateral and suprasylvian, are shown in Figure 11a. Naming of many of the extrastriate visual areas is based on their relative locations along or near one of these sulci. For example, dorsally, just adjacent to the peripheral lower field representation of V3, lies an area along the anterior lateral gyrus, area ALG (Symonds and Rosenquist, 1984). Other areas have been named as an extension of the alternative numbering scheme adapted from Brodmann (1909) that refers to areas V1–V3 as areas 17, 18 and 19 (see Payne and Peters, 2002). These areas, 20 and 21, are found adjacent to the middle and ventral parts of area 19 (V3), and have been split into two areas each – 20a, 20b, 21a, and 21b (Rosenquist, 1985; Tusa and Palmer, 1980). However, the liberal subdividing of these regions results in areas with only incomplete representations of the contralateral visual hemifield (see Kaas and Krubitzer, 1991). Just anterior to the two ventral areas, 20a and 20b, is the posterior suprasylvian area, PS, which also contains an incomplete retinotopic map in that it primarily represents the lower field (Updyke, 1986). While ALG, is among the smallest extrastriate areas described in cats, areas 5 and 7, which are also named based on similarities to architectonically defined regions of Brodmann (1909), are among the largest (after V2 and V3). Anterior to the dorsal portion of V3, in a similar region of posterior parietal cortex as visual areas reported in the flying fox (OP) and tree shrew (TAL; see Section 3.22.4.1),

areas 5 and 7 have only crude retinotopic organizations and contain bimodal neurons responding to visual and somatosensory stimulation (see Manger *et al.*, 2002b). Ventrally in the lateral suprasylvian sulcus is the aptly named area VLS, situated somewhat between areas 21a and 21b. On the opposite, dorsal bank of the suprasylvian sulcus, VLS is bordered by area DLS. While there is some evidence for retinotopic organization of VLS and DLS, area EPp, immediately anterior to DLS, is not retinotopically organized (see Payne, 1993).

Just medial to VLS and DLS, but still along the lateral suprasylvian sulcus there is a large complex of visual areas that roughly corresponds in location to the Clare–Bishop area, a region first identified as visually responsive over 50 years ago (Clare and Bishop, 1954). This complex has been split into as few as two areas (see Sherk, 1986a, 1986b; Shipp and Grant, 1991) and as many as four regions as portrayed in Figure 11 (see Rosenquist, 1985). The four areas are named PMLS, AMLS, PLLS, and ALLS, based on their posterior (P) or anterior (A) locations on the medial (M) and lateral (L) banks of the lateral suprasylvian (LS) sulcus. This region of extrastriate cortex, particularly PMLS, has been studied in detail and comparisons have been made to the direction selective MT, or V5, of primates (see Grant and Hilgetag, 2005; Payne, 1993; also see Section 3.22.5.6). Lastly, the anterior ectosylvian visual area (AEV), located several millimeters anterior to the nearest visual area is unique in that it sits surrounded by cortical areas responsive to modalities other than vision (see Sereno and Allman, 1991). Perhaps because of its unusual isolation, it is helpful to include visual as part of its name.

The cat also contains a rather large visual pulvinar complex in the thalamus (Figure 6c), as we have seen for other visual species. The large size of the cat pulvinar, or lateral posterior-pulvinar complex, reflects the expansiveness of extrastriate visual cortex with which it is extensively interconnected. The LP-pulvinar can be divided into subdivisions of the lateral posterior region including lateral (LPl), intermediate (LPi) and medial (LPm), and the pulvinar-proper (e.g., Hutchins and Updyke, 1989), based on connection patterns with visual cortex and differences in architecture (see Lyon *et al.*, 2003b; Casanova, 2004). The lateral subdivision of the lateral posterior region, LPl, is the only subdivision interconnected with early visual areas V1 and V2. It also connects with the complex of areas in and around the PMLS region, and areas 19, 20, and 21. The medial division, LPm (sometimes considered the inferior subdivision, LPi), also connects with areas 19–21, the PMLS region, as well as posterior parietal areas 5 and 7, and AEV. Likewise the pulvinar-proper connects to the same areas as LPm, but does not connect with AEV. LPi has been distinguished architectonically from LPm as staining darkly for both substance P (Hutsler and Chalupa, 1991) – a marker associated with SC inputs – and AChE (Graybiel and Berson, 1980). This dark staining for AChE is similar to the dorsal subdivision of the tree shrew pulvinar (Figure 6b; see Lyon *et al.*, 2003a).

Other than the multiple areas forming the PMLS complex, the majority of the extrastriate areas in cat, have been identified in their mustelan cousin, the ferret, in a series of retinotopic mapping experiments by Manger and colleagues (Innocenti *et al.*, 2002; Manger *et al.*, 2002a, 2002b, 2004). However, it has been suggested that areas similar to those found in the cat PMLS complex are also present in ferrets (Manger, 2005), so the two species appear to share the same number of cortical visual areas. Because there is a rather substantial overall difference in brain size between the two species, it is perhaps surprising that the number of extrastriate visual areas is comparable. Manger (2005) has used these observations as support for the idea that, within orders, there is little interspecies variability in cortical organization. However, we have already seen substantial differences between squirrels and smaller, less visual rodents (see Section 3.22.3). Yet, rodent species vary significantly in their emphasis on vision, and it can be argued that the entire carnivore order is highly visual, as visual cortex is also large in other carnivore species such as the mink (see McConnell and LeVay, 1986). Perhaps, this is what drives the similarity in visual cortex organization in carnivores. Another issue to consider is that V1 and V2 in both cats and ferrets is highly modular as revealed through connectivity patterns and optical imaging (Gilbert and Wiesel, 1983; Kisvarday *et al.*, 1997; Weliky *et al.*, 1996; White *et al.*, 1999). Yet, one of the more striking anatomical modules found in cats and primates, the CO blobs in V1, are not present in ferret V1. Perhaps at this level, cats and ferrets differ in complexity as differences in V1 modular organization exist between tree shrews and squirrels as well (see Section 3.22.4.2).

3.22.5.2 Prosimian Visual System

Prosimian primates – lemurs and lorises – having split over 50 Mya from other primates are considered to have retained many early primate features (see Fleagle, 1988). Thus, understanding prosimian cortical organization provides insights into the organization that may have been present in the earliest primates. Only a few prosimian species have been

studied, with the galago or bush baby (*Otolemur garnetti*) examined in the most detail. As we have seen for the mammals covered previously in this review, V1 is easily distinguishable as a large, densely myelinated region in the caudal-most portion of the prosimian cortex. As in cats, staining for the metabolic enzyme, CO, reveals regularly distributed blobs throughout V1 (Casagrande and Kaas, 1994). Earlier reports found a lack of CO blobs in certain prosimian species (see Sereno and Allman, 1991). Because CO blobs are present in all studied monkeys and apes, but not in every prosimian species, this left open the possibility that blobs evolved independently in monkeys and prosimians (see Preuss and Kaas, 1996). However, the absence of CO blobs in earlier preparations may have been due to technical difficulties, as subsequent studies demonstrated that CO blobs are a common feature in prosimian V1 (Preuss and Kaas, 1996).

In galagos, retinotopic maps of V1 show an orderly representation of the contralateral visual hemifield, with a larger portion of cortex representing central vision (Rosa *et al.*, 1997). In addition, galago V1 exhibits intrinsic patchy connectivity (Cusick and Kaas, 1988b; Preuss *et al.*, 1993; Lyon and Kaas, 2002c) and contains an orderly orientation preference map (Xu *et al.*, 2005). Immediately adjacent to V1 lies V2 (see Figure 12), which can be identified through myeloarchitecture as well (Krubitzer and Kaas, 1990; Collins *et al.*, 2001; Lyon and Kaas, 2002c). Galago V2 resembles V2 in other species as it represents a compressed, rough mirror image of the representation of the visual field in V1 (Rosa *et al.*, 1997), and receives feedforward inputs from and sends feedback to V1 (Collins *et al.*, 2001; Symonds and Kaas, 1978; Tigges *et al.*, 1973).

A second extrastriate cortical area common to all primates is the MT area (see Kaas and Lyon, 2001). First described in New World monkeys (Allman and Kaas, 1971b), homologous area was subsequently identified in prosimians using similar criteria (Allman *et al.*, 1973; Tigges *et al.*, 1973; Symonds and Kaas, 1978). Whether a homologous area is present in other mammals is a matter of debate (see Section 3.22.5.6; Payne, 1993; Kaas, 2002; Rosa and Tweedale, 2005).

In prosimians, as many as 12 more extrastriate visual areas (Figure 12) are discernable through feedback connections to V1 (Cusick and Kaas, 1988b; Krubitzer and Kaas, 1993; Lyon and Kaas, 2002a; Preuss *et al.*, 1993), V2 (Collins *et al.*, 2001), and MT (Krubitzer and Kaas, 1990). One of these 12 extrastriate areas, V3, had not been identified in prosimians (Allman *et al.*, 1979; Beck and Kaas, 1998a; Collins *et al.*, 2001; Rosa *et al.*, 1997) until recently, when several tracer injections placed in estimated retinotopic locations of V1 proved instrumental in dividing extrastriate visual cortex of the galago (Lyon and Kaas, 2002a). As a result, for example, the dorsal medial area, DM, originally identified through microelectrode mapping of retinotopy (Allman *et al.*, 1979, Rosa *et al.*, 1997), has

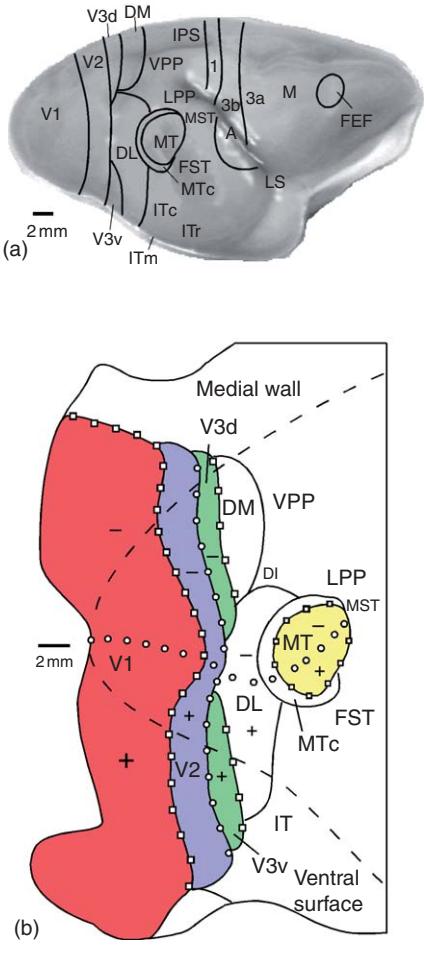

Figure 12 The organization of visual cortex in prosimian primates. a, The locations of several visual and nonvisual areas are shown on a digital photograph of the cortical surface of an intact galago brain. The intraparietal (IPS) and lateral (LS) sulci are clearly visible, while the superior temporal sulcus is present as a slight dimple ventral to MT. b, A flattened cortical view of the relative sizes and locations of galago visual areas. V1 (red) and V2 (blue) are likely homologous to similar areas present in other mammals, while primate areas V3 (green) and MT (yellow) may be unique to the primate order. The homology of primate V3 and MT to areas present in nonprimates is a matter of debate (see Sections 3.22.5.5 and 3.22.5.6). Nevertheless, the existence of V3 and MT, as well as several other visual areas (DL, DM, DI, MTc, MST, IT) in prosimian primates indicates that these areas were present early in primate evolution. For abbreviations and detailed descriptions of galago visual areas see Section 3.22.5.2. a and b, Modified from Lyon, D. C. and Kaas, J. H. 2002a. Connectional evidence for dorsal and ventral V3, and other extrastriate areas in the prosimian primate, *Galago garnetti*. *Brain Behav. Evol.* 59, 114–129.

been slightly displaced by V3 from its original location adjacent to the anterior border of dorsal V2 (see Figure 12; Lyon and Kaas, 2002a). Anterior to DM and dorsal to MT, at least two more areas have been identified the lateral and ventral areas of posterior parietal cortex, LPP and VPP (Beck and Kaas, 1998a; Lyon and Kaas, 2002a). Ventral to DM, along the anterior border of the ventral two-thirds of V3 is the dorsal lateral visual area, DL (Cusick and Kaas, 1988b), a possible homologue of macaque monkey V4 (see Stepniewska *et al.*, 2005). Some studies suggest that prosimian DL can be split into caudal and rostral subdivisions as proposed for both New and Old World monkeys (Cusick and Kaas, 1988a; Lyon and Kaas, 2002c; Stepniewska *et al.*, 2005). Anterior to the ventral half of DL, at least three areas in inferior temporal cortex (IT) can be distinguished based on clusters of labeled cells in different relative locations – the caudal (ITc), medial (ITm), and rostral (ITr) areas, as described for New World monkeys (Weller and Kaas, 1987). Surrounding MT, three more areas, the MT crescent (MTc), the area of the fundus of the superior temporal sulcus (FST), and the medial superior temporal area (MST) are also distinguishable.

Some of the available evidence suggests that galagos and perhaps prosimians in general represent a scaled-down version of the organization of monkey visual cortex (see Kaas, 2004a, 2004b). For example, monkey V1 is larger and contains a greater representation of central vision (Van Essen *et al.*, 1984; Gattass *et al.*, 1987; Rosa *et al.*, 1997). Furthermore, while both monkeys and prosimians have CO blobs in V1, the monkey – but not galago – V2 also contains distinct cytoarchitectonic bands (see Sections 3.22.5.3 and 3.22.5.4; however, see Preuss and Kaas, 1996). In addition, the pulvinar nucleus, which is a distributor of visual information to all of visual cortex, is larger in monkeys than in prosimians and can be divided into several subcompartments based on architectonic criteria and cortical projection patterns (Beck and Kaas, 1998b; Stepniewska, 2003). Despite these differences, the number and locations of visual areas described for prosimians is very similar to those described in monkeys (see Figures 12 and 13; Kaas, 1997a, 2004a, 2004b; Kaas and Lyon, 2001; Van Essen, 2004; Rosa and Tweedale, 2005).

3.22.5.3 New World and Old World Monkey Visual Systems

Several species of New World monkeys have been examined from the very small 300g marmoset monkey and moderately sized 1kg squirrel monkey, to the large, macaque-sized cebus monkey. Various species of the macaque monkey genus have been the Old World monkey of choice. Weighing as much as 15kg and boasting a brain size as large as 10 times that of the tiny marmoset (80g vs. 8g; see Rosa and Tweedale, 2005), macaques are used as the primary model for comparisons to the human visual system (Brewer *et al.*, 2002; Fize *et al.*, 2003; Orban *et al.*, 2004; Sereno and Tootell, 2005; Tootell *et al.*, 2003; Van Essen, 2004). Emphasis on the macaque monkey as a human model is due in part to historical factors and the ready availability of this species (Rosa and Tweedale, 2005). Yet, despite the size differences and the 10 million years of evolution that separate the New World and Old World monkey lineages, several studies indicate that the organization of their visual cortex is quite similar (Casagrande and Kaas, 1994; Lyon and Kaas, 2002b, 2002c; Lyon *et al.*, 2002; Rosa and Tweedale, 2005). This is particularly true for the early, caudal visual areas that have been studied in more detail in both New and Old World species (see Kaas and Lyon, 2001; Kaas, 2004a). Therefore, this section will review studies on visual cortex organization in both New and Old World monkeys.

Monkey visual areas V1 and V2 are well established as the two largest visual areas in primate cortex (see Figures 13 and 14). V1 and V2 are found at the caudal end of cortex and are likely homologous to V1 and V2 of all mammals (see Sections 3.22.2 and 3.22.5.4). Of all the remaining extrastriate visual areas proposed to exist in monkeys, area MT (see Section 3.22.5.6) is the only one that is unanimously accepted (see Felleman and Van Essen, 1991; Kaas and Lyon, 2001). MT, also referred to as V5, was first described as a densely myelinated retinotopically organized region located several millimeters anterior to V2 in the middle temporal lobe of New World owl monkeys (Allman and Kaas, 1971b) and as a region on the posterior bank of the superior temporal sulcus (STS) in macaque monkeys that received direct projections from V1 (Zeki, 1971). Subsequently, MT of macaques was also shown to be retinotopically organized (Gattass and Gross, 1981; Van Essen *et al.*, 1981). Additional work demonstrated that MT is likely to be a common primate feature (see Kaas and Lyon, 2001; Kaas, 2004a). Whether primate MT evolved from an area common to nonprimates is a matter of debate (Kaas, 2002; Rosa and Tweedale, 2005; see Section 3.22.5.6.1). Studies on the functional properties of MT show that it plays a major role in the processing of binocular

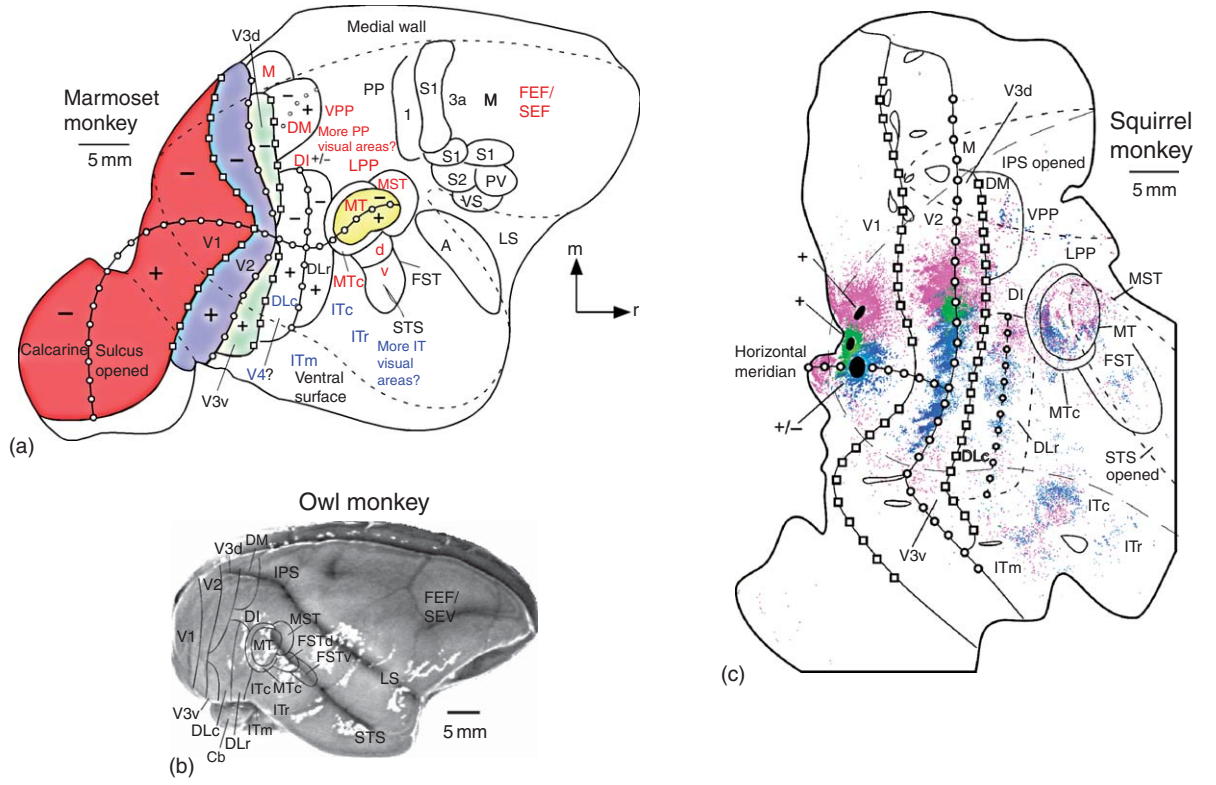

Figure 13 The organization of visual cortex in New World monkeys. a, The locations and sizes of visual areas in New World monkeys are shown on a flattened sheet of an entire cortical hemisphere from a marmoset monkey. Like other highly visual species V1 (red shading) and V2 (blue shading) are quite large, and retinotopically organized (see schematic in Figure 4a). V3 (green shading) is present as two distinct dorsal (d) and ventral (v) divisions, each representing a compressed mirror image of the retinotopic organization in dorsal and ventral portions of V2. Several more retinotopic areas (DLc, DLr, DI, DM, and MT) are present in extrastriate cortex (indicated by +/–). Areas lettered in red, fed predominantly through feed-forward projections from direction selective area MT, are thought to comprise the dorsal stream of visual processing related to motion and spatial processing, whereas areas lettered in blue, fed predominantly by V4 (Dlc/DLr), are related to the processing of color and form (see Section 3.22.5.3). SEF, supplementary eye field; FEF, frontal eye field. b, The positions of several areas relative to the sulcus pattern on the cortical surface are shown on a digital photograph of an owl monkey brain. Intraparietal sulcus (IPS), lateral sulcus (ILS), superior temporal sulcus (STS). c, The pattern of retrogradely labeled neurons following injections (black ovals) of three tracers into different retinotopic locations of V1 in a single case is shown on a flattened reconstruction of the caudal half of squirrel monkey cortex. Colored dots represent individually labeled neurons from the corresponding tracer injection. An injection of fast blue (blue) was made on the representation of the horizontal meridian (line of circles, see schematic in Figure 4a), thus labeled neurons in extrastriate cortex were found near known locations of the horizontal meridian. For example, the horizontal meridian is located at the V2/V3 and DLc/DLr borders, as well as the caudal border of MT. Injections of diamidino yellow (green) and cholera toxin subunit-b (CTB; purple) were placed near the horizontal meridian, but slightly medial, in cortex representing progressively more peripheral vision. As a result, the labeled cells in extrastriate cortex from each of the two injections were located progressively more medial along the horizontal meridians at the V2/V3 and DLc/DLr borders. Interestingly compared to the smaller marmoset monkey V3 which is split into dorsal and ventral divisions (a), V3 in squirrel monkeys is a continuous strip. Additional retrogradely labeled neurons were found in several other cortical areas, indicating that a wide region of visual cortex provides feedback to V1. For abbreviations and detailed description of the organization of New World monkey visual areas see Section 3.22.5.3. a, Modified from Lyon, D. C. and Kaas, J. H. 2001. Connectional and architectonic evidence for dorsal and ventral V3, and dorsomedial area in marmoset monkeys. *J. Neurosci.* 21, 249–261. b, Modified from Lyon, D. C., Xu, X., Casagrande, V. A., Stefansic, J. D., Shima, D., and Kaas, J. H. 2002. Optical imaging reveals retinotopic organization of dorsal V3 in New World owl monkeys. *Proc. Natl. Acad. Sci. USA* 99, 15735–15742; Based on data from Lyon, D. C. and Kaas, J. H. 2002c. Evidence from V1 connections for both dorsal and ventral subdivisions of V3 in three species of New World monkeys. *J. Comp. Neurol.* 449, 281–297. c, Modified from Lyon, D. C. and Kaas, J. H. 2002c. Evidence from V1 connections for both dorsal and ventral subdivisions of V3 in three species of New World monkeys. *J. Comp. Neurol.* 449, 281–297, with permission from John Wiley and Sons.

disparity and the direction and speed of visual stimuli (Albright, 1984; Born and Bradley, 2005; Felleman and Kaas, 1984; Maunsell and Van Essen, 1983b), and is likely a primary source of input to the motion and spatial processing areas in the dorsal stream of visual processing (Maunsell and Van Essen, 1983a; Shipp and Zeki, 1995; Ungerleider and Desimone, 1986).

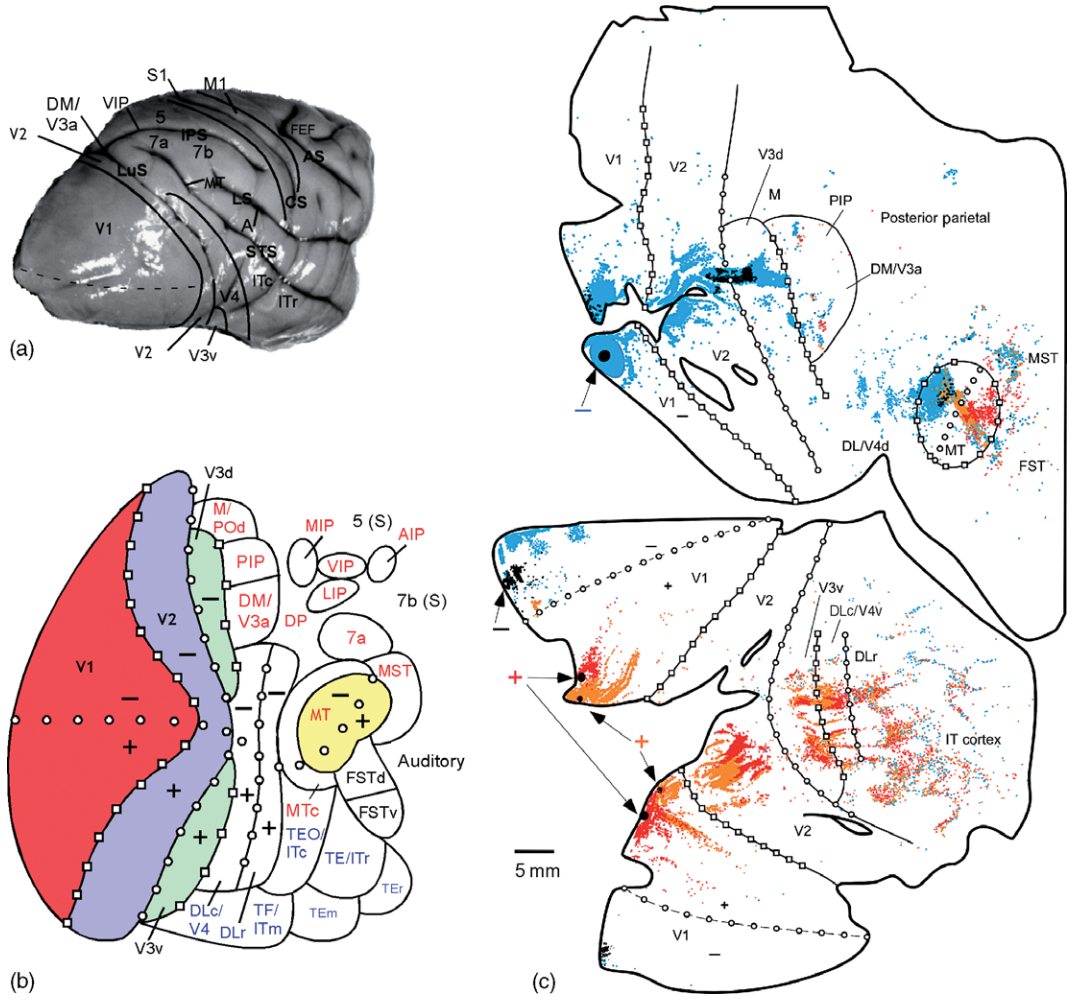

Figure 14 The organization of visual cortex in Old World macaque monkeys. a, The positions of several areas relative to the sulcus pattern on the cortical surface are shown on a digital photograph of a macaque monkey brain. The majority of V2, all of V3 and V3a, are buried within the lunate sulcus (LuS). Area MT and its satellites are buried within the dorsal extent of the superior temporal sulcus (STS), whereas much of the higher-order ventral stream areas in inferotemporal cortex (IT) are located within the ventral extent of the STS. Higher-order dorsal stream areas such as the ventral intraparietal area (VIP) are buried within the intraparietal sulcus (IPS). AS, arcuate sulcus; CS, central sulcus; FEF, frontal eye field. b, The relative locations and sizes of visual areas in macaque monkeys are shown on a flattened representation of the caudal half of cortex. Many visual areas reported for the macaque are likely homologous to those proposed for New World monkeys (see Figure 13), especially in the caudal extent of visual cortex, including areas V1, V2, V3, V4 (DLc/DLr), MT, MTc, MST, FSTd/v, DM (V3a), POd (M), DP (DI). However, several more areas have been described in the higher-order stations of the dorsal and ventral processing streams. It is uncertain whether these areas represent an expansion of visual cortex in Old World monkeys because (1) similar areas have been described in larger New World cebus monkeys (see Rosa and Tweedale, 2005) and (2) few studies have looked at the detailed organization of higher-order visual cortex in the more commonly studied smaller New World monkey species. TE, temporal cortex; TEm, middle subdivision of TE; TEr, rostral subdivision of TE; TEO, temporal occipital cortex. c, The pattern of retrogradely labeled neurons following injections (black ovals) of four tracers into different retinotopic locations of V1 in a single case is shown on a flattened reconstruction of the caudal half of macaque cortex. Colored dots represent individually labeled neurons from the corresponding tracer injection. Two injections were placed in the upper visual field representation in ventral V1, one (orange) nearer the representation of the vertical meridian (line of squares) found at the V1/V2 border, and the other nearer the representation of the horizontal meridian (line of circles; see schematic in Figure 4a). In adjacent ventral cortex, a series of mirror reversals of the retinotopic locations of the V1 injections was found in the pattern of retrogradely labeled cells in extrastriate areas V2, V3, DLc (V4), and DLr. A similar pattern of labeled cells resulted in dorsal visual cortex following two tracer injections in the lower visual field representation of dorsal V1. Retinotopic patterns of retrogradely labeled cells from the dorsal and ventral V1 injections were found in MT as well. A crude retinotopic pattern of connections with MST was also present. Labeled neurons in areas V3a and PIP were sparse, yet indicated a crude retinotopy. Few cells, if any, were labeled in PP, indicating that higher-order dorsal stream areas do not provide feedback to V1. In contrast, numerous cells were labeled in IT cortex (nonretinotopically), indicating that, like New World monkeys, higher-order ventral stream areas provide feedback to V1. For abbreviations and detailed description of the organization of macaque monkey visual areas see Sections 3.22.5.2 and 3.22.5.3. c, Modified from Lyon, D. C. and Kaas, J. H. 2002b. Evidence for a modified V3 with dorsal and ventral halves in macaque monkeys. *Neuron* 33, 453–461, with permission from Elsevier.

Probably the greatest factor contributing to the establishment of V1, V2, and MT as valid visual areas is that these are the only areas that can be reliably identified through architectonic criteria, such as stains for myelin and CO (see Figures 15 and 16). The numbers, locations, and sizes of the myriad of remaining, proposed extrastriate visual areas in monkey cortex remain uncertain. In some cases it is simply a difference in area size and terminology (DL vs. V4; see Stepniewska *et al.*, 2005), while in other cases it is a reported difference in connectivity and function (dorsal V3 vs. ventral V3; see Section 3.22.5.5). As a result, several different organizational schemes of monkey visual cortex have been proposed (see Kaas, 1997a; Rosa, 1997; Van Essen, 2004). Methods used to identify areas play a large role in the observed variability. For example, fine-scale methods such as microelectrode mapping have been used to demonstrate several retinotopically organized areas in the third tier of visual cortex (Allman and Kaas, 1975; Rosa and Schmid, 1995); conversely a single area, V3, is revealed through techniques capable of measuring activity of larger regions of cortex simultaneously, such as intrinsic signal optical imaging (Lyon *et al.*, 2002; Xu *et al.*, 2004) and fMRI (Brewer *et al.*, 2002; Fize *et al.*, 2003). While others have provided comparisons of the many proposals of monkey visual cortex organization (Kaas, 1997a; Rosa, 1997; Van Essen, 2004), the goal of this section is to present a composite representation (see Figures 13 and 14) of the various versions to allow for straightforward comparisons of New and Old World monkeys, and to provide a scheme that can be readily compared to those presented for other mammals.

Other than areas V1, V2, and MT, a conservative estimate of the number of proposed visual areas in monkeys is slightly greater than 20 (see Figures 13a and 14b). Areas early in the cortical hierarchy located more caudally have been identified through similar methodologies in both New and Old World monkeys. Retrograde tracing studies that focused on the feedback connectivity patterns of these areas to V1 have served as a useful means for comparison across species, revealing many similarities in organization (see Figures 13c and 14c). On the other hand, cortical areas located more distantly from V1, for example in PP, have been studied more extensively in macaque monkeys (see Andersen, 1995; Andersen *et al.*, 1985b; Colby and Duhamel, 1991; Lewis and Van Essen, 2000a, 2000b), making comparisons with New World monkeys more difficult.

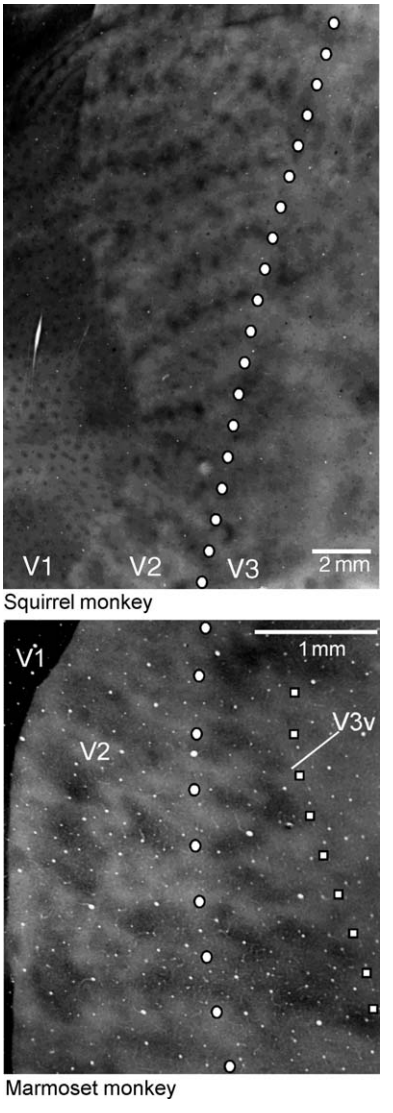

Figure 15 When processed for the presence of cytochrome oxidase (CO), cortex manually flattened and cut tangential to the cortical surface shows a regular pattern of dark (high concentration of CO) and light (low concentration of CO) modules in early visual areas of monkeys. As shown in squirrel monkey cortex (top panel), in V1, the dark CO patches form small, discrete blobs, whereas in V2 the blobs are strung together to form stripes, or bands. The V2 bands are characterized as either 'thick' or 'thin', and have been linked to the dorsal and ventral visual processing streams, respectively (see Section 3.22.5.4.1). Though less distinct than the bands in V2, dark CO bands are often visible in V3 (bottom panel). Whether these bands are associated to either of the visual processing streams remains to be determined, but it has been shown that band-like patches of labeled neurons become labeled in V3 following tracer injections into dorsal stream areas DM and MT (Lyon and Kaas, 2001). Squirrel monkey, reproduced from Lyon, D. C. and Kaas, J. H. 2002c. Evidence from V1 connections for both dorsal and ventral subdivisions of V3 in three species of New World monkeys. *J. Comp. Neurol.* 449, 281–297, with permission from John Wiley & Sons. Marmoset monkey, reproduced from Lyon, D. C. and Kaas, J. H. 2001. Connectional and architectonic evidence for dorsal and ventral V3, and dorsomedial area in marmoset monkeys. *J. Neurosci.* 21, 249–261, copyright 2001 by the Society for Neuroscience, with permission.

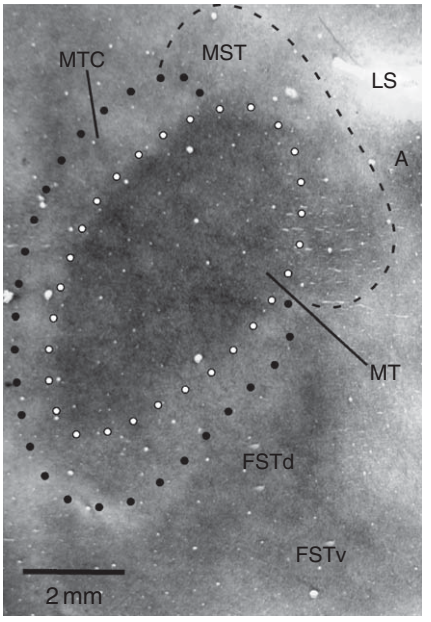

Figure 16 In flattened cortical preparations, dense myelination easily distinguishes primate area MT from surrounding cortical areas. Furthermore, in this section through the superficial layers of cortex dark and light patches within MT indicate a modular organization of dense and light myelination. In addition to differences in myelination, several forms of modular organization within MT have been revealed through a variety of techniques (see Sections 3.22.5.3 and 3.22.5.6 for more details of MT and surrounding cortical areas). Reproduced from Lyon, D. C. and Kaas, J. H. 2001. Connectional and architectonic evidence for dorsal and ventral V3, and dorsomedial area in marmoset monkeys. *J. Neurosci.* 21, 249–261, copyright 2001 by the Society for Neuroscience, with permission.

In addition to the identification of discrete cortical areas, primate visual cortex can be segregated into the dorsal and ventral streams of visual processing (see Figures 13a and 14b). The identification of these processing streams derives from lesion studies in cats and primates, including humans, revealing a segregation of function into separate cortical pathways (see Ungerleider and Mishkin, 1982; Ungerleider and Pasternak, 2004). Motion and spatial information are processed in a stream of neighboring visual areas found more dorsally within occipital and parietal cortex, the dorsal or parietal stream. Conversely, color and form processing is most pronounced in neighboring ventral regions within occipital and temporal cortex, the ventral or temporal stream. The higher-order areas comprising the two streams are fed in varying degrees by early caudal visual areas, V1, V2, V3, V4 and MT (Van Essen and De Yoe, 1995).

V3, considered a provider of inputs to both streams (see Gegenfurtner *et al.*, 1997), is located immediately adjacent to V2 as a narrower strip of cortex that was first identified through its connectivity with V1 (Cragg, 1969; Zeki, 1969). While a V3-like area has been identified in several other mammalian species as discussed throughout this article and elsewhere (see Rosa, 1999), the organization and even the very existence of primate V3 remains controversial (Kaas and Lyon, 2001; see Section 3.22.5.5). Monkey V3 has a retinotopic map representing a condensed mirror image of the visual field representation in V2 as revealed through microelectrode mapping (Gattass *et al.*, 1988), intrinsic signal optical imaging (Lyon *et al.*, 2002; Xu *et al.*, 2004), fMRI (Brewer *et al.*, 2002; Fize *et al.*, 2003), and connections with primary visual cortex (Lyon and Kaas, 2001, 2002b, 2002c). Importantly, V3 can be identified in flat mounted sections through myelo- and cytochrome architecture (Figure 15; Lyon and Kaas, 2001, 2002c; Sincich *et al.*, 2003; Xu *et al.*, 2004). In this regard, V3 joins the company of the three well-established areas – V1, V2, and MT. Though less reliably demonstrated, the CO architecture in V3 reveals modular light and dark bands. These bands are like those found in V2, yet thicker and less differentiated (Lyon and Kaas, 2001; Xu *et al.*, 2004). Connection patterns with well-established areas such as MT reveal a band-like pattern of modular organization in V3 as well (Lyon and Kaas, 2001).

Often associated with the ventral stream, V4, or DL, lies adjacent to V3. This region of cortex is less distinguishable through staining procedures, but has been identified principally through microelectrode mapping and connection patterns with well-established cortical areas (Gattass *et al.*, 1988; Piñon *et al.*, 1998; Stepniewska *et al.*, 2005). Issues as to the dorsal and ventral extents of DL/V4 remain unresolved. Some researchers have the area extending well onto the ventral cortical surface, adjacent to the entire extent of ventral V3 and even extending as far as the ventral extreme of V2 (Gattass *et al.*, 1988), whereas others have proposed a truncated version based on a change in connectivity patterns for tracers injected more ventrally (Stepniewska *et al.*, 2005). The ventral DL/V4 border illustrated in Figures 13 and 14 is intermediate to the proposals contrasted above. This border is derived from studies on the connection patterns from tracer injections placed in different retinotopic locations in V1 of New and Old World monkeys, including the peripheral representation of the upper visual quadrant, which is likely to be found near the ventral-most regions in areas V3 and V4 (Lyon and Kaas, 2002b, 2002c). As MT is often viewed as a distributor of feedforward inputs to dorsal stream

areas, V4 is considered to be a main distributor to ventral stream areas (see Figure 13a; Shipp and Zeki, 1995; Van Essen and De Yoe, 1995).

The dorsointermediate, dorsomedial, and medial areas (DI, DM, and M, respectively), are consecutively located dorsal to DL/V4. In the organizational scheme presented here, the dorsalmost area M lies adjacent to the dorsal-most portion of V2. Area M, first described in New World owl monkeys (Allman and Kaas, 1971a), may be a homologue of the dorsal parietal occipital area (POd) or the posterior intraparietal area (PIP) described in macaques (Colby *et al.*, 1988; Felleman and Van Essen, 1991). Ventral to M, areas DM and DI are displaced from the outer border of V2 by dorsal V3. Earlier descriptions based on studies of New World monkeys placed these areas immediately adjacent to V2 (Allman and Kaas, 1975; Rosa and Schmid, 1995); however, subsequent connectional and architectonic evidence has shown that these areas, particularly DM, are in the location of V3a as described in macaques (Lyon and Kaas, 2001, 2002b, 2002c; Van Essen and Zeki, 1978). Whether DI in macaques lies ventral to DM/V3a, or V3a can be split into two regions, the ventral of which may correspond to DI, is uncertain. Connections with V1 show separate clusters of cells in the dorsal and ventral halves of V3a, termed PIP or M dorsally and V3a (Figure 14c) or DM ventrally. However, it may be that the ventral portion corresponds to DI and the dorsal portion to DM/V3a. This interpretation is more consistent with results found in similar experiments of New World monkeys where connections between V1 and M were not found (Lyon and Kaas, 2002c).

In the vicinity of the dorsal extent of the STS (the exact location relative to the STS depends on the size of the monkey; see Figures 13b and 14a), MT is easily identified through its myelo- and cytoarchitecture (Figure 16). Particularly in flattened cortex preparations, MT serves as a reliable point of comparison for the examination of architecture, connection patterns, and microelectrode mapping of visual areas in the immediate vicinity. Sometimes referred to as the 'MT-satellites' several areas surrounding MT have been identified (Allman and Kaas, 1974; Desimone and Ungerleider, 1986; Kaas, 2004a; Krubitzer and Kaas, 1990; Rosa, 1997; Rosa and Elston, 1998). The MTc surrounds much of the dorsal, posterior, and ventral borders of MT (Allman and Kaas, 1974; Rosa and Elston, 1998) and can be differentiated from MT as a retinotopically organized arc of moderately myelinated cortex and through its unique pattern of connections with the ventral division of area FST (Kaas and Morel, 1993; Lyon and Kaas, 2001; Rosa and Elston, 1998). FST, first identified in macaques (Desimone and Ungerleider, 1986), extends from the ventral border of MT and MTc and can be split into separate dorsal and ventral areas, FSTd and FSTv (Krubitzer and Kaas, 1990; Kaas and Morel, 1993). The MST area is located adjacent to the representation of the peripheral visual field in MT, along its medial anterior border (Maunsell and Van Essen, 1983a; Desimone and Ungerleider, 1986; Ungerleider and Desimone, 1986; Rosa and Elston, 1998) and receives inputs from functionally distinct modules in MT (Berezovskii and Born, 2000).

Based on connectivity patterns (Felleman and Van Essen, 1991) and the evidence for roles in the processing of increasingly complex moving stimuli, such as spiral motion (Duffy and Wurtz, 1995), the MT satellites can be considered the second stages of the dorsal stream after MT. Dorsal to MT, areas in PP represent a third stage in the dorsal stream as they receive the bulk of their input from the MT satellites. In New World monkeys, based on feedback projections to V1, DM and the SC (Collins *et al.*, 2005; Krubitzer and Kaas, 1993; Lyon and Kaas, 2001, 2002c), only two areas within PP have been identified, LPP, located just dorsal to MT, and VPP, dorsal to LPP and just anterior to DM. Initial macaque monkey studies identified similar regions, area 7a (Andersen *et al.*, 1985a, 1985b; Motter and Mountcastle, 1981) just dorsal to MT, and the ventral area of the intraparietal sulcus, VIP (Maunsell and Van Essen, 1983a). Subsequent studies have proposed several more visual areas in PP. While none of these areas shows any precise retinotopic organization, contributing to the uncertainty, connection studies placing injections directly into different regions of the PP and functional mapping studies have led to the identification of at least four areas located medial, ventral, lateral, and anterior in the intraparietal (IP) sulcus – areas MIP, VIP, LIP and AIP (Figure 14b; see Colby and Duhamel, 1991; Andersen, 1995; Lewis and Van Essen, 2000a).

Despite uncertainty as to the exact number of areas in PP, this region seems greatly expanded compared to the amount of PP processing vision in the cat (see Payne, 1993). Neurons in this region of monkey cortex are highly selective for complex spatial perception (Siegel and Read, 1997) and are heavily involved in deciding where next to move

the eyes (Duhamel *et al.*, 1992) – cortex involved in eye movements is less prominent in cats (see Payne, 1993). Accordingly, these areas, as well as MT and some of its satellites, are heavily interconnected with eye movement fields in prefrontal cortex (FEF; Schall *et al.*, 1995). In addition, neurons in some areas are multimodal, responding to both visual and somatosensory stimuli (Duhamel, 2002), while neurons in AIP play a visual role in the guidance for the grasping of objects (Fogassi *et al.*, 1996; Jeannerod *et al.*, 1995) – a feature unlikely to be of as much use to carnivores.

Areas comprising the ventral stream are located ventral to MT in the IT, and have little interconnections with areas in PP (Baizer *et al.*, 1991). Like the areas in PP, retinotopic organization of areas in IT is coarse at best (Boussaoud *et al.*, 1991; Desimone and Gross, 1979). Even so, the IT region can be subdivided into at least four areas based on connectivity patterns and functional differences (see Figure 14b; Baizer *et al.*, 1991; Buffalo *et al.*, 2005; Distler *et al.*, 1993; Weller and Kaas, 1987). V4 at the head of the ventral stream is fed through V2 projections originating in CO light interbands and CO dark thin bands (Felleman *et al.*, 1997; Shipp and Zeki, 1995; Van Essen and De Yoe, 1995; Xiao *et al.*, 1999). In turn, V4 projects to adjacent area TEO/ITc (where TEO = temporal occipital cortex; Nakamura *et al.*, 1993). These areas can also be identified through direct feedback connections to V1 (Figure 14c; Lyon and Kaas, 2002b, 2002c; Rockland and Van Hoesen, 1994). The segregated V4 inputs are thought to give rise to the specialized object and color processing of cells in the ventral stream. TEO, for example, has been implicated in color selectivity (Tootell *et al.*, 2004) and the perception of objects (Brincat and Connor, 2006), while areas further anterior in the hierarchy are selective for complex objects such as faces (Perrett *et al.*, 1982, 1984).

3.22.5.4 V1 and V2 in Cats and Primates

As we have seen earlier in the article, V1 and V2 are present in most mammals and are considered to have been retained from an early common ancestor (see Section 3.22.2). And, as described for other highly visual mammals, such as tree shrews (see Section 3.22.4.2), cats, ferrets, prosimians, and monkeys have patchy, long-range, intrinsic connections within V1 and V2 (Rockland and Lund, 1982; Gilbert and Wiesel, 1983; Casagrande and Kaas, 1994). Also, as seen for tree shrews, correlation of the connection patterns to functional maps of orientation preference show that like-orientation domains are preferentially connected (Kisvarday *et al.*, 1997; Malach *et al.*, 1993; Schmidt and Lowel, 2002). While these features are found in tree shrews and ferrets, as well as primates and cats, V1 and V2 of the complex visual systems of cats and primates have independently evolved additional features of modular organization. Most notable is the regular distribution of CO blobs in V1 (Casagrande and Kaas, 1994; Horton and Hubel, 1981; Matsubara and Boyd, 2002; Murphy *et al.*, 1995).

While several features have been attributed to CO blobs, such as the processing of color in primates (Livingstone and Hubel, 1988, 1984), these interpretations remain controversial (see Sincich and Horton, 2005). The simplest explanation for blobs is that they arise from increased activity brought about by direct thalamic connections (Livingstone and Hubel, 1982), as CO is a marker for higher metabolic activity (Wong-Riley, 1979) and thalamic afferents provide strong driving inputs (Reid and Alonso, 1996). Blobs, which are most prominent in superficial layer 3, coincide with the termination zones of K cell geniculate afferents (Casagrande, 1994). The lighter interblob regions lack geniculate afferents. Consistent with the interpretation of thalamic afferents resulting in increased CO activity, layer 4C, the main geniculate input zone, receives dense nonpatchy geniculate afferents from M and P cells and as a result stains uniformly dark for CO. Patchy geniculate afferents in cat have also been correlated with the blobs (Matsubara and Boyd, 2002).

3.22.5.4.1 Early parallel processing From the early work in cats and primates, the concept emerged that three ganglion cell types (X, Y, and W) give rise to parallel processing streams relayed through the LGN – X, Y, and W geniculate cells in cats, and the P, M, and K geniculate cells in primates – to V1 (see The Evolution of Parallel Visual Pathways in the Brains of Primates; Stone *et al.*, 1979; Casagrande and Xu, 2004). In primates, the parallel channels remain segregated in their projections to V1 and this was thought to play a crucial role in the emergence of the dorsal and ventral processing streams in higher-order cortex (Livingstone and Hubel, 1988; Ungerleider and Mishkin, 1982; Ungerleider and Pasternak, 2004; Zeki and Shipp, 1988; see Section 3.22.5.3). M cells can be characterized by features that lead to motion and spatial perception – fast conducting axons, high temporal resolution, and sensitivity to low-contrast stimuli – characteristic

of visual areas in the dorsal stream. In contrast, P cells exhibit features that could lead to the perception of form and color (high spatial resolution and chromatic contrast), characteristics of the ventral stream. In cats, there is less segregation of the X and Y channels in V1 (Casagrande and Xu, 2004; Humphrey *et al.*, 1985; Payne and Peters, 2002). Nevertheless, there is support for dorsal and ventral streams in visual cortex of cats and ferrets (Lomber *et al.*, 1996a, 1996b; Manger *et al.*, 2002b, 2004; Payne, 1993).

While the parallel geniculate inputs to V1 in primates remain much more segregated than the geniculostriate projections of cats, there is clear evidence that mixing of the streams occurs at subsequent processing stages within V1 of primates (Callaway, 1998, 2005; Casagrande and Kaas, 1994; Merigan and Maunsell, 1993; Sincich and Horton, 2005). Thus, it is unclear whether the segregation of geniculostriate projections is relevant to subsequent outputs to the dorsal and ventral streams. This convergence within V1 is consistent with receptive fields becoming more complex as information flows through the visual hierarchy, but it leads to a complicated picture of the emergence of the dorsal and ventral streams (see Van Essen and De Yoe, 1995). Nevertheless, most evidence points towards some segregation of the functional streams – dorsal stream areas receive an M dominated relay, whereas the ventral stream receives a relay containing P and M signals. However, recent findings have shown a convergence of M and P geniculostriate projections that are in turn relayed directly to dorsal stream area MT, indicating that M and P signals are mixed even within the dorsal stream (Nassi *et al.*, 2006).

A major evolutionary modification present in V2 of monkeys, compared to cats, tree shrews, and prosimians is the three architectonically distinct bands, or stripes, that can be revealed through CO architecture (Krubitzer and Kaas, 1990; Livingstone and Hubel, 1982; Tootell *et al.*, 1985). These compartments have distinct connection patterns and functional selectivity of their neurons (see Sincich and Horton, 2005) that feed higher-order areas in the dorsal and ventral streams (see Section 3.22.5.3; Shipp and Zeki, 1995; Van Essen and De Yoe, 1995). The thick CO bands are reported to receive input from the M dominated stream in V1, whereas interbands and thin CO bands receive inputs from a mixture of M and P sources. The thick bands are thought to feed subsequent dorsal stream areas, whereas interbands and thin bands feed areas in the ventral stream. While the CO staining is a fairly reliable marker there is some question as to its functional relevance. While band location can be correlated with inputs from V1 and projections to areas V4 and MT, the dark CO bands in V2 may actually result from thalamic afferents arriving from the pulvinar nucleus (Levitt *et al.*, 1995).

3.22.5.5 The Controversy over Monkey V3

Is V3 a feature common to most mammalian visual systems? As we have seen for V2, the size, the relative position, feedforward projections from and feedback projections to V1, and a compressed mirror reversal of the retinotopic organization of V1 are the main reasons for considering V2 as part of the common mammalian plan. This is true even for cat and ferret V2, where, through divergent evolution, V2 has become more primary-like. Using the same criteria, a comparison across species shows that an area exists in a similar location, adjacent to the anterior border of V2, represents a compressed mirror image of the retinotopic organization of V2, and receives feedforward projections from V1 and V2, while providing feedback to these areas. While these criteria point toward a homology of V3 across mammals, there are some obstacles to overcome. While many of these obstacles have been addressed in earlier sections (3.22.2.3 and 3.22.4.1), a remaining obstacle in accepting V3 as a homologous region across most mammals, is that even in the most visually complex order, the primates, there is a debate over the existence of V3 (see the discussion below; Kaas, 2005b; Kaas and Lyon, 2001; Rosa and Manger, 2005).

Since the early retinotopic mapping experiments by Allman and Kaas (1975), the existence of a V3 in primates has been questioned (see Kaas and Lyon, 2001; Lyon and Kaas, 2002c). One issue, deriving from work on New World monkeys, is whether several visual areas, in place of a V3, can be found immediately adjacent to V2 (Allman and Kaas, 1975; Rosa and Schmid, 1995; Lyon and Kaas, 2001, 2002c; Lyon *et al.*, 2002; Rosa *et al.*, 2005). A second issue, derived from studies on Old World monkeys, is whether there are functional and connectional differences between the dorsal and ventral halves of V3 (Van Essen, *et al.*, 1986; Lyon and Kaas, 2002b), resulting in the identification of two separate areas.

In Old World monkeys the dorsal and ventral halves of V3, V3d and V3v, contain the representation of the lower and upper visual fields, respectively (Gattass *et al.*, 1988). A reported difference in the neuronal properties and projection patterns from

V1 led to a split of V3 into two separate areas (Burkhalter *et al.*, 1986; Felleman and Van Essen, 1987; Van Essen *et al.*, 1986). V3d became V3, representing only the lower visual quadrant, whereas V3v became the ventral posterior area, VP, representing only the upper visual quadrant. The functional properties of neurons may not be relevant to the homology of V3 in other species – as the function of V2 can differ between species. Nevertheless, it is unusual that functional properties such as chromatic sensitivity and direction selectivity would differ between the upper and lower visual fields of a single area. However, the functional characteristics of neurons in V3v have only been examined in one study (Burkhalter and Van Essen, 1986), so confirmation of the proposed differences in V3v and V3d neurons is needed. This is particularly important because several studies have yielded different characterizations of the response properties of neurons just within V3d (Baizer, 1982; Felleman and Van Essen, 1987; Gegenfurtner *et al.*, 1997; Zeki, 1978). Part of such differences in characterizations is due to different criteria used to define the characteristics of the neurons (see Gegenfurtner *et al.*, 1997), and part is likely due to the difficulty of accessing V3d in the macaque monkey, as it is completely buried within the lunate sulcus. It is also unusual for the connection patterns to differ between the two visual fields of a single area (see Kaas, 1996; Zeki, 2003). Accordingly, a re-examination of the connection patterns has revealed the missing connections. Whereas, initially, it was reported that V3d was interconnected with V1, but that V3v was not (Van Essen *et al.*, 1986), more recent work has revealed that both V3d and V3v are interconnected with V1 (Lyon and Kaas, 2002b). Thus, the controversy over V3 in Old World monkeys has become more settled (see Kaas, 2005b; Van Essen, 2004).

For New World monkeys, differences in interpretation over the size and location of V3 continues. The main issue in New World monkeys concerns the organization of cortex just anterior to the dorsal extent of V2. Early microelectrode mapping experiments in owl monkeys found the representation of the upper visual quadrant immediately adjacent to dorsal V2 (Allman and Kaas, 1975). As dorsal V2 represents the lower visual quadrant, the existence of an adjacent upper field representation argued against the idea of a V3 strip of cortex mirroring the V2 retinotopy. This new region was demonstrated to have a complete retinotopic map occupying cortex similar in size to area MT. Subsequent experiments in the smaller marmoset monkey also found an upper field representation immediately adjacent to dorsal V2, but the resulting DM contained an unconventional split representation of the horizontal meridian (Rosa and Schmid, 1995). More recently, however, connection patterns with V1 in four species of New World monkeys found no evidence for an upper field representation near the anterior border of V2 (Lyon and Kaas, 2001, 2002c). Instead, the observed connections from upper field V1 were displaced at least 1–2 mm anterior to the dorsal V2 boundary. Along with evidence from cytochrome and myeloarchitecture, dorsal V3 was identified as a strip of cortex displacing DM from dorsal V2. Counter to these connection patterns, however, a subsequent study placing injections anterior to dorsal V2 did show connections with the upper field representation in marmoset V1 (Rosa *et al.*, 2005). However, injection sites were centered nearly 2 mm from the outer V2 border. Thus, the upper field connections likely arose from the upper field location of DM.

In addition to the conflicting reports of the upper field connectivity of DM, it has been argued that the microelectrode maps of this dorsal region clearly demonstrate an upper field representation immediately adjacent to the dorsal V2 border (Rosa and Schmid, 1995; Rosa *et al.*, 2005; Rosa and Manger, 2005; Rosa and Tweedale, 2005). Yet, careful examination of the published data reveals some ambiguities, and there are additional results that are rather perplexing. First of all, the evidence for an upper field representation immediately adjacent to V2 (Rosa and Schmid, 1995) can be interpreted in another way, especially in light of more recent connection studies (Lyon and Kaas, 2001, 2002c). The receptive fields of recording sites considered as part of the upper field were centered very near the outer border of V2 representing the horizontal meridian, as a result much of the recorded receptive fields of individual neurons were found in the lower visual field as well the upper (Rosa and Schmid, 1995). As most of these recording sites were located near the V2 border they were taken as evidence for upper field representation immediately adjacent to V2. However, as the V2–V3 border represents the horizontal meridian it is not surprising that the receptive field would partially overlap with the upper field, and thus this evidence in itself cannot be used to argue against a V3.

While this issue is open to interpretation, there are other findings reported from the retinotopic maps of DM, such as an irregular progression from the central to peripheral visual field and the representation of two separate horizontal meridians (Rosa, 2002;

Rosa and Schmid, 1995), that lead one to question the reliability of the microelectrode technique as a means for such issues, especially in regions that have been difficult to define. Alternatively, techniques such as intrinsic signal optical imaging offer a fairly high spatial resolution but, unlike microelectrode mapping, allow the recording of neural activity from a large array of cortex simultaneously (see Figure 9). This procedure is helpful in reducing any ambiguities that may arise through the reconstruction of electrode tracks in several coronal or parasagitally cut sections. In owl monkeys, optical imaging of neural activity revealed only the representation of the lower visual field adjacent to V2 (Lyon *et al.*, 2002). The resulting retinotopic map revealed a slightly compressed mirror-image representation of the retinotopy in dorsal V2, consistent with a dorsal V3 (Figure 17). Also consistent with a dorsal V3, the V2–V3 border was marked by the representation of the horizontal meridian, while the outer V3 border was marked by the vertical meridian. These results are also consistent with the retinotopic connections of this region to V1 in owl monkeys, three other New World monkey species, and two species of Old World macaque monkeys (Lyon and Kaas, 2001, 2002b, 2002c). In addition, the observed retinotopy of dorsal V3 in owl monkeys is similar to the retinotopic organization revealed in macaque monkey V3 through microelectrode mapping (Gattass *et al.*, 1988) and fMRI (Brewer *et al.*, 2002; Fize *et al.*, 2003).

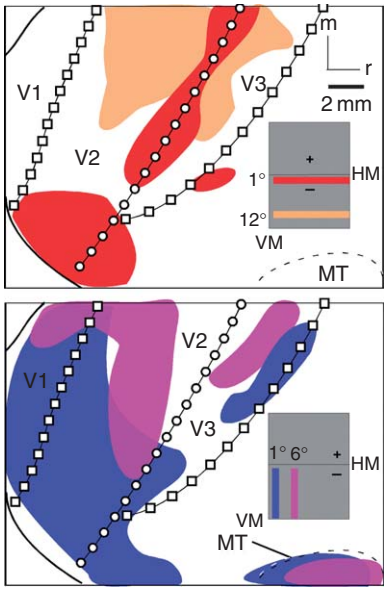

Figure 17 Reconstructions of the retinotopic organization of monkey V2 and V3. These summary diagrams are reconstructed from data in Lyon, D. C., Xu, X., Casagrande, V. A., Stefansic, J. D., Shima, D., and Kaas, J. H. 2002. Optical imaging reveals retinotopic organization of dorsal V3 in New World owl monkeys. *Proc. Natl. Acad. Sci. USA* 99, 15735–15742.

3.22.5.6 The Middle Temporal Area, MT

Primate area MT exhibits several features that make it the most prominent extrastriate area, outside of V2. As revealed through connection patterns, MT is strongly interconnected with V1, V2, and V3 (Born and Bradley, 2005; Lyon and Kaas, 2002b, 2002c; Shipp and Zeki, 1989a, 1989b), receiving inputs perhaps dominated by the M geniculate pathway (Merigan and Maunsell, 1993; Van Essen and De Yoe, 1995), but also inputs from the P pathway (see Nassi *et al.*, 2006). In addition, MT receives direct but sparse input from the K geniculate cells (Sincich *et al.*, 2004; Stepniewska *et al.*, 1999) and receives fairly dense disynaptic projections from the SC (Lyon *et al.*, 2005). In turn, MT is a primary provider of inputs to many of the areas in PP that form the dorsal stream of visual processing (Maunsell and Van Essen, 1983a; Ungerleider and Desimone, 1986; Shipp and Zeki, 1995). Another distinctive feature of MT is its dense and patchy myelination (Figure 16; Allman and Kaas, 1971b; Krubitzer and Kaas, 1990) and blob-like staining for the metabolic enzyme CO (Tootell *et al.*, 1985; Lyon and Kaas, 2001).

Functionally, MT contains a precise first-order representation of the contralateral visual hemifield (Allman and Kaas, 1971b; Rosa and Elston, 1998) similar to that found in V1 and a systematic representation of direction preference that is arranged in a columnar fashion (Albright *et al.*, 1984) that is in many ways similar to the orientation columns of V1 (see Albright and Desimone, 1987). Columns are also seen in MT architecture and connection patterns. V1 projections to MT terminate in a modular arrangement (Rockland, 1989) and MT cells projecting to other extrastriate areas, such as DM, FST, and MST, are also arranged in small, modular clusters (Kaas and Morel, 1993; Krubitzer and Kaas, 1993; Berezovskii and Born, 2000). The internal architecture of MT revealed through stains for CO (Tootell *et al.*, 1985) and myelin (Krubitzer and Kaas, 1990) contains intermittent dark and light regions comparable in size to MT functional columns. Interestingly, callosal projections from MT have been correlated with the myelin dark regions (Krubitzer and Kaas, 1990). Subsequent studies have indicated that similar functionally defined modules may be correlated to differences in MT architecture and patchy intrinsic connectivity (Born and Tootell, 1992; Malach *et al.*, 1997). More recently, Berezovskii and Born (2000) have

shown that MT regions responsive to local motion cues project preferentially to the dorsal half of MST, whereas MT regions more responsive to global motion cues project preferentially to the ventral half of MST and to FST.

3.22.5.6.1 Is MT found in other mammals? Whereas there is a similar V3-like area found in most mammalian species, it is even less certain whether there is an MT-like area across species. MT is a prominent region in primate visual cortex and thought to have emerged early in primate evolution. One theory suggests that tree shrew TD, rather than a V3-like area, is a potential MT homologue and that MT gradually drifted further anterior from the V2 border as cortex evolved in successive primate lines – prosimians, New World monkeys, and Old World monkeys (Northcutt and Kaas, 1995). Like MT, tree shrew TD shows an increase in myelination compared to surrounding regions and is heavily connected with V1. However, there are several extrastriate areas in primate that are connected with V1, including V3 which is also moderately myelinated (Lyon and Kaas, 2001). Additionally, there are several other extrastriate areas in the tree shrew that lie more anterior to V2 and could represent an MT-like area based on other criteria. The most prominent area, TI, is connected with V2 rather than V1, but it does stain conspicuously dark for myelin and lies very close to primary auditory cortex (Lyon *et al.*, 1998). The dark myelination of primate MT is probably its most distinctive feature, and like tree shrew TI, primate MT is found just posterior to auditory cortex (see Lyon and Kaas, 2001). What is sorely lacking is any evidence of functional similarities between MT and TD or TI, as in tree shrew there are no reports on the functional properties of neurons outside of V1.

In other species, areas in location similar to TD have also been proposed as MT homologues. A squirrel MT homologue, area ML-L, has been proposed on the basis of functional similarities to primate MT (Paolini and Sereno, 1998) and it is in a similar location to tree shrew TD. A region anterior to V2 in the flying fox has also been proposed as a homologue to MT based on its myelination, retinotopic organization, and input from V1 (see Kaas and Preuss, 1993). However, like the tree shrew, this proposed MT homologue has been considered a homologue to V3 in other interpretations (Rosa, 1999). While the data in support of an MT homologue is limited for these species, a significant body of evidence has implicated the cat PMLS complex as a homologue to MT and its surrounding satellites (see Grant and Hilgetag, 2005; Hilgetag and Grant, 2000; Payne, 1993). Indeed, PMLS and MT share many similarities. Both regions are located well anterior to V2 in temporal cortex and are comprised of neurons specialized for direction discrimination. In addition, they both have orderly retinotopic maps, stain darkly for myelin, receive feedforward projections from V1, V2, and V3, and provide feedforward projections from similar cortical layers to the surrounding motion processing areas. Furthermore, Matsubara and Boyd (2002) report similarities in the origin of the projections from V1 to the PMLS region in cat and MT in monkey, showing that cells located in CO blobs within the layer 4B project to these areas. Despite the great number of similarities, a cladistic approach makes it difficult to conclude that PMLS is an MT homologue because the evidence for an MT homologue in intervening species is so limited (Kaas, 2002). Nevertheless, MT-like regions have been proposed for species in sister groups to both cats (flying foxes) and primates (tree shrews and squirrels) leaving open the possibility that primate MT emerged from an area common to many mammals.

3.22.6 Conclusions

Despite detailed evidence reviewed here on the organization of visual cortex in several mammalian taxa, at this point we know very little about how differences in mammalian visual cortex organization evolved (see Streidter, 2005). As presented in this article, we can look at the organizational schemes of several different mammalian orders from the small nonvisual insectivores to the large and highly visual carnivores and primates. Still, because detailed evidence is lacking, we can say little about homologies between extrastriate visual areas. Should we care whether cat visual areas are homologous to the primate? Is an understanding of whether brain structures are homologous or homoplaseous critical to understanding how the brain functions? For, despite uncertainty of the homology between species, is it not unreasonable to use neuronal properties of, for example, cat PMLS to help us understand how monkey and human brains process motion? If area PMLS is homologous to MT, will that help us understand the brain any better? More likely, understanding the brain will help us determine whether areas are homologous.

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3.23 The Evolution of Crossed and Uncrossed Retinal Pathways in Mammals

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Glossary

contralateral (crossed) axons	Retinal axons that cross the midline and project to higher targets on the opposite side of the brain from which they originate.
ipsilateral (uncrossed) axons	Retinal axons that do not cross the midline and that project to higher targets on the same side of the brain from which they originate.
optic chiasm	Retinal ganglion cell axons from each eye project through the optic nerves and make up this X-shaped commissure; here, fibers from each eye project to one or the other side of the brain and re-sort to form the optic tracts.
retinal axon decussation	Retinal ganglion cell axons from each eye diverge from one another in the optic chiasm, to cross or remain on the ipsilateral side of the brain.
retinal ganglion cells (RGCs)	The only cell class that projects axons from the retina to the brain, conveying information from the photoreceptors in the outer nuclear layer and from interneurons such as bipolar, horizontal, and amacrine cells in the inner nuclear layer. The axons of RGCs form the optic nerve.

3.23.1 Anatomical Basis of Binocular Vision

3.23.1.1 Types of Retinal Fiber Decussation in the Optic Chiasm of Vertebrates

Strictly speaking, all animals with two eyes have binocular vision, but the terms binocular vision and stereoscopic vision are usually reserved for animals that possess a large area of binocular overlap and this overlap is utilized to code depth. In animals with good binocular vision, the eyes are forward facing and each eye receives a slightly different perspective on a scene. Visual information from each eye is sent to both sides of the brain because retinal fibers from the nasal regions of the retina cross the brain midline and fibers from the temporal retina do not cross the midline and instead project to the same side of the brain. Once this information reaches the visual cortex, the two different images perceived by each side of the brain are integrated into one cohesive mental image. The differences in perspective transmitted through each eye allow the brain to triangulate distance much more accurately, resulting in improved depth perception (Bishop and Pettigrew, 1986; Figure 1; see The Role of Vision in the Origin and Evolution of Primates, The Evolution of Visual Cortex and Visual Systems).

Retinal ganglion cells (RGCs) are the last cells in the retina to collect visual information within each eye and then project their axons toward the main visual nuclei in the central nervous system, the lateral geniculate nucleus in the thalamus, and the superior colliculus, or optic tectum in lower vertebrates, in the midbrain (Figure 2). The structure in which RGC axons from each retina partially decussate or cross is the optic chiasm and it is located in the ventral diencephalon (Figure 2). Thus, one important function of hemidecussation is to obtain inputs from the same part of the visual field perceived by each retina and to transmit this

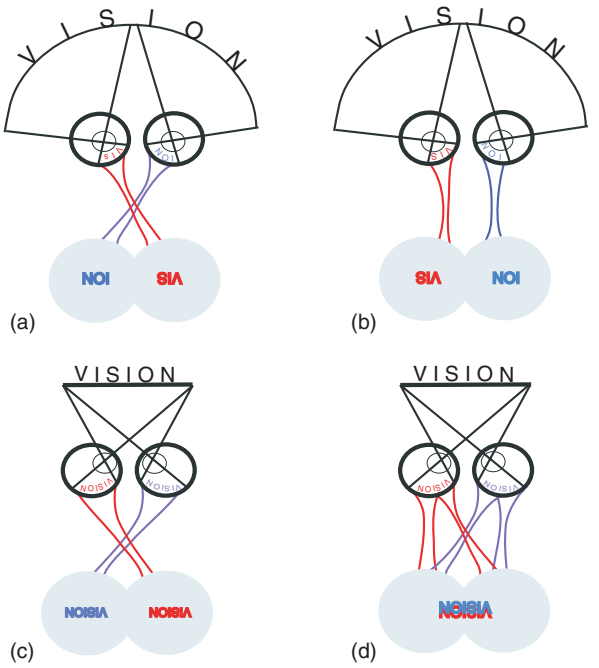

Figure 1 Crossed and uncrossed visual pathways. Images that form on the retina are inverted by the lens of the eye. In animals without stereoscopic vision, eyes placed more laterally allow panoramic view and predator scanning. Complete crossing of retinal fibers at the chiasm is necessary to restore a congruent image of the outside visual world (the word VISION is re-formed in (a)). b, If no fiber crossing occurs, IONVIS would be perceived. c, In animals with forward-facing eyes, if complete crossing occurs, as in lower vertebrates, stereoscopic information would be compromised, as in albino animals of many species. d, In most mammals, partial fiber crossing is necessary to fuse both retinal images homotopically in the brain.

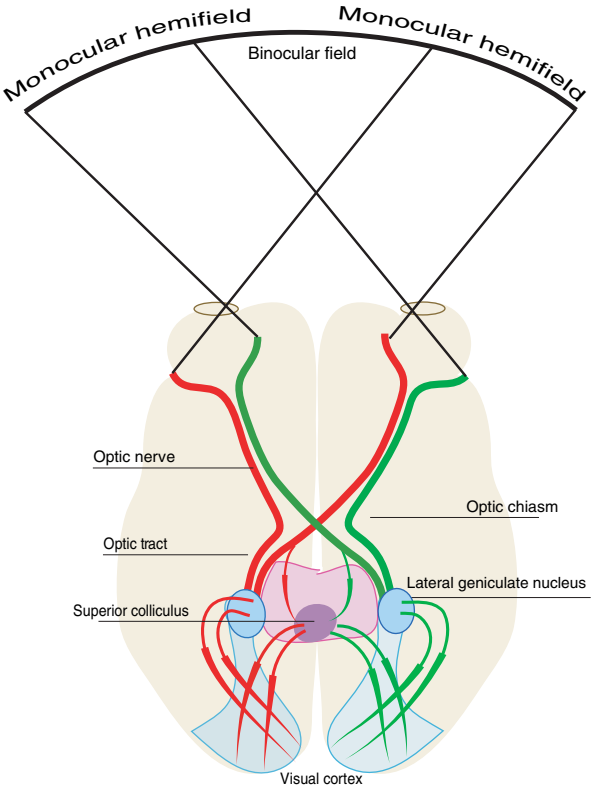

Figure 2 The visual pathway from retina to primary visual cortex, in human brain. The binocular field is that portion of the total visual field within which the monocular fields overlap. The higher visual centers in the brain – the superior colliculus, lateral geniculate nucleus, and visual cortex – in each hemisphere receive input from both eyes. Visual information is perceived by the two left halves of the retina (red) and ends up in the left half of the brain. In the same manner, axons from the right half of each eye (green) bring information to the right occipital lobe. This occurs because approximately half the optic nerve fibers cross at the chiasm and the remainder are uncrossed.

information to the same location in targets that are located further centrally.

Other optic decussations occur in lower vertebrates, for example, in the isthmus complex at the pontine–midbrain junction, interconnected with the optic tectum, and in the form of retinoretinal projections (Thanos, 1999). In amphibians and reptiles, the isthmic complex projects topographically to the contralateral as well as the ipsilateral tectal lobe. The isthmo-optic nucleus also projects centrifugally to the retina. This projection develops after birth, once visual activity has commenced, and appears to be required for the further refinement of tectal maps by sensory activity (Constantine-Paton and Cline, 1998; Schmidt and Edwards, 1983). The development of retinoretinal projections and the centrifugal projection from the isthmo-optic nucleus may be interdependent (Thanos, 1999).

In this article, we focus on the decussation of RGC axons in the ventral diencephalon during formation of the optic chiasm, a process that takes place during embryonic development and that is apparently independent of visual stimuli. Partial decussation of the fibers from each eye occurs in most mammals. The majority of RGC axons originating from the nasal retina cross the midline to enter the optic tract on the opposite side of the brain, whereas axons from the temporal or ventrotemporal (VT) segment of the retina, depending on the species, do not cross (Chalupa and Lia, 1991; Polyak, 1957; Stone *et al.*, 1973). The number of uncrossed fibers is proportional to the size of the binocular visual field, which in turn depends on the extent to which the eyes are located in a frontal position. In animals with frontally placed eyes, such as humans or monkeys, the uncrossed component reaches approximately 40% of all RGCs (Chalupa and Lia, 1991; Stone *et al.*, 1973). Cats, ferrets, horses, rabbits, and mice have different degrees of separation of the eyes in the head and show a gradually decreased uncrossed component, which ranges from 30% in cats to approximately

5% in mice, which have laterally placed eyes and poor binocular vision (Jeffrey, 2001). In lower vertebrates, amphibians such as the much-studied *Xenopus* are an interesting case, since a subpopulation of ganglion cells in the VT retina projects ipsilaterally beginning at metamorphosis, when the laterally located eyes become positioned frontally (Hoskins and Grobstein, 1985; Mann and Holt, 2001; see The Evolution of Vertebrate Eyes).

Birds and fish have laterally located eyes and panoramic vision and do not have an uncrossed projection except for a minor early ipsilateral projection that is transient (see The Role of Vision in the Origin and Evolution of Primates). The totally decussating fibers from each retina intercalate in the chick optic chiasm but the bundles of fibers from each eye overlay one another in fish (Drenhaus and Rager, 1992; O'Leary *et al.*, 1983; Polyak, 1957; Thanos *et al.*, 1984; Figure 3).

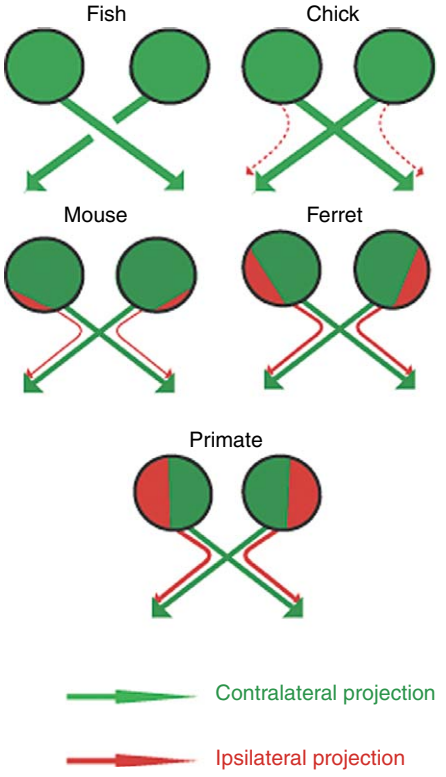

Figure 3 Crossed and uncrossed pathways throughout evolution. In fish, all RGCs project contralaterally and retinal axons do not intermingle at the chiasm. In chick and other birds, retinal axons project contralaterally but there is a small transient ipsilateral projection that disappears during maturation (red dashed lines). Binocular vision is partially developed in mammals such as mouse or ferret, with most of the retinal axons crossing the midline (green) but with an appreciable number of axons projecting ipsilaterally (red); binocularity is fully developed in those mammals with frontally located eyes, such as felines or primates. In this figure, line thickness approximates the proportions of fibers that project to ipsilateral or contralateral targets.

For many species of prey, such as cows or horses, the wider field of view provided by side-facing eyes and monocular vision provides better adaptation, reducing the chance that a predator could approach them unaware. In contrast, in many hunting animals, binocular vision is a common feature, as it is more important for predators to accurately determine the distance between themselves and their prey. Primates have good stereoscopic vision and rely on it when navigating complex three-dimensional environments. Most complex visual tasks, such as reading, detecting camouflaged objects, and eye–hand coordination, are performed more effectively with two eyes rather than with one, even when the visual display has no depth.

In sum, the retinal origin of the fibers that cross the midline or remain on the same side of the brain via the optic chiasm is relatively conserved across species, but the size of the uncrossed projection is related to the degree of binocularity, which, in turn, relates to the position of the eyes on the head.

3.23.1.2 Visual Defects Resulting from Anomalies in Optic Chiasm Formation

Albinos and individuals that suffer the nondecussating retinal–fugal fiber, or achiasmatic, syndrome represent interesting anomalies related to retinal fiber decussation. In general, the albino defect causes an approximately 50% decrease in the normal number of uncrossed axons, irrespective of the species (Jeffery, 1997; Rice *et al.*, 1995), whereas in achiasmatic individuals, all retinal axons are uncrossed (Victor *et al.*, 2000). The gene mutated in the most prominent form of albinism is tyrosinase, a key enzyme in melanin synthesis, but other gene mutations causing defects in melanin biosynthesis can also lead to the absence of pigment in the retinal pigment epithelium and subsequently a reduction of ipsilateral fibers (Incerti *et al.*, 2000). It is not understood how tyrosinase, or any other component of the melanogenic pathway, influences the projection of RGC axons. It has been shown that spatiotemporal aspects of neurogenesis are altered in albino mice; such alterations could influence the specification of the RGC projection phenotype (see below) (Rachel *et al.*, 2002). Interestingly, in achiasmatic humans, and to a lesser extent in albinos, the altered mapping and sensory input are almost corrected later in the visual pathways by interhemispheric communication (Victor *et al.*, 2000).

3.23.1.3 Optic Chiasm Development

Even with its very small ipsilateral component, the mouse is the most commonly used mammalian model for studying how the optic chiasm and the partial retinal decussation develop. The formation

of the optic chiasm in mouse occurs in two sequential steps. In a first phase, pioneer axons arising from the dorsocentral retina leave the optic cup through the optic disk at embryonic gestational day 12 (E12) and navigate into the optic stalk to enter the developing ventral diencephalon. The majority of these pioneering axons then cross the midline and grow in close relationship with an inverted V-shaped array of early neurons, establishing the position of the optic chiasm along the anterior–posterior axis of the brain (Figure 4). A small proportion of these axons continue to extend on the same side of the brain, distant from the midline, and form an early uncrossed projection that is thought to be transient. In a second phase, at approximately E14, retinal axons arising from the VT crescent in the retina arrive at the midline and turn back to the same side of the brain from which they originate, whereas axons from the rest of the retina cross the midline (Guillery *et al.*, 1995; Mason and Sretavan, 1997; Figure 4). It is unclear how the first step gives rise to the second phase, but ipsilateral axons from the peripheral VT retina generated in the second phase are considered to constitute the permanent uncrossed component. In a third and final phase, RGCs with an uncrossed projection cease to be generated after E17, and thereafter, until E19, RGCs born in the VT retina, as well as in the rest of the retina, tend to have a crossed projection (Dräger, 1985; Figure 4).

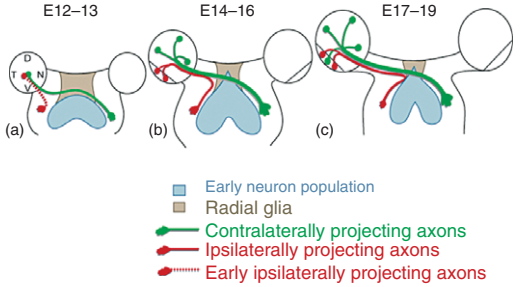

Figure 4 Development of the optic chiasm. The formation of the optic chiasm takes place in three phases. a, In an early phase of RGC axon out growth (E12–13), the earliest generated RGC axons originating from the dorsal-central retina grow in close relationship to the inverted V-shaped array or CD44 neurons (blue) at the developing ventral diencephalon and grow contralaterally across the midline. A population of RGC axons, thought to be transient, turn lateral to the early neurons and do not approach or cross the midline, but directly enter the optic tract. b, By E14–16, a group of RGC axons originating from the VT retina have reached the midline but they turn away from it to project into the ipsilateral optic tract, whereas axons originating from other regions of the contralateral retina have crossed the midline to project into the same optic tract. c, In a final phase, E17–19, axons from the VT quadrant remain as an ipsilateral projection, but additional RGCs intermingled with the uncrossed RGCs in the VT retina now project contralaterally. D, dorsal; N, nasal; T, temporal; V, ventral.

Several aspects of the course of retinal fibers deserve mention. Whereas fibers leave the retina and enter the optic nerve in a rough retinotopic organization, this order is lost and new rearrangements with reference to the topographic origin of retinal fibers occur near the chiasm (Chan *et al.*, 1999). Second, where the fibers rearrange, growing retinal axons shift from being organized by interfascicular glia to an environment populated by radial glia. Third, the youngest fibers to join the optic nerve and chiasm change their position at this glial transition, such that they grow among glial end-feet at the pial surface, in the lateral chiasm. At the midline region, growth cones extend dorsally to cross or turn away from the midline. The growth cones then grow ventrally again as they project into the optic tracts (Guillery *et al.*, 1995; Reese *et al.*, 1997).

The focus on the chiasm midline began when tracing analyses revealed the course of fibers of retinal axons that diverged or turned away from the midline of the optic chiasm (Godement *et al.*, 1990). Only fibers from the VT retina, e.g., those with an ipsilateral projection, are tipped with highly complex growth cones, similar to those seen *in vitro* at a border of inhibitory substrate (Mason and Erskine, 2000, 2004). Time-lapse studies of retinal axon dynamics indicated that, in fact, growth cones arising from all parts of the retina pause at the midline and develop growth cone forms that are more complex than those seen in the optic nerve or tracts, where rapid, straight-ahead growth occurs (Godement *et al.*, 1994; Mason and Wang, 1997). The cellular composition of the midline region was then investigated to determine the cellular basis for inhibition of extension of the uncrossed fibers, and a slowing down of growth of the crossing fibers.

The midline region of the ventral diencephalon in which the optic chiasm develops contains two cellular specializations that have been implicated in regulating RGC axon guidance. One cell population is a group of radial glia with cell bodies positioned at the floor of the third ventricle, with their fibers straddling the midline and end-feet on the pia of the ventral diencephalon. During the establishment of the permanent ipsilateral projection (middle period, Figure 4b), all retinal growth cones enter this glial palisade before crossing or turning back from the midline. As seen by ultrastructural analysis, the lamellae of the growth cones interdigitate with the radial glia processes, suggesting that interactions between these cells play a critical role in directing RGC axon pathfinding (Marcus and Mason, 1995; Mason and Sretavan, 1997).

The second group of cells located at the ventral diencephalon during optic chiasm formation is an

early born population of diencephalic neurons (Mason and Sretavan, 1997; Mason and Wang, 1997; Sretavan *et al*., 1994). These neurons constitute a V-shaped template of cells around which retinal axons express specific carbohydrate epitopes such as stage-specific embryonic antigen-1 (SSEA-1)), CD44 (a hyaluronan-binding protein), and others (Kaprielian *et al*., 2001; Marcus *et al*., 1995; Sretavan *et al*., 1994; Figure 4). Experiments in which these cells have been perturbed provide evidence that they are essential for proper growth around the chiasm, even though the growth appears to be around the confines of the cell population (Sretavan *et al*., 1994). In lower vertebrates, with totally crossed pathways, there are equivalent ensembles of cells, e.g., early born neurons that constitute the tract of the postoptic commissure and midline glia, but less is known about their epitope expression and retinal growth cone relationships (Maggs and Scholes, 1986; Wilson *et al*., 1988).

In vitro experiments have shown that when cocultured with cells dissociated from the optic chiasm region, axons from the VT retina grow less well than axons from other areas of the retina (Wang *et al*., 1995, 1996). These *in vitro* experiments demonstrated that the midline glia and the early neuron population express signals that are repulsive for uncrossed RGC axons and less so for crossed RGC axons, as predicted by the different growth cone forms on retinal axons during divergence from the chiasm midline.

In summary, the growth pattern of divergence within the radial glial palisade and growth around the contours of the early born neurons, as well as the differential growth inhibition on dissociated chiasm cells, implicate midline radial glia and early born neurons as prime candidates for patterning the optic chiasm. In the following section, we review the molecular cues expressed on these cells of the chiasmatic region that serve as growth and inhibitory signals for proper channeling of axons and placement of the X of the optic chiasm and that produce the differential response of retinal axons to cross or avoid the midline – all critical for proper optic chiasm formation.

3.23.2 Molecular Mechanisms of Retinal Axon Guidance at the Optic Chiasm

3.23.2.1 Molecules That Shape the Placement and Integrity of the Chiasm

A number of guidance factors that play a role in axon guidance at the midline have been localized to the area in which the chiasm forms. However, rather than directing the crossing and/or avoidance of the midline to implement retinal axon divergence, several of these factors play a role in ensuring proper placement of the chiasm and general fasciculation of retinal fibers, without specifically being selectively repulsive or supportive of different subpopulations of retinal axons.

The secreted glycoproteins, the Slits, and their receptors, the Robos, lead to repulsive interactions that mediate midline crossing in *Drosophila* and in the ventral spinal cord of vertebrates (Kidd *et al*., 1999; Long *et al*., 2004). Slits and Robos are expressed in the early born neurons in the ventral diencephalon and radial glia in the preoptic area rostral to where the chiasm forms, as well as in the retina during the formation of the optic chiasm (Erskine *et al*., 2000), but the Robo receptors are not localized selectively in RGCs that give rise to crossed or uncrossed pathways. Mice in which both Slit1 and Slit2 are missing exhibit routing errors at the optic chiasm level, including multiple decussations and straying into the ventral diencephalon (Plump *et al*., 2002). However, an ipsilateral projection forms in the double knockouts, suggesting that Slits channel axons into a repulsion-free corridor, normally positioned at a precise point along the anteroposterior axis, rather than acting as midline gatekeepers for uncrossed versus crossed axons. Likewise, in zebra fish, Robo receptor nulls show extreme errors in fiber routing, with axons wandering and multiple chiasmata being formed (Fricke *et al*., 2001).

A second molecular family thought to play a role in the routing of retinal axons at the chiasm, based on localization and experimental perturbation, is the proteoglycans (PGs). Chondroitin sulfate proteoglycans (CSPGs) are expressed by the early born, CD44-SSEA-positive neurons in the ventral diencephalon (Chung *et al*., 2000) and have an age-dependent effect on RGC axon guidance. Enzymatic removal of CSPGs at E13.5 leads to axon stalling or misrouting and many axons fail to cross the midline. When CSPG removal is performed at later stages, only ipsilateral axons are affected. Heparan sulfate PGs, including neurocan and phosphacan, are localized to the chiasm region and optic tract and have been proposed to participate in fiber sorting rather than divergence *per se* (Leung *et al*., 2003, 2004). In the visual pathway of zebra fish, PGs have also been implicated in optic tract fiber sorting (Lee and Chien, 2004; Lee *et al*., 2004). This study and others based on genetic models (Kantor *et al*., 2004) have indicated that PGs can regulate the effect – either positive or negative – of known guidance receptors and cues, such as in the Robo/Slit and Semaphorin families (Lee and Chien,

2004). It remains to be tested whether or not these guidance cues interact with PGs in the chiasm, and if so, what their effect on divergence is.

Other guidance factors and morphogens that are prominent in other midline loci, such as the floor plate of the spinal cord, have been analyzed during optic chiasm formation. Sema5A functions to channel RGC axons within the optic nerve (Oster *et al.*, 2003), but the semaphorins have not been studied in detail at the optic chiasm.

Sonic hedgehog seems to restrict RGCs to the proper dorsoventral position around the chiasm midline in the earliest phase of axon growth, as studied in the chick (Trousse *et al.*, 2001).

Thus, many of the guidance molecules that function in other systems do not appear to directly mediate retinal axon divergence at the chiasm, even though they facilitate several aspects of retinal axon navigation in the ventral diencephalon.

3.23.2.2 Axon Guidance Factors That Mediate Divergence at the Chiasm

The guidance family whose function in retinal axon divergence is more defined is the B subclass of Ephs and their ephrin ligands (Klein, 2004). Ephs and ephrins are membrane proteins that mediate repulsion and regulate many processes during embryonic development and they are key in the formation of topographic projections of retinal axons in the optic tectum/superior colliculus. EphrinAs occupy a domain just ventral to the optic chiasm, where they may restrict retinal axons from growing into inappropriate regions of the hypothalamus (Marcus *et al.*, 2000). EphrinAs do not specifically instigate a differential response between contra- and ipsilateral axons. In contrast, there is ample evidence that ephrinBs direct retinal axon divergence at the optic chiasm. The retinal projection in *Xenopus* is completely crossed but at metamorphosis, an uncrossed retinal projection develops as a result of a new wave of RGC neurogenesis in the VT retina (Marsh-Armstrong *et al.*, 1999). An ephrinB is present at the chiasm coincident with the formation of this uncrossed component at metamorphosis, and premature misexpression of an ephrinB in the ventral diencephalon induces an ectopic ipsilateral projection (Nakagawa *et al.*, 2000).

In mouse, ephrinB2 is a prominent player in directing divergence at the chiasm. The uncrossed RGC axons from the VT retina are especially sensitive to ephrinB2, resulting in inhibition of their extension through the midline (Williams *et al.*, 2003). Importantly, these data support previous results describing direct interaction between retinal growth cones and midline glial cells because ephrinB2 is expressed in the radial glia population located in the chiasmatic region (Williams *et al.*, 2003; Figure 5).

3.23.2.3 Molecular Differences between Uncrossed and Crossed RGCs

The discovery of ephrinB2 as mediator of inhibition of uncrossed axons at the chiasm midline encouraged

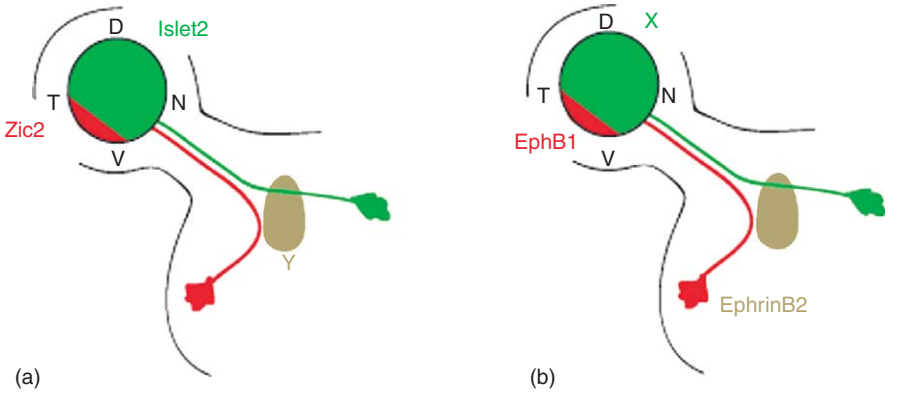

Figure 5 Molecular players mediating the retinal projections through the optic chiasm. a, Transcription factors: Zic2 is expressed by RGCs located specifically in the VT retina (red), which gives rise to the uncrossed retinal projection. Islet2 is expressed only in crossed RGCs (green) located in a pattern complementary to Zic2-positive cells. Islet2 does not specify contralateral cells, since mice deficient in Islet2 function still conserve most of the contralateral RGCs, but it could negatively regulate Zic2. A set of transcription factors localized at the ventral diencephalon (Y, brown) appear also to be important for specification of the cellular/molecular cues of the chiasm midline and to be crucial to retinal axon divergence. b, Axon guidance factors: Uncrossed RGC axons expressing EphB1 (red) turn away from ephrinB2-expressing midline glia near the midline (brown), whereas crossing RGCs (green) traverse the ephrinB2 zone. It is unknown whether the crossing axons express a complementary axon guidance molecule (X) functioning actively to overcome the inhibitory cues or whether they use an entirely different molecular mechanism to cross the midline.

the search for a specific receptor for ephrinB2 involved in this process. Of all the possible Eph receptors that could interact with ephrinB2, EphB1 is the only one that is highly expressed in the VT retina in RGCs with an ipsilateral trajectory during the period of their outgrowth. Furthermore, in mice lacking EphB1, the ipsilateral retinal projection is severely diminished, suggesting that this receptor is both specific and required for the formation of the uncrossed projection (Williams *et al*., 2003; Figure 5).

Thus, ephrinB2 at the chiasm and EphB1 at the retina appear to be the principal axon guidance molecules that direct retinal axon divergence at the chiasm midline. But what controls the finely tuned spatial and temporal specificity of the expression of these guidance molecules? The emerging view in other models used to study neural identity and axon trajectory is that each neuronal subtype possesses an intrinsic capacity to detect its own unique path soon after it becomes postmitotic (Shirasaki and Pfaff, 2002). This ability is conferred by the action of specific transcription factors that regulate programs of axon guidance, through the control of expression of receptors to guidance factors by binding to specific DNA domains.

Zic2, a zinc finger transcription factor involved in early neural patterning, has been identified as the first regulatory gene directly involved in axon divergence at the optic chiasm (Herrera *et al*., 2003). Zic2 is expressed postmitotically in RGCs that project ipsilaterally but not in contralaterally projecting RGCs, during axonal extension through the optic chiasm. Zic2 is crucial for directing the ipsilateral retinal projection, since genetically modified mice expressing low levels of this protein (Zic2 knockdown mice) show a severe reduction in the number of uncrossed axons. Interestingly, Zic2 expression in the VT retina is conserved in both mammals and amphibians, precisely mirroring the extent of binocularity. In ferret, the number of cells expressing Zic2 in the VT retina is greater than in mouse, according to the greater proportion of uncrossed axons. In *Xenopus*, Zic2 expression is upregulated at metamorphosis, coinciding with the late development of the uncrossed component, whereas chick lacks an ipsilateral projection and, accordingly, Zic2 is not expressed in any part of the retina. Of interest is that in albino mice, which have fewer uncrossed axons than pigmented mice, the number of Zic2-expressing RGCs is reduced, exactly matching the reduced size of the uncrossed component.

Zic2 expression in the VT retina matches the spatiotemporal expression of EphB1 (Pak *et al*., 2004). Despite the close spatiotemporal relationship between Zic2 and EphB1, whether Zic2 regulates EphB1 in the VT retina or is simply expressed in a parallel program remains to be tested. However, whereas Zic2 and EphB1 are co-expressed in the VT retina during the formation of the permanent uncrossed component, EphB1 – but not Zic2 – is present in the dorsocentral retina when the early transient ipsilateral projection forms (see Figure 4), suggesting that EphB1 expression at this time may be controlled by transcription factors other than Zic2.

EphB1 and ephrinB2 are axon guidance signals that mediate repulsion. Since retrograde labeling experiments in EphB1 null mice show that normally ipsilaterally projecting RGCs project contralaterally, it is reasonable to think that EphB1/ephrinB2 repulsive signaling is sufficient for guiding the turning of the ipsilateral projection at the midline. However, there are some ipsilaterally projecting cells remaining in the EphB1 mutant that appear not to respond to the absence of EphB1 when encountering the midline. Moreover, Zic2 knockdown mice exhibit a phenotype that does not perfectly match that of mice lacking EphB1. In both $Zic2^{kd/kd}$ and EphB1 mutants, there is a strong reduction in the number of fibers that project ipsilaterally (Williams *et al*., 2003), but in $Zic2^{kd/kd}$ mice, there is an additional defasciculation phenotype in which retinal axons wander from the distal optic nerve (Herrera *et al*., 2003), suggesting that Zic2 may mediate axon patterning by coordinating the regulation of multiple guidance genes. Further experiments will be required to clarify whether or not other forces apart from the inhibitory-EphB1/ephrinB2-mediated response of ipsilateral axons, for instance, an opposing program for crossing the midline, participate in divergence of uncrossed axons.

3.23.2.4 The Crossing Pathway

As described above, the proper expression of some axon guidance molecules, such as Slits/Robos and PGs, is required for contralateral axon crossing of the midline, in terms of fasciculation and placement of the cross-point. At the transcriptional level, one report identifies the LIM homeobox transcription factor Islet2 as being exclusively expressed by RGCs outside of the VT crescent. This pattern is complementary to that of Zic2 expression. Curiously, mice lacking Islet2 exhibit an increase in the number of ipsilateral axons arising from an expansion in the domain of ipsilateral RGCs in the VT retina, rather than a total loss of the contralateral projection (Pak *et al*., 2004). Concomitantly, the number of Zic2-positive cells increases. Based on these findings, Islet2 is thought to repress the Zic2-mediated

ipsilateral pathfinding program rather than acting as a specific determinant of contralateral RGCs. One interesting hypothesis arising from these results is that the Islet2/Zic2 border might determine the location of line of decussation, the boundary between crossed and uncrossed RGC populations, that changes with the degree of binocularity.

3.23.2.5 Other Regulatory Genes That Affect Optic Chiasm Formation

A number of other regulatory genes expressed in the developing retina have been suggested to play a role in retinal axon guidance at the optic chiasm.

The Pou4f2 (also called Brn3b) gene is expressed postmitotically in RGCs in which axonogenesis has commenced (Erkman *et al.*, 2000). The number of ipsilaterally projecting axons increases in Pou4f2$^{-/-}$ mice and this misrouting is partially prevented when Pou4f3 (also called Brn3c) is also removed (Wang *et al.*, 2002). Thus, Pou4f2/Pou4f3 could control the production of ipsilaterally projecting RGCs. However, the fact that these two genes are not expressed exclusively in the retinal regions giving rise to ipsi- or contralaterally projecting RGCs, along with the additional pathfinding defects observed at several decision points along the visual pathway in Pou4f2 KO mice (Wang *et al.*, 2002), argues for a more general function of these proteins in axon guidance, rather than the direct control of divergence at the optic chiasm.

Vax2-deficient mice show a reduction in the ipsilateral projection (Barbieri *et al.*, 2002; Mui *et al.*, 2002) and Pax2 null mice have a complete absence of contralateral projections (Torres *et al.*, 1996). Foxg1 or Foxd1 mutant mice also display alterations in the ratio of uncrossed versus crossed axons (Herrera *et al.*, 2004; Pratt *et al.*, 2004). All of these genes are expressed early in eye development, before RGCs differentiate, suggesting a role in morphogenesis and regional specification of the eye, and in turn, affecting events upstream of mechanisms directly controlling axon divergence, such as Zic2 and EphB1.

3.23.3 Patterning the Retina and Patterning the Chiasm: The Relationship between Them

Among the transcription factors expressed in the retina that regulate the ipsi/contralateral growth programs, the Fox genes are of interest. Foxd1 (also known as BF-2) is expressed in the VT retina but in a wider domain than Zic2. Foxd1 seems to act upstream of Zic2 and EphB1, since in mice null for Foxd1, these two proteins are not expressed in RGCs in the VT retina (Herrera *et al.*, 2004). In addition, Fox genes, and likely others, contribute to the formation of the optic chiasm itself. Foxg1 and Foxd1 are expressed in opposing rostrocaudal domains in the region of the ventral diencephalon, in which the optic chiasm forms (Marcus *et al.*, 1999). The Foxd1 domain includes the region that early retinal axons traverse to establish the optic chiasm, whereas Foxg1 is located more rostrally. Thus, both genes seem to play a role in the regionalization of the ventral brain during chiasm formation. In the Foxd1-deficient ventral diencephalon, Foxg1 expression invades the Foxd1 domain, Islet1 and Zic2 expression is diminished, and Slit2 prematurely expands (Herrera *et al.*, 2004). Thus, Foxd1 appears to play a dual role in the establishment of the binocular visual pathways: first, in the specification of the VT retina, acting upstream of proteins directing the ipsilateral pathway, and second, in the patterning of the developing ventral diencephalon where the optic chiasm forms.

Zic2, like Foxd1, is also expressed in the chiasm. The alteration of Zic2 expression in retinal explants *in vitro* is sufficient to change the behavior of RGC neurites in response to cues provided by chiasmatic cells, arguing that Zic2 acts primarily in the retina. However, as we have seen, both Fox and Zic genes are expressed in the presumptive chiasm and eyecup early in development before the uncrossed pathway forms, and mutations in Zic2 produce holoprosencephaly, a condition in which the halves of the forebrain are fused, occasionally associated with cyclopia in humans (Brown *et al.*, 1998). The relationship between the two loci of expression of these genes, both retina and ventral diencephalon, merits further analysis. It is intriguing that in the early development of *Xenopus*, a small group of cells migrates from one hemisphere to the other (Jacobson and Hirose, 1978). These cells contribute to the region where the chiasm will form as well as to the retinal region that will ultimately give rise to the uncrossed pathway. It is not known whether the migration of this cell group across the midline is related to the separation of the hemispheres, to the determination of the proportion of axons that remain in their own hemisphere, or even to the position of the eyes in the head. Whether the formation of the optic chiasm is an event unrelated to the specification of RGC subtypes is also unclear. Investigations on the derivation of subregions of the eye fields, the organization of the terrain on which the optic chiasm forms, and the designation of eye position in the head should reveal how binocularity is patterned.

In conclusion, progress has been made in understanding how the uncrossed pathway diverges

from the crossed pathway and in appreciating that the RGCs with a crossed and uncrossed trajectory may have different molecular profiles that encode for crossing or avoidance of the midline in the case of the uncrossed population. Zic2 and EphB1 are expressed concomitantly in the VT retina, the site of origin of the uncrossed RGCs. Gain- and loss-of-function experiments *in vitro* and *in vivo* strongly argue that this transcription factor and guidance receptor, respectively, constitute major determinants of the uncrossed pathway, with ephrinB2 acting as a key inhibitory ligand on the midline radial glia. Future experiments will reveal whether there is a link between Zic2 and EphB1. Furthermore, it appears that Islet2 plays a role in repressing the ipsilateral program. A crucial question is whether crossing axons actively overcome inhibitory cues or use a different type of mechanism, such as molecularly-mediated cell adhesion or other molecular receptor–ligand interactions, to specifically mediate extension to traverse the midline. Finally, some of the genes and molecular factors critical to patterning of the retina are also important for regionalization of the terrain on which the optic chiasm forms. Now that several molecular signatures have been ascribed to the crossed and uncrossed pathways, investigations of other genes involved in the process of patterning of the the binocular projection will be facilitated.

Additional Note

In a recent report (Williams *et al*., 2006), we show that the cell adhesion molecule Nr-CAM is expressed by RGCs that project contralaterally, but is critical only for the guidance of late-born RGCs within the VTC (Figure 4c). Blocking Nr-CAM function causes an increase in the size of the ipsilateral projection, and reduces neurite outgrowth on chiasm cells in an age- and region-specific manner. We also demonstrate that EphBl/ephrinB2-mediated repulsion and Nr-CAM-mediated attraction comprise distinct molecular programs that each contribute to the proper formation of binocular visual pathways.

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3.24 Evolution and Development of Eye-Specific Layers in the Lateral Geniculate Nucleus

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Glossary

afferent Neural fiber projecting from one brain region and terminating in another.

axon guidance cue A molecule that acts on axons of developing neurons to alter the progression/stability/morphology of the growth cone as it migrates through the environment, thereby guiding the axon towards its target.

cDNA plasmid A circular double-stranded DNA molecule that contains a coding region for one or more mRNA molecules without introns.

center-surround receptive field The spatial visual receptive field characteristic of retinal ganglion cells and LGN cells, consisting of a central spot of one response type (ON or OFF) and a surrounding annulus of the opposite response type.

contralateral Located on or affecting the opposite side of the body.

cytoarchitecture The arrangement of cells in a tissue, especially the arrangement of nerve-cell bodies in the cerebral cortex.

Eph The largest subfamily of receptor tyrosine kinases (RTKs) important in mediating cell–cell communication and regulating cell attachment, shape, and mobility.

electroporation A significant increase in the electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electrical field. It is usually used in molecular biology as a way of introducing some substance into a cell, such as loading it with a molecular probe, a drug that can change the cell's function, or a piece of coding DNA.

enucleation Removal of the eye.

ephrin An axon guidance cue; the ligand for the Eph receptors.

lateral geniculate nucleus (LGN) Thalamic nucleus that receives input from the retina and projects to the primary visual cortex.

line of projection A line of cells across the LGN that receives input from a given point in visual space.

immunotoxin Consisting of an antibody linked to a toxin, this chimeric protein targets a specific cell type, is bound and taken into the cell, and then kills the cell by the action of the toxin molecule.

ipsilateral Located on or affecting the same side of the body.

nasal Of, relating to, or near the nose.

nicotinic acetylcholine receptor Ionotropic receptor present in many brain areas, usually excitatory in nature.

ocular dominance columns Interdigitated bands of neurons in primary visual cortex that receive visual input from primarily one or the other eye.

optic chiasm The part of the brain where the optic nerves partially cross from one side of the body to the other.

retinal ganglion cell Retinal neuron that receives information from photoreceptors via bipolar neurons and projects to many targets in the central nervous system.

retinogeniculate Relating to the projections from the retina to the LGN.

retinorecipient nucleus	A brain region receiving input from ganglion cells of the retina.
retinotopy	Organization of a visual brain area in a way that adjacent points in the visual field (that fall on adjacent points on the retina) are processed by neurons in adjacent parts of that area.
starburst amacrine cell	A cholinergic type of retinal amacrine neuron thought to be important in regulating spontaneous patterns of activity in the developing retina.
superior colliculus	A structure of the midbrain receiving visual and other sensory information that is involved in the generation of eye and head movements.
temporal	Of, relating to, or near the temples of the skull.

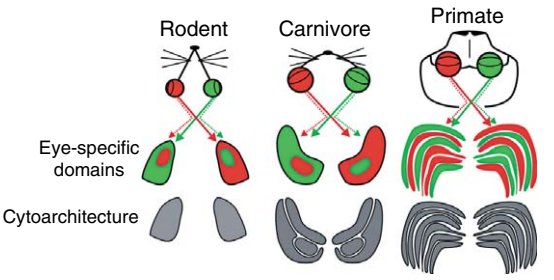

Figure 1 Differences in the degree and pattern of eye-specific connections to the LGN across orders. The top row shows the approximate position of the left eye (depicted in red) and right eye (in green) in the head of a prototypical rodent, carnivore, and primate. Note that the eyes are more lateralized in the rodent than in the carnivore, and eyes are more lateralized in the carnivore than in the primate, which has very frontally placed eyes. The middle row shows the pattern of eye-specific domains (axonal terminations from the retinas) in the LGN in red and green. The bottom row shows the degree and pattern of eye-specific cytoarchitecture (cellular lamination) in the LGN in gray. Rodents have no obvious cellular lamination in the LGN whereas carnivores and primates do.

3.24.1 Introduction

In mammals, the neural connections that transmit visual information from the left and right eyes are generally segregated from one another. A striking example of this is found in the lateral geniculate nucleus (LGN) of many species wherein ganglion cell axons arising from the right and left eyes are organized into a highly stereotyped arrangement of nonoverlapping domains (Jones, 1985). In some species, these eye-specific axonal domains are mirrored by eye-specific cellular layers: groups of postsynaptic neurons separated by cell-sparse, interlaminar zones. There are three basic types of LGN organization: poorly segregated inputs from the two eyes and no cellular lamination, well-segregated inputs but no cellular lamination, and well-segregated inputs with cellular lamination. In placental mammals, the degree of segregation of afferents and the number of eye-specific domains increases with the degree to which the two eyes reside in front of the skull, and thus the degree of binocular vision (see Visceral Afferents). Also, eye-specific cellular layers are generally found only in highly binocular animals. For instance, in insectivores with highly lateralized eyes, such as the hedgehog, there is poor segregation of inputs and no cellular lamination. In mice, which have lateralized eyes such that a small region of the visual field is viewed by both eyes, there is a single region of the LGN that receives axons from the contralateral eye, another region that receives axons from the ipsilateral eye, and no overt eye-specific cellular lamination (Figure 1). By contrast, the LGN of a carnivore such as the ferret has good segregation of inputs into two major eye-specific layers with clear corresponding cellular layers (Figure 1), while the LGN of the highly binocular macaque monkey contains three separate axonal domains per eye and six corresponding eye-specific cellular layers (Figure 1). In marsupials, the relationship between eye placement and LGN lamination pattern may not hold. Within marsupials, all three basic types of LGN organization are seen. There has not been a careful study of the placement of the eyes in the head among different species of marsupials, but in general all marsupials have relatively laterally placed eyes. Within one suborder of marsupials, polyprotodonts, there is a relationship between lifestyle and LGN organization similar to that seen in placental mammals, with carnivores having more complex LGNs than herbivores. However, within the other suborder, diprotodonts, this relationship breaks down. Most diprotodonts studied are herbivores, yet have very complex LGNs with as many as ten distinct retinal recipient layers. Thus, it is clear that other phylogenetic factors, in addition to eye placement and lifestyle, must also be involved in the evolution of LGN organization (reviewed by Kahn and Krubitzer, 2002).

It is noteworthy that eye-specific segregation is present in all of the 25+ retinorecipient nuclei (Muscat *et al.*, 2003), but in non-LGN retinorecipient nuclei, the shape and positioning of eye-specific domains is somewhat variable. In the LGN, however, eye-specific projections are highly stereotyped. Also, eye-specific cellular layers are only found in the LGN. To date, neither the segregation of axons from the two eyes nor the presence of eye-specific cellular layers has been directly linked to any particular aspect of visual

processing. It is tempting to speculate that the mere presence of these features indicates a role for them in visual processing, especially in the LGN of carnivores and primates where eye-specific patterning is so stereotyped within species (see The Role of Vision in the Origin and Evolution of Primates). However, it is equally possible that these features are epiphenomena – byproducts of developmental events that bear no purposeful impact on vision itself – as has been argued for ocular dominance columns in visual cortex (reviewed by Adams and Horton, 2005).

Regardless of their possible function (or lack thereof) in visual processing, eye-specific axonal domains and eye-specific cellular layers in the LGN are classic model systems for exploring how afferent targeting occurs during development, and how this targeting relates to anatomical and functional differentiation of cells within postsynaptic structures. Here we discuss what is currently known about the cellular and molecular mechanisms underlying development of eye-specific connections to the LGN in rodents, carnivores, and primates. In doing so, we address which of these mechanisms likely evolved to induce varying degrees of eye specificity in the LGN. Where relevant, we speculate about the possible role of eye-specific connections in visual processing.

3.24.2 Development of Eye Specificity in the LGN

Numerous studies have shown that the development of eye-specific retinogeniculate projections emerges from a state in which axons from the two eyes initially overlap (macaque: Rakic, 1976; ferret: Linden *et al.*, 1981; cat: Shatz, 1983; rat: Jeffery, 1984; mouse: Godement *et al.*, 1984). In addition, all of these experiments concluded that eye-specific segregation occurs before vision is possible. In some species, such as the monkey or cat, this is because eye-specific segregation occurs *in utero*, whereas in other species where segregation occurs postnatally (such as in the mouse or the ferret) photoreceptors do not become light responsive until after segregation is complete (Linden *et al.*, 1981; Godement *et al.*, 1984; Akerman *et al.*, 2002; Tian and Copenhagen, 2003). In all mammalian species where it has been examined, ocular segregation in the LGN appears competition-dependent; if one eye is removed at the stage of development when axons from the two eyes overlap, projections from the remaining eye become distributed throughout the LGN (rat: Lund *et al.*, 1973; macaque: Rakic, 1981; cat: Chalupa and Williams, 1984; ferret: Guillery *et al.*, 1985a). What mechanisms mediate binocular competition in the LGN? One popular hypothesis is that correlated activity of retinal ganglion cells within the same eye out-compete inputs from the other eye because there is increased synaptic efficacy from groups of ganglion cell axons arising from the same eye onto single LGN neurons, relative to axons arising from different eyes onto single LGN neurons. Because eye-specific LGN layers emerge before the onset of vision, such correlations must arise spontaneously. Maffei and colleagues reported the presence of correlated spontaneous ganglion cell activity in the embryonic rat retina (Galli and Maffei, 1988; Maffei and Galli-Resta, 1990). Others have shown that spontaneous retinal activity also occurs during the period of eye-segregation in ferrets. The activity recorded in the ferret retina during eye-segregation consists of waves of excitation that sweep across the retina, engaging neighboring retinal ganglion cells (RGCs) to fire in synchrony (Meister *et al.*, 1991; Wong *et al.*, 1993). These waves are driven by acetylcholine (ACh) acting through nicotinic receptors present on retinal ganglion cells (Feller *et al.*, 1996). The pharmacologic cholinergic agonist, Epibatidine (EPI), blocks this spontaneous retinal activity (by receptor desensitization) when acutely applied at high concentrations *in vitro* (Penn *et al.*, 1998), and binocular injections of EPI from postnatal day 1 (P1) to P10 completely prevent eye-specific segregation in the LGN, indicating that eye-specific segregation requires retinal activity. If EPI is injected into only one eye from P1 to P10, axons from the EPI-treated eye retract to occupy a smaller-than-normal region of the LGN, and the projection from the other eye expands. This indicates that ocular segregation in the LGN relies on activity-mediated binocular competition. Further reinforcing the binocular competition model, Stellwagen and Shatz (2002) showed that if activity is elevated in both eyes, there is no effect on patterning of eye-specific retinogeniculate projections. However, if wave activity is increased only in one eye, axons from the more active eye acquired more LGN territory than the projection from the normally active, untreated eye. These results indicate that the relative level of activity in the two eyes is a key parameter for patterning of eye-specific projections.

3.24.2.1 Which Aspects of Retinal Activity Drive Eye-Specific Segregation?

It is worth noting that although many experiments have shown activity blockades in the retina or the

LGN can prevent ocular segregation in the LGN (Sretavan *et al.*, 1988; Shatz and Stryker, 1988; Penn *et al.*, 1998; Cook *et al.*, 1999; Huberman *et al.*, 2002; Stellwagen and Shatz, 2002; Rossi *et al.*, 2001), when all spiking activity is abolished one cannot conclude that the pattern of activity is important in the segregation process. Unfortunately, in the experiment where retinal activity levels were elevated rather than eliminated (Stellwagen and Shatz, 2002), correlated ganglion cell activity was maintained. To test the hypothesis that correlated firing of retinal ganglion cells drives eye-specific segregation, we injected an immunotoxin that kills starburst amacrine cells, into the eye of P0 ferrets. Dual cell patch-clamp recordings showed that, in control ferrets, the spontaneous spiking activity and membrane potential changes of neighboring ganglion cells were significantly correlated, whereas the spiking and membrane potential activity of ganglion cell pairs from toxin-treated retinas were not. Importantly, despite the marked perturbation in ganglion cell activity patterns caused by starburst amacrine cell depletion, the mean firing rate of ganglion cells was not significantly elevated or reduced. What is the effect of eliminating correlated retinal activity on eye-specific segregation in the LGN? Surprisingly, axonal projections to the LGN of immunotoxin-treated ferrets were indistinguishable from those observed in control ferrets (Huberman *et al.*, 2003). Thus, the correlated firing of neighboring ganglion cells does not appear critical for eye-specific retinogeniculate segregation. This conclusion may differ across species. In macaques, modern axonal tracing techniques show that eye-specific segregation occurs before the stage when ganglion cell axons form synapses in the LGN (Huberman *et al.*, 2005a) and there is no correlated activity in the macaque retina during this stage of development (Warland *et al.*, 2006). Thus, as in the ferret, retinal waves are unlikely to drive eye-specific segregation in the macaque LGN. By contrast, in transgenic mice that lack the beta-2 subunit of the nicotinic acetylcholine receptor (β2nAChR), correlated waves of activity are disrupted only in the first postnatal week and eye-specific segregation fails to occur, indicating that waves may be important for eye-specific segregation in mice (Torborg *et al.*, 2005). Importantly, however, the β2nAChR knockout mice lack activity in approximately 50% of the ganglion cells, and it is unknown whether the LGN cells themselves are silenced. Therefore, it remains unclear if it is indeed lack of retinal waves or, alternatively, reduced activity levels in the retina and/or LGN that prevented eye-specific segregation in these knockouts. Experiments that employ retinal-specific knockouts of wave activity, and in which overall levels of retinal activity are unaltered, are necessary to resolve these issues. Another consideration is that the β2nAChR knockouts exhibit severely altered retinotopy of ganglion cell projections to both the superior colliculus and the LGN (McLaughlin *et al.*, 2003; Grubb *et al.*, 2003). Thus, it may not be surprising that eye-specific projections are disrupted in these mice. In speculating why different species would rely more or less on correlated activity in the retina for patterning of eye-specific projections to the LGN, it is worth noting that in carnivores and primates (such as ferrets and macaques) that have well-differentiated and stereotyped eye-specific LGN layers, ganglion cells projecting to each eye-specific domain arise from distinct regions of the retina. Therefore cues unique to different retinal regions, rather than activity, could drive eye-specific patterning in the LGN. In rodents, however, axons from throughout the entire contralateral eye project to the LGN. These eye-specific domains are less stereotyped and there is no cellular lamination, so retinotopic cues and activity may act in concert to influence eye-specific patterning without the need for additional bona fide eye-specific layer patterning cues. We consider these possibilities in more detail below.

3.24.2.2 What Cues Regulate Spatial Patterning of Eye-Specific Axonal Projections?

A striking feature of eye-specific inputs to the LGN (especially in carnivores and primates) is their remarkably stereotyped shape, size, and position. For example, in carnivores such as ferrets and cats, the axons from the contralateral eye always occupy the innermost LGN and axons from the ipsilateral eye always project to the more outer LGN. Although activity-dependent models can explain how inputs from the two eyes segregate from one another, they cannot explain how the ipsilateral eye axons always segregate into the same regions of the LGN. The same is true for the six-layered primate LGN; axons from the two eyes intermingle before segregating into their respective eye-specific territories, but the order of the layers is always the same (e.g., the ventral-most region is always innervated by ganglion cells from the contralateral eye) and there is remarkable symmetry of eye-specific projections in the two LGNs, even at very early stages of eye-specific differentiation (Rakic, 1976; Huberman *et al.*, 2005a). Simply put, there must be a bias for one or the other eye to win a given piece of LGN real estate.

3.24.2.3 What Mechanisms Could Dictate the Spatial Location that Axons from the Two Eyes Segregate into?

We carried out experiments in ferrets in which we prevented the segregation of retinogeniculate inputs (by silencing spontaneous retinal activity in both eyes with EPI) and then allowed these animals an extended period of recovery, during which spontaneous retinal activity returned to normal (Huberman *et al.*, 2002). We then assessed the effects of this manipulation on the pattern retinogeniculate inputs. As shown previously, in normal P1 ferrets, ganglion cell axons from the two eyes overlap extensively and by P10, they are segregated (Linden *et al.*, 1981). As seen by Penn *et al.* (1998) in ferrets that received binocular EPI injections from P1 to P10, inputs from the two eyes remain overlapped in the LGN. In ferrets that received binocular intravitreal injections of EPI from P1 to P10, but were then allowed to survive until P25 or older (hereafter called EPI-recovery ferrets) retinogeniculate afferents end up completely segregated. However, unlike control ferrets, the spatial pattern of eye-specific retinogeniculate projections is highly aberrant. There are multiple ipsilateral projections of various shapes, positions, and sizes, and these are distributed over a significantly greater than normal extent of the LGN. This indicates that there is something special about the developmental time window in which retinogeniculate segregation normally occurs for proper patterning of eye-specific inputs to the LGN. It is possible that the pattern of spontaneous retinal activity present from P1 to P10 provides an instructive cue for development of this feature. Indeed, the pattern of spontaneous retinal activity is different from P1 to P10, than it is during the recovery phase from P10 to P25 (Wong *et al.*, 1993). However, as noted in the introduction, ganglion cells send eye-specific projections to approximately 25 subcortical nuclei and yet, none of these nuclei contain the highly regular spatial patterning of eye-specific inputs witnessed in the LGN, despite the presence of normal P0–P10 retinal waves. For example, during the same developmental stage when retinogeniculate afferents segregate into eye-specific domains in the LGN, retinal activity induces ganglion cell axons to segregate into randomly positioned eye-specific clusters in the rostral superior colliculus (Thompson and Holt, 1989). Also, in studies where retinal inputs destined for the LGN were rewired into the medial geniculate nucleus (MGN), axons from the two eyes segregate into eye-specific patches that do not resemble the normal spatial organization of inputs to the LGN (Angelucci *et al.*, 1997). Spontaneous retinal activity is normal throughout development in these rewired animals and yet normal eye-specific domains fail to develop in novel targets, indicating that patterning of ganglion cell axons into stereotyped eye-specific regions in the LGN is controlled by cues that are intrinsic and unique to the LGN, rather than by patterns of retinal activity. Moreover, it is hard to imagine how activity could give rise to highly stereotyped eye-specific layers since activity is likely to differ across animals, and yet eye-specific domains in the LGN always form in the same location, size, shape, and orientation. Thus, patterning of eye-specific inputs to the LGN almost certainly relies on the presence of molecular signals that bias the location and boundaries of the regions into which afferents from one or the other eye segregate.

3.24.2.4 Ephrin-As Pattern Eye-Specific Axonal Domains in the LGN

The idea that molecular cues direct sorting of binocular inputs into their stereotyped pattern of eye-specific layers in the LGN has been proposed repeatedly (Williams *et al.*, 1994; Crowley and Katz, 1999, 2000; Chapman, 2000), but until recently the identity of those cues remained elusive. What sort of axon guidance cues might contribute to patterning of eye-specific inputs to the LGN? One clue is that, unlike in rodents where ganglion cells from the 'entire' retina project to the contralateral LGN, in carnivores and primates the contralateral retinal projection arises strictly from ganglion cells in the nasal retina and the ipsilateral projection from ganglion cells in the temporal retina. Thus, a candidate axon guidance cue that could mediate eye-specific patterning are the ephrin-As and their receptors (EphAs). Ephrin-As are known to regulate topographic mapping of the nasal-temporal retina in the SC and LGN of lower vertebrates and mice (Cheng *et al.*, 1995; Drescher *et al.*, 1995; Nakamoto *et al.*, 1996; Frisen *et al.*, 1998; Feldheim *et al.*, 1998, 2000; Feldheim, 2004; see The Evolution of Vertebrate Eyes). There are obvious differences between retinotopic maps (which are smooth and continuous) and eye-specific LGN layers (which have abrupt borders). However, the well-established role of ephrin-As in nasal-temporal mapping as well as on inter- and intra-areal pathfinding in other projection systems (Dufour *et al.*, 2003) leads us to hypothesize that in species where eye-specific layers obey the nasal- versus temporal-retina distinction, ephrin-As mediate patterning of eye-specific projections.

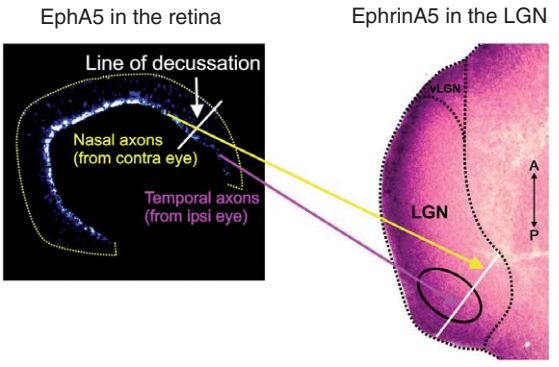

Figure 2 Model for ephrin-A induced targeting of eye-specific retinogeniculate projections. Ferret retina labeled for EphA5 mRNA shows a central → peripheral gradient of EphAs (high abundance of the transcript appears in white). The line of decussation is shown. Axons to the left of the line of decussation would project to the contralateral LGN (nasal axons), axon to the right of the line of decussation would project to the ipsilateral LGN (temporal axons). Here the inputs from two eyes into the same LGN are depicted as arising from the same retina. Ephrin-A5 mRNA appears in an outer → inner gradient in the LGN (high abundance transcript appears in purple). The axons from these two populations of ganglion cells send axons to the 'same' line of projection (white line in the LGN) but 'different' eye-specific layers. The relatively higher levels of EphA5 in the contralateral-projecting ganglion cells (yellow arrow) combined with the outer → inner gradient of ephrin-As in the LGN, causes these eye axons to project to the more inner LGN than the ipsilateral-projecting ganglion cells that view the same location in visual space (purple arrow). See Huberman *et al.* (2005b) for details.

Using *in situ* hybridization to detect mRNAs and affinity probe binding to detect proteins, we observed the presence of an outer → inner gradient of ephrin-A5 in the early postnatal ferret LGN (Figure 2). We also examined the pattern of EphAs in the developing ferret retina and observed a central greater than peripheral (central → peripheral) gradient of EphA5 (Figure 2). Therefore, there are relatively higher levels of EphAs expressed in the crossed (nasal) versus uncrossed (temporal) ganglion cell axons that converge on a single line of projection (Figure 2). Since ephrin-As have consistently been shown to be repellant toward ganglion cell axons expressing relatively higher levels of EphAs (Cheng *et al.*, 1995; Drescher *et al.*, 1995; Nakamoto *et al.*, 1996; Frisen *et al.*, 1998; Feldheim *et al.*, 1998, 2000, 2004; Brown *et al.*, 2000), the central → peripheral gradient of EphAs in the retina, combined with the outer → inner gradient of ephrin-As in the LGN therefore leads to a scenario whereby the contralateral-eye domain (expressing higher levels of EphA) always maps to the inner LGN (where there are lower levels of ephrin-A), whereas the ipsilateral-eye domain (expressing lower levels of EphA) maps to the outer LGN (where there are higher levels of ephrin-A) (Figure 2). To test directly whether ephrin-A:EphA interactions regulate eye-specific layer formation, we developed an *in vivo* retinal electroporation strategy to overexpress cDNA plasmids in ganglion cells of postnatal ferrets (Huberman *et al.*, 2005b). Axons of ganglion cells transfected with control plasmids were targeted normally in the P10 LGN. By contrast, ferrets that were electroporated with EphA5 on P1 showed markedly perturbed retinogeniculate projections. Axons from the ipsilateral eye were displaced to the inner LGN and into territory dominated by the contralateral eye. This resulted in ipsilateral-eye input to the LGN that was significantly expanded along the axis perpendicular to eye-specific layers (i.e., along lines of projection). In addition, axons from the contralateral eye were found in the region of the P10 LGN normally only occupied by axons from the ipsilateral eye. We also traced single retinogeniculate axons in control and EphA5 electroporated ferrets. In every control ferret examined, ganglion cell axons from temporal portion of the ipsilateral retina were restricted to the outer portion of the ipsilateral LGN, as expected for animals of this age (Hahm *et al.*, 1999). By contrast, ganglion cell axons labeled from the temporal retina of EphA5 electroporated ferrets at both ages extended much further along across the outer–inner axis of LGN than was observed in controls (Huberman *et al.*, 2005b).

By examining the time-course of ephrin-A and EphA expression in normal ferrets, we found that, whereas ephrin-A ligands are robustly expressed in the P0–P3 LGN, by P5 their levels are reduced conspicuously. Our previous work in EPI-recovery ferrets showed that normal development of stereotyped eye-specific inputs to the LGN is restricted to the early postnatal period when eye-specific segregation normally occurs, suggesting there may be a critical period for eye-specific layer formation (Huberman *et al.*, 2002). To test if expression of ephrin-As in the LGN contributes to this critical period, we electroporated ferrets with EphA5 at P5. Remarkably, despite the robust overexpression induced by retinal electroporation at this age, there was no detectable effect on patterning of eye-specific retinogeniculate projections. These results (Huberman *et al.*, 2005b) and those of an accompanying paper in mouse (Pfiffenberger *et al.*, 2005) represent the first evidence for axon guidance cue-based targeting of eye-specific projections. Also, Lambot *et al.* (2005), showed that EphA receptors are present in the fetal human retina, in patterns similar to that observed in ferrets (central → peripheral). Of course, ephrin-As likely represent only one

of several (and perhaps many) cues that nasal and temporal retinal axons rely on to pathfind to their stereotyped locations in the LGN. As mentioned above, in mice ephrins act as retinotopic cues, whereas in more binocular species ephrins may have additional, specialized roles in eye-specific patterning in the LGN. Regardless, it is clear that changing distribution patterns of mapping molecules such as ephrins could explain in part the varying number and patterns of eye-specific terminations seen across species. We now consider this hypothesis in more detail.

3.24.2.5 Eye-Specific Cellular Lamination

As mentioned in the introduction, primates and carnivores have eye-specific cellular layers in the LGN. Several studies have addressed the developmental relationship between axons from the two eyes and patterning of cellular layers in the LGN (Brunso-Bechtold and Casagrande, 1981; Casagrande and Condo, 1988). In the LGN of carnivores, cells form cytoarchitectural layers that lie in direct register with the layers formed by the terminals of retinal afferents (Linden *et al.*, 1981; Stryker and Zahs, 1983; Zahs and Stryker, 1985; Hutchins and Casagrande, 1990; Hahm *et al.*, 1999). Retinal axons appear to instruct development of cellular layers in the LGN: the development of cellular layers occurs after afferents from the two eyes segregate and if the pattern of retinogeniculate afferents is rendered abnormal, the cytoarchitecture of the LGN directly reflects these abnormal inputs. For example, in monocularly or binocularly enucleated animals, eye-specific cellular laminae do not develop (Brunso-Bechtold and Casagrande, 1981; Rakic, 1981; Guillery *et al.*, 1985a, 1985b; Sretavan and Shatz, 1986; Garraghty *et al.*, 1988; Morgan and Thompson, 1993) and in coat-color mutants, where the density of the ipsilateral-eye projection to the LGN is reduced, the cellular laminae mirror the reduced ipsilateral input and the associated abnormal topography of the retinal projections (Guillery, 1969, 1971; Guillery and Kaas, 1971). We observed that, despite the presence of well segregated eye-specific domains in the LGN of the EPI-recovery animals, the LGN completely lacked normal patterns of cellular lamination. Clusters of cells surrounded by cell-sparse regions are occasionally visible, but comparison of these clusters with the pattern of retinogeniculate afferents in the same tissue sections reveals that they do not correspond to eye-specific termination zones of ganglion cell axons. Because these animals lacked retinal activity from P0 to P10 but normal activity was present thereafter, this indicates that early postnatal retinal activity is required for patterning of eye-specific cellular layers. We also sought to determine whether disrupting the pattern of retinal afferent lamination alters the physiology of LGN neurons; we performed multiunit extracellular recordings in the LGN of EPI-recovery animals. All cells encountered were monocular, indicating that functional as well as anatomical segregation of eye-specific inputs to the LGN occurred following the termination of the EPI treatment. Cells in the LGN of the treated animals exhibited ON- or OFF-center responses typical of normal ferrets (Stryker and Zahs, 1983; Zahs and Stryker, 1985) and normal center-surround receptive field organization was present. Surprisingly, in the EPI-recovery animals, the topographic representation of the binocular visual field was mapped normally, even across the boundaries of eye-specific domains. Thus, dramatically disrupting the organization of eye-specific lamination does not affect the gross topographic representation of visual space in the LGN. This finding is unexpected given the widely varying pattern of eye-specific layers both between and within the LGNs of the EPI-recovery animals, and it indicates that neither stereotyped patterning of eye-specific axonal termination in the LGN nor eye-specific cellular layers are required for normal topographic mapping.

3.24.3 Which Developmental Mechanisms Might Explain the Variation in Eye-Specific Lamination Seen Across Species?

Given that the degree of eye-specific differentiation in the LGN of placental mammals scales directly with the degree of binocular vision, we now consider how the position of the eyes in the skull (the major determinant of the degree of binocular vision) could impact the number, shape, and location of eye-specific domains in the LGN. It is important to note that the percentage of ganglion cells that take an uncrossed route at the chiasm is in direct proportion to the degree of binocular vision. For instance, in rodents, which have minimal binocular vision, only approximately 5–8% of retinal ganglion cells in each eye project ipsilaterally, whereas in carnivores and primates 12–50% of retinal ganglion cells project ipsilaterally. So it follows that the developmental mechanisms that influence the number of ganglion cells that project ipsilaterally will directly impact the degree of eye-specific differentiation in LGN. Recent evidence indicates that ganglion cells expressing the ephrin-B receptor, EphB1, take an

uncrossed route at the chiasm because the repellant ligand, ephrin-B2, is expressed at the chiasm (Williams *et al.*, 2003). As described above, in ferrets (Huberman *et al.*, 2005b) and also in humans (Lambot *et al.*, 2005), the expression of EphAs in the retina is central → peripheral, whereas in the less binocular mouse, EphAs are expressed in a temporal → nasal gradient. Because repellant ephrin-A ligands are expressed in the LGN in a manner sufficient to induce stereotyped patterning of eye-specific layers (Huberman *et al.*, 2005b), the distribution of ephrin-As and ephrin-Bs, and their receptors, are important determinants of patterning of eye-specific differentiation in the LGN. Ephrin-Bs dictate how many cells from the two eyes project to the same LGN, and ephrin-As dictate where the axons from each eye will project to within the LGN. Retinal activity-mediated binocular competition might then act to sharpen the eye-specific boundaries induced by ephrin signaling. How could the position of the eyes in the skull dictate patterns of ephrin expression in the retina and/or LGN? Lambot *et al.* (2005) proposed an ingenious hypothesis that there is a (currently unidentified) molecule that is expressed in a fixed location within the skull and outside the eye and that this putative molecule influences the location of peak expression of both EphAs and EphBs in the retina. In their model, the rotation of the eye from the side to the front of the skull would change the location of this fixed signaling center relative to the neural retina, thereby changing the patterns of ephrins accordingly. The identification of this putative signaling molecule, examination of its expression patterns across species, and ultimately, its manipulation, will be required to determine the validity of this model. It is unknown what factors determine whether eye-specific cellular layers form in the LGN. Mice have well-segregated eye-specific axonal inputs to the LGN, but no corresponding cellular layers. Is this because the contralateral projection to the LGN of mice arises from ganglion cells throughout the retina and therefore cannot be molecularly distinguished from ipsilaterally projecting ganglion cells? At present, the answer to this question remains unknown. Experiments that focus on the possible function of eye-specific cellular layers may lend insight to this issue.

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3.25 Evolution of Gustation

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Glossary

cyprinid	Pertaining to freshwater fish. All fish in this family are egg-layers.
papillae	A bump occurring in various animal tissues and organs. Taste buds are found in circumvallate, foliate, and fungiform papillae.
taste buds	Small sensory organs that contain gustatory receptor cells, basal cells, and supporting cells. Taste buds in humans are found in the epithelia of the tongue, palate, and pharynx. They are innervated by the chorda tympani nerve (a branch of the facial nerve), the glossopharyngeal nerve, and the vagus nerve.
taste cells	Neuroepithelial cells found in taste buds.
tetrapod	A vertebrate animal having four feet, legs, or leglike appendages.
TRP channels (transient receptor channels)	Calcium-permeable channels. They can be gated by a variety of chemical and physical stimuli. A subpopulation of taste cells contain TRPM5 channels and many pain fibers contain TRPV1 channels, which are activated by capsaicin, the component in chile pepper that produces a burning sensation.

3.25.1 Introduction

The sense of taste allows mammals to discriminate between nutrient-rich stimuli and aversive, potentially toxic compounds. In vertebrates with a developed central taste system, it is fundamental to appropriate feeding behavior and survival (see Evolution of Taste). We show that the development of the mammalian brain made possible the formation of complex associations in the gustatory–reward cortices between the perceptual features of taste stimuli and the internal, physiological state of the organism. Supported by an intricate circuitry containing several distributed interacting pathways, mammalian feeding behavior became adaptive and efficient.

3.25.2 Peripheral Taste System

In vertebrates, the sense of taste is mediated by specialized epithelial cells arrayed in specific sensory end organs, the taste buds. Taste buds first appear phylogenetically coincident with the vertebrate lineage. In contrast, in invertebrates, the sense of taste is mediated via bipolar sensory neurons that have a distal process reaching the surface of the epithelium and a central process extending directly into the central nervous system, indicating a nonhomology with respect to vertebrates (Finger and Simon, 2000).

Several morphological features of taste buds are shared throughout the vertebrate lineage. They consist of proliferative basal cells, centrally situated elongated cells, and flattened edge cells that form the lateral boundary of the taste bud and the transition to extragemmal epithelium (Murray and Fujimoto, 1969; Finger and Simon, 2000). Taste buds occur mainly within the oropharynx but can also be found in some species, in the epiglottis and lips. In some cases, they are found across the entire body surface (see below). Nevertheless, in all cases, at specific regions of the taste bud the epithelial (taste) receptor cells make synapses with primary sensory neurons from the facial (VII), glossopharyngeal (IX),

and vagal (X) cranial nerves (CNs) (Norgren, 1990; Finger and Simon, 2000). In most species, taste buds have the overall shape of an onion and can contain 20–100 cells, with widths ranging from 30 to 100 mm (Duncan, 1964; Finger and Simon, 2000).

In tetrapods (all of which have tongues), taste buds are located on the tongue as well as in the mouth and throat (Butler and Hodos, 1996). In some amphibians, such as the frog, the tongue is a soft organ covered by 400–500 scattered fungiform papillae, most of which contain disk-shaped taste buds with no pores (Rapuzzi and Casella, 1965), comprising distinct supporting cells that do not synapse onto sensory fibers (Osculati and Sbarbati, 1995). In birds, some avian taste buds are situated deep in the epithelium and have a long taste pore, called the taste canal (Ganchrow and Ganchrow, 1987). It is not clear whether this could be generalized to all birds, but in general they seem to have fewer taste buds than tetrapods (Butler and Hodos, 1996). Taste buds in bony and cartilaginous fishes contain three distinct cell types (elongated cells bearing small microvilli, elongated cells bearing a thick microvillus, and serotonergic basal cells) that synapse onto either other taste cells or sensory nerve fibers (Finger and Simon, 2000). In some fishes, taste buds are located in areas other than the mouth and throat. For example, in cyprinids (which include carps and goldfishes), taste cells are present across the entire body surface, allowing these animals to taste their environment while searching for nutrients (Butler and Hodos, 1996).

In mammals, taste buds normally comprise a collection of 50–100 elongated epithelial cells and a comparatively smaller number of proliferative basal cells (Kruger and Mantyh, 1996). Each taste bud contains several distinguishable types of elongated taste cells, based on morphological and biochemical features, first revealed by Murray (1973) in his studies on rabbit taste receptor cells. Among these features, one way to characterize these taste cell types is to study which proteins they express. Briefly, type I cells express GLAST, a glial glutamate transporter (Lawton *et al.*, 2000), suggesting glial function; type III cells can be characterized by the expression of SNAP25, a synaptic membrane protein, which indicates transmission of information to the central nervous system (Finger, 2005). Studies suggest that this is the only cell type that forms synapses and the transmitter is ATP (Finger *et al.*, 2005). Type II cells are especially interesting in that they express the entire transduction cascade for sweet, bitter, and umami chemoreception, including the downstream transduction-related molecules phospholipase Cβ2 (PLCβ2) and IP3R3 (Miyoshi *et al.*, 2001). The downstream product of these transduction pathways appears to be the TRPM5 channel (see Figure 1 and Perez *et al.*, 2002; Zhang *et al.*, 2003).

In any event, these different cell types express taste receptors on the apical surface corresponding to the considered five basic modalities of mammalian gustatory senses: sweet, sour, bitter, salty, and umami (often described as the taste of protein, as elicited by monosodium glutamate and 5′ nucleotide monophosphate) (Scott, 2005; see Figure 2).

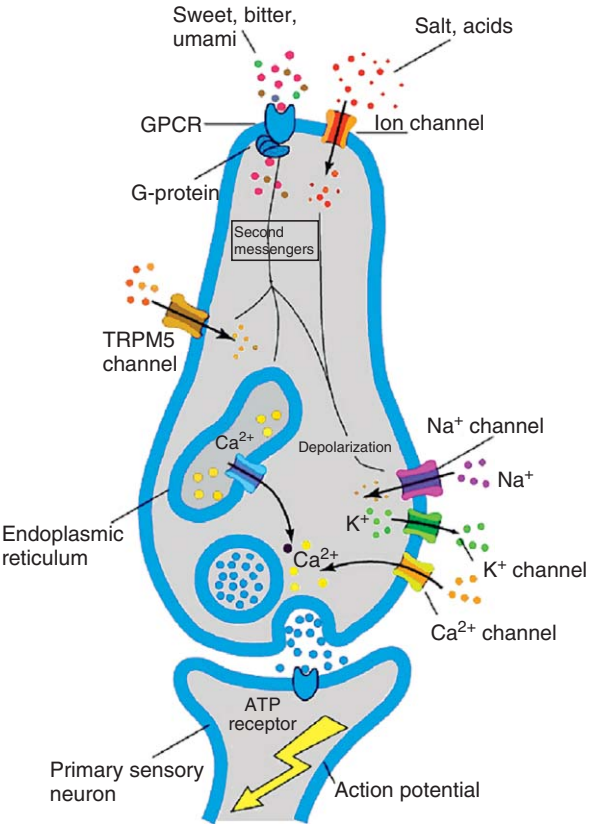

Figure 1 Schematic representation of sensory transduction in taste cells. Ion channels detect the presence of salty (NaCl) and sour (HCl) tasting compounds, whereas G-protein-coupled receptors respond to umami, sweet, and bitter tasting compounds (see Figure 2). All of these receptors are located in the apical domain of taste cells, which is separated from the basolateral domain by tight junctions. The components of the internal signaling cascade that is coupled to taste receptor molecules (including G-proteins and associated second-messenger molecules) are also preferentially expressed in the apical domain. Voltage-gated Na^+, K^+, and Ca^{2+} channels mediating the release of neurotransmitter from presynaptic specializations at the base of the cell onto sensory fibers are located in the basolateral domain, as well as the endoplasmic reticulum, which is also involved in regulating Ca^{2+} intracellular concentration. Communication between taste cells and primary sensory fibers is mediated by the neurotransmitter ATP and possibly serotonin. Another channel that is involved in G-protein-coupled receptor-mediated responses and taste cell depolarization is TRPM5.

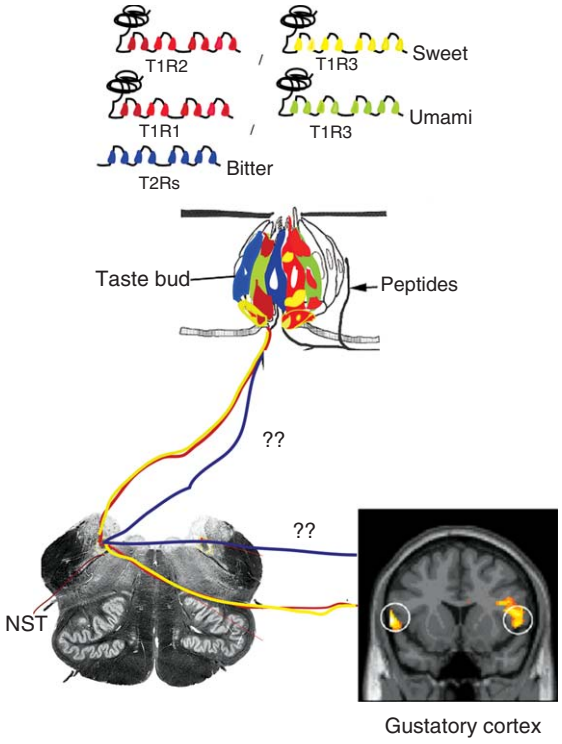

Figure 2 Diagram depicting families of taste receptors recognizing sweet, bitter, and umami substances. The heteromer T1R2/T1R3 responds to compounds that produce a sweet taste (e.g., sucrose, glucose), whereas T1R1/T1R3 responds to receptors that are activated by compounds that produce the umami taste (e.g., monosodium glutamate). The T2R family recognizes compounds that produce bitter taste sensations (e.g., caffeine, quinine). These signals seem to be transduced separately to the central nervous system through specialized gustatory neurons (labeled lines) that elicit different behaviors. Illustrated are these neurons' projections to the solitary nucleus and its tract (NST) and further projections to the primary gustatory cortex. This separation appears to persist at higher levels in the gustatory pathway although more definitive evidence is needed (question marks). The peptidergic general sensory perigemmal neurons are also shown. These neurons have receptors for molecules such as capsaicin (TRPV1) and provide information regarding the pungency of foods.

Salty (NaCl) taste uses the amiloride-sensitive sodium channel, ENaC (Lindemann, 2001). Other salts may permeate taste cells via a TRPV1 splice variant (Lyall *et al.*, 2004). Sour taste, which is represented by the hydronium concentration, uses a variety of pathways depending on whether it is a strong or weak acid (Lyall *et al.*, 2004; DeSimone *et al.*, 2001; Caicedo *et al.*, 2002). The other standard modalities are mediated by G-protein-coupled receptors. Sweet and umami detection are mediated by the T1R receptor family (see The Evolution of the Sweetness Receptor in Primates). Three genes, T1R1, T1R2, and T1R3, control their expression in taste cells. Evidence indicates that T1R receptors function as heterodimers, in that the T1R1/T1R3 combination in rodents is broadly tuned to amino acid detection (in humans it is narrowly tuned to glutamate) and T1R2/T1R3 to sweet detection (Li *et al.*, 2002). The T2R receptor family, comprising approximately 30 members, is known to be necessary and sufficient for the perception of bitter taste (Mueller *et al.*, 2005). T2Rs are of high behavioral relevance, since they mediate the detection and consequent rejection of potentially poisonous or toxic substances. Sequence polymorphisms between T2Rs of mice (Kim *et al.*, 2003) and nonhuman primates (Parry *et al.*, 2004) with respect to those expressed in humans were linked to different bitter sensitivities in these species. Species-specific sensitivities for sugars have also been shown (e.g., mice vs. human; Zhao *et al.*, 2003). This indicates ongoing evolutionary diversification of T1R and T2R receptors and a role in dietary adaptation and nutrient selection (Parry *et al.*, 2004).

3.25.3 The Mammal Central Taste System

3.25.3.1 Anatomy

Rodents and primates (including humans) constitute the most studied cases of central gustatory processing. The chemosensory information from CN VII primarily involves the sense of taste, whereas CNs IX and X convey chemosensory information that drives the swallowing and gaping reflexes (Markison *et al.*, 1996). Also, general sensory fibers from CNs V, IX, and X provide textural and thermal responses as well as information from irritating chemosensory stimuli. In all these species, CNs VII, IX, and X transmit electrical signals that convey the chemical properties and quantity of tastants to the rostral division of the nucleus of the solitary tract (NST) of the medulla, the principal visceral sensory nucleus of the brainstem. In the rat, second-order fibers (i.e., NST afferents) project ipsilaterally to the gustatory parabrachial nuclei (PBNs) in the pons, proceeding then to the parvicellular part of the ventroposterior medial nucleus of the thalamus (VPMpc). In primates, the NST projection fibers bypass the PBN only to join the central tegmental tract and synapse directly into the VPMpc (Pritchard *et al.*, 2000), whereas the PBN seems to be dedicated to convey general visceral information (e.g., from the vagus) to specialized thalamic nuclei including VPM (Pritchard *et al.*, 1989, 2000).

Thalamic afferents then project (reciprocally) to the gustatory cortex (Scott and Plata-Salaman, 1999). In the rat, it was found that parabrachial fibers reach some forebrain areas including the

lateral hypothalamus and the central nucleus of the amygdala, giving gustatory information direct access to motivational and reinforcement-related structures including the dopaminergic system (through direct projections from the central nucleus of the amygdala; Fudge and Haber, 2000).

The primary taste cortex in macaques can be defined in terms of VPMpc afferents (Scott and Plata-Salaman, 1999). Pritchard *et al*. (1986) have studied the efferent projections of the VPMpc of the monkey, *Macaca fascicularis*, with tritiated amino acid autoradiography. Two discrete cortical areas were characterized as a target of VPMpc projections. First, labeled cells were located in the ipsilateral insular–opercular cortex adjacent to the superior limiting sulcus and extending as rostrally as the caudolateral orbitofrontal cortex. Moreover, further projections were located within the primary somatosensory cortex (SI), in the precentral gyrus subjacent to the anterior subcentral nucleus (i.e., a precentral extension of SI). This area is anterior to the VPM projection sites representing somatosensory information and is adjacent to or overlaps with cortical somatotopic sites for the face and oral cavity (Jain *et al*., 2001). Thus, this area might be a target of VPM and VPMpc projection fibers and thus implement the convergence in the cortex of the somatosensory and gustatory aspects of stimuli delivered in the mouth (see below).

Scott and Plata-Salaman (1999) define the anterior limit of the primary taste cortex in the macaque as the junction of the orbitofrontal and opercular cortices, from which it extends 4.0 mm posteriorly. The mediolateral extension is defined ~16–19 mm lateral to the midline in an average adult macaque. The dorsal limit is defined as ~6 mm above the lateral fissure. The insular cortex, in the depth of the Sylvian fissure, has been divided into four rostrocaudal subdivisions (Cipolloni and Pandya, 1999): the most rostral portion has been designated the insular proisocortex; adjacent to it is the agranular subdivision of the insula, followed caudally by the dysgranular and the granular insular areas. In these terms, the VPMpc nucleus projects to the opercular and insular regions of the granular and dysgranular insula and extends to adjacent agranular portions of the insula.

One of the projections from this primary taste cortex is to the central nucleus of the amygdala where gustatory information reaches the basal forebrain, lateral hypothalamus (Scott and Plata-Salaman, 1999), and dopaminergic cells in the substantia nigra pars compacta and ventral tegmental area (Fudge and Haber, 2000). Fibers also project anterior to the dysgranular caudolateral orbitofrontal region (which is defined as a secondary taste cortical area by Baylis *et al*., 1995). This transition zone, including the more anterior parts of the primary taste cortex and the adjoining caudolateral orbitofrontal cortex, was also named area G by Carmichael and Price (1996). Taste neurons in the caudolateral orbitofrontal cortex form connections laterally with visual areas in the inferior temporal cortex and, importantly, converge with more medial cells receiving projections from primary olfactory cortex, which have implications for the perception of flavor. Taste-responsive cells in the caudal orbitofrontal cortex project to the caudate nucleus, where taste information is distributed throughout the striatum, and lateral hypothalamus (Öngur *et al*., 1998; Scott and Plata-Salaman 1999), which in turn communicates directly with the central nucleus of the amygdala. The central nucleus of the amygdala in turn projects back to the NST (Price and Amaral, 1981). The described circuit could then form a complex neural network integrating information about the identity of individual tastants with their hedonic and motivational properties.

3.25.3.2 Electrophysiology

In rodents and monkeys, taste cells have been sampled across the central gustatory pathway by electrophysiological techniques and this may reveal some species-specific features. For example, in rats, NST taste-responding cells seem to be modulated by physiological need and satiety signals (e.g., gastric distention; Glenn and Erickson, 1976). However, NST taste cells in primates are unaffected by satiety, as shown, for example, by reversing the incentive value of glucose in a sensory-specific satiety type of experiment (Yaxley *et al*., 1985). This apparent distinction between the rodent and primate cases might be partially accounted for by the fact that in primates NST projection fibers bypass the PBN, where visceral and physiological information could be preferentially processed.

Top-down regulation is an important feature of taste processing in that stimulation of the hypothalamus or the central nucleus of the amygdala can modulate responses to tastants in NST and parabrachial nuclei (Cho *et al*., 2002; Li *et al*., 2005). This is significant since both the amygdala and the hypothalamus receive projections from cortical taste areas and could thus work as an intermediate for cortical modulation of taste processing at the brainstem level. Notice that these top-down pathways also exist in primates (Price and Amaral, 1981).

In the primates, despite its name, only a small proportion of cells in the primary taste cortex do

actually respond exclusively and consistently to taste stimuli (Scott and Plata-Salaman, 1999; ~6.5%), whereas a higher proportion (~23%) responded during tongue or jaw movements, for example. This suggests that the primate primary taste cortex might be encoding simultaneously taste and oral somatosensory properties of (intraoral) stimuli. These recording studies then constitute an early indication that multisensory encoding might occur in the primary taste cortex.

In the primary gustatory cortex (in primates, including both frontal opercular and dysgranular insula), the responses of taste-related neurons are multisensory and are more broadly tuned than in NST and VPMpc (Sewards and Sewards, 2001). Interestingly, in the rodent case, Katz *et al.* (2001, 2002) have shown that when time is accounted for as a source of variability, the taste specificity of the responses increased from approximately 10%, when only the average activity is considered, to 41% of the recorded gustatory cells, suggesting that encoding of temporal information is a central feature of taste processing. In this regard, Katz *et al.* (2002) have also shown that neurons that exhibit synchronous activity may also contribute to the identification of tastants.

Single-cell recording studies of the secondary taste cortex (orbitofrontal cortex) were able to evidence more clearly the distributed and multimodal characteristics of taste processing in primates. The role of the primate orbitofrontal cortex in reward processing has been consistently established by a number of different lines of evidence. In nonhuman primates, there is strong evidence at the single-neuron level that the orbitofrontal cortex responds as a function of the reward value of taste (Rolls *et al.*, 1989), olfactory (Critchley and Rolls, 1996), and visual stimuli (Critchley and Rolls, 1996). This shows that vision, a sensory modality especially developed in primates, can also provide inputs for association with taste perceptual information (see Primate Brain Evolution in Phylogenetic Context).

In the specific case of neurons responding to the reward value of taste stimuli, neurons in the macaque monkey orbitofrontal cortex have been shown to respond in a sensory-specific satiety manner (Rolls *et al.*, 1989). In addition, reward-related learning and expectation appear to be represented at the single-neuron level in the primate orbitofrontal cortex (Schultz *et al.*, 2000), probably involving the midbrain dopaminergic system. Thus, the findings detailed above provide evidence that a part of the primate taste cortex could support the simultaneous encoding of several sensory features of taste stimuli, including stimulus identity, multisensory combinations (olfactory, somatosensory), and reward value (see The Loss of Olfactory Receptor Genes in Human Evolution, Evolution of the Somatosensory System – Clues from Specialized Species).

3.25.4 Functional Neuroimaging

Single-cell recording studies are limited to a relatively small number of samples from a single cortical area. To understand the dynamics between multiple brain areas representing taste–reward pathways, one may use bundles of electrodes implanted in each area (Nicolelis *et al.*, 2003). This field is in its infancy with respect to gustatory processing. However, advances in human functional neuroimaging techniques, such as functional magnetic resonance imaging (fMRI; for a description of its physiological basis, see Logothetis *et al.*, 2001), allowed for more general descriptions of taste processing in humans.

Human studies indeed confirmed that gustatory areas homologous to those of primates (as defined by anatomical studies) are responsive to unimodal taste stimuli in humans, including the anterior insula/frontal operculum, the orbitofrontal cortex, and the amygdala (Small *et al.*, 1999; O'Doherty *et al.*, 2001; de Araujo *et al.*, 2003a). This includes responses to glucose, NaCl (O'Doherty *et al.*, 2001), umami (de Araujo *et al.*, 2003a), caffeine, and citric acid (Schoenfeld *et al.*, 2004). In particular, the de Araujo *et al.* (2003c) study revealed activations for taste (sucrose) in all homologous areas in the ascending central taste pathway receiving first- or second-order projections from the VPMpc: the frontal operculum/insula complex, the orbitofrontal cortex, the amygdala, and the ventral forebrain, which most likely included anterior parts of the hypothalamus (see Figure 3).

Studies with human subjects provide evidence that gustatory cortices not only respond to the major perceptual categories of taste, but also support the encoding of the multisensory aspects of taste stimuli. In a study using taste and retronasal olfactory stimuli (and their combinations), de Araujo *et al.* (2003c) have shown that taste and olfactory inputs in the human brain converge in particular in the far anterior (putatively agranular) insular cortex. This region of the far anterior (agranular) insula is close to the part of the insular cortex where it adjoins the caudal orbitofrontal cortex.

A homology between the rodent and primate cases with respect to the central anatomy of taste and olfactory integration has been previously suggested and, thus, it is being proposed here that this homology would extend to humans to encompass at least three mammal species. In fact, Shi and Cassell (1998) reported that in rats both the granular and

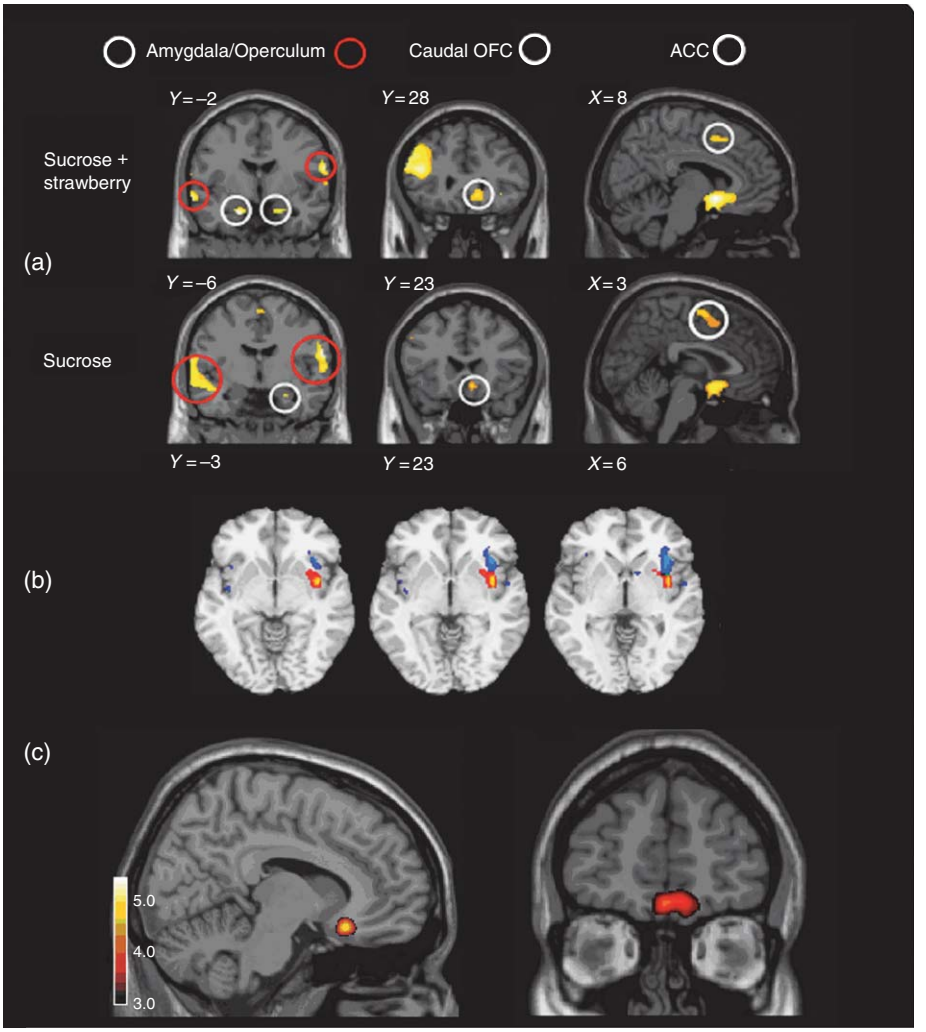

Figure 3 Increases in activity level in human gustatory brain areas as detected by functional magnetic resonance imaging. a, Activations produced by 0.5 M sucrose (bottom row) and sucrose combined with strawberry odor (top row) were observed in most of the central gustatory areas: insular/operculum, medial (rostral and caudal) orbitofrontal cortex, amygdala, and forebrain (which might include the hypothalamus and some parts of the thalamus). This shows that cortical gustatory areas support multisensory maps involving taste representations. b, Insular areas of the human brain responding to both pure tastants (sucrose) and water in the mouth (blue) and correlating with hydration states (red). c, Left, medial orbitofrontal cortex region, where it borders the subgenual cingulate cortex, that responds to water in the mouth only when thirst is present, thus indicating representations in the taste cortex of the internal state of the organism. Right, an anterior medial orbitofrontal cortex area in which activity correlates with the subjective pleasantness of a taste/olfactory mixture. Adapted from de Araujo, I. E., Kringelbach, M. L., Rolls, E. T., and McGlone, F. 2003b. Human cortical responses to water in the mouth, and the effects of thirst. *J. Neurophysiol.* 90, 1865–1876 and de Araujo, I. E., Rolls, E. T., Kringelbach, M. L., McGlone, F., and Phillips, N. 2003c. Taste–olfactory convergence, and the representation of the pleasantness of flavor, in the human brain. *Eur. J. Neurosci.* 18, 2059–2068.

the dysgranular zones in the posterior insula are part of the gustatory cortex (see also Cechetto and Saper, 1987). Moreover, based on the projection patterns among the granular, dysgranular, and agranular parts of the rat insula (as well as on the projection patterns from the VPMpc; Cechetto and Saper, 1987), Shi and Cassell (1998) claimed that the dysgranular insular cortex constitutes a secondary taste association cortex (in contrast to the lower-order granular zone). They further hypothesized that the agranular part of the insular cortex is a tertiary taste association cortex supporting flavor perception (given its afferent projections from the olfactory bulb and piriform/endopiriform cortices). Sewards and Sewards (2001) proposed thus that this homology between the rodent and primate cases holds also for the secondary (dysgranular) and tertiary (agranular) taste association area. In particular, the agranular insula and the adjoining caudal part of the orbitofrontal cortex would support flavor perception given the convergence of olfactory and taste inputs in these areas. Further regions of

taste/retronasal odor convergence found in this neuroimaging study (de Araujo *et al.*, 2003c) include the amygdala, the ventral forebrain, and the anterior cingulate cortex, which is targeted by regions of the anterior insula, possibly including taste cortex (Vogt and Pandya, 1987). The findings above thus indicate that several parts of the central taste system allow for combinations between taste and odor to form flavor percepts; moreover, its anatomical bases seem comparable across distinct mammal species.

As mentioned, it has been found in monkeys that a representative number of neurons in the primary taste cortex respond to oral somatosensory/motor stimulation (Scott *et al.*, 1986; Ogawa, 1994). In fact, it has been shown in humans (de Araujo and Rolls, 2004) that activation of the anterior insular (putative primary) taste cortex by oral viscosity stimuli occurs in such a way that brain activation in this region was proportional to the log of the viscosity of the oral tasteless stimuli (carboxymethyl cellulose), providing evidence of somatosensory/gustatory integration in the primary taste cortex. It is known that in more posterior regions of the insular cortex in owl monkeys (Jain *et al.*, 2001), the caudal part of the face representation in area 3b extends anterior beneath the central sulcus and above the upper bank of the lateral sulcus. The representation of the oral cavity is located rostral to this region extending to the orbitofrontal cortex (Manger *et al.*, 1996; Jain *et al.*, 2001). The de Araujo and Rolls (2004) study used quantitative variation of texture features of intraoral stimuli by manipulating viscosity and found activation of the midinsular and anterior insular cortices.

Another example of activations in the human primary taste cortex that are independent of the major perceptual categories of taste is activations to water in the mouth, when subtracted from activations produced by artificial saliva at the same viscosity (de Araujo *et al.*, 2003b). This corroborates previous electrophysiological studies in macaques showing that water in the mouth activates neurons in the primary taste cortex in the anterior insula and adjoining frontal operculum (Scott *et al.*, 1986; Yaxley *et al.*, 1990). Thus, not only the stimulation of taste receptors by prototypical tastants, but also substances generally relevant to behavior and survival, seem to elicit responses in the mammal gustatory cortices.

In fact, to guide feeding behavior and maintain energy homeostasis, mammal brains must not only represent the sensorial aspects of an intraoral stimulus, but also combine these with the internal state of the organism, in that they must ascribe the stimulus motivational value. Human studies also provide evidence that the reward value of taste is represented in the gustatory cortices, in particular in the orbitofrontal, insular, and anterior cingulate cortices. Small *et al.* (2001) found that the caudomedial part of the orbitofrontal cortex and a region of the midinsula represent the changing reward value of a food eaten to satiety. Interestingly, the same pattern of responses was found in responses to water in the mouth at different levels of hydration: activity in the mediocaudal orbitofrontal cortex and midinsula is modulated by the physiological state (thirst) of the body (de Araujo *et al.*, 2003b).

The finding that the midinsula and adjoining posterior insular areas respond to water in the mouth in a (thirst) state-dependent way is in agreement with the viscerotopic map of the rat insular cortex as proposed by Cechetto and Saper (1987). Their results suggest an anterior–posterior distribution of visceral representations in the rat insula, with special visceral (taste) projections situated preferentially in more anterior areas, whereas general visceral (including gastric mechanoreceptor-responsive and cardiopulmonary units) were distributed more posteriorly and dorsally. The human insula might thus reproduce such topography by combining special visceral (taste) and general visceral inputs in insular regions.

In addition to the current motivational value of a taste stimulus, the secondary taste cortex, the orbitofrontal cortex, and the adjacent anterior cingulate cortex area also represent the degree to which subjects ascribe reinforcing properties to gustatory-related stimuli. For example, correlations with consonance and pleasantness ratings for the smell and taste combinations were found in a medial anterior part of the orbitofrontal cortex (de Araujo *et al.*, 2003c), the pleasantness ratings for a food eaten to satiety were correlated with activity in the medial orbitofrontal cortex (Small *et al.*, 2001), and the (subjective) rewarding properties of water in the mouth under different hydration states were correlated with activity in the medial orbitofrontal cortex and in the far anterior cingulate cortex (de Araujo *et al.*, 2003b). Moreover, the orbitofrontal cortex is also involved in encoding the reward value of visual signals predicting taste stimulus receipt. In a classical conditioning paradigm, where a previously neutral cue was associated with receipt of glucose, expectation of the pleasant taste produced activation in particular in the amygdala and orbitofrontal cortex (equivalent results were found for a cue predicting receipt of an unpleasant taste, saline; O'Doherty *et al.*, 2002), also evidencing the ability of the human cortex to associate visual and taste representations.

The evidence described above indicates that the mammal taste cortex can serve a more general

purpose other than simply representing the end line for ascending taste information. It seems rather that this is a byproduct of a more general function, namely, to encode information about stimuli relevant for survival, be they taste stimuli (sugars), nutrient-rich stimuli with particular textures (fat), or clean water. Thus, it should be involved in generating behavior through back-projections to the noncortical regions of the taste system, such as the hypothalamus and the brainstem.

3.25.5 The Mammal Taste System in the Context of Vertebrate Evolution

Mammals first appeared approximately 210 Mya during the first interval of the Mesozoic era, approximately at the same time as crocodiles and dinosaurs (e.g., Rougier and Novacek, 1998). A characteristic feature of all living mammals is a 1–3 mm thick, multilayered sheet of neural tissue situated between more lateral olfactory areas and medial hippocampal areas, the isocortex (Northcutt and Kaas, 1995). Although there are different views on how the mammalian isocortex might have evolved from their nonmammalian ancestors (e.g., outgroup vs. recapitulation hypotheses; Northcutt and Kaas, 1995), it seems clear that it resulted in more complex cortical processing and much higher associative power. Aboitiz *et al.* (2003), for example, argued that the mammalian isocortex appeared by means of a dorsalizing effect during the early development of the pallium of the first mammals. This would have resulted in the formation of a hippocampal–dorsal cortex circuit supporting complex olfactory-based representations of space. The ability to form such complex representations and to use them to guide behavior would be then a hallmark of mammalian evolution.

In fact, when compared to other tetrapods, the multilayered cortex seems to account for most of the specificity in mammalian sensory processing. Comparative data on gustatory processing are very scarce. It nevertheless seems clear that in nonmammalian tetrapods, CN fibers provide taste-related information to the ascending gustatory pathway arising in the nucleus of the solitary tract that then projects to the parabrachial region, which in its turn projects extensively to the forebrain, as in the case of the lizard *Varanus exanthematicus* (Ten Donkelaar and De Boer-Van Huizen, 1981). The forebrains of reptiles and mammals are similar in that the dorsal surface of their cerebral hemisphere is formed by a pallium with three major segments: an olfactory (laterally situated) cortex, a limbic cortex (dorsomedial), and an intermediate cortical tissue that in the mammal case corresponds to the isocortex, but in reptiles and birds consists of part of the dorsal cortex and the dorsal ventricular ridge (Ten Donkelaar, 1999) (see Evolution of Vertebrate Olfactory Subsystems). In any case, in all tetrapods, gustatory information (as well as other modalities) reaches the telencephalon, and the intermediate pallial segment receives sensory projections from the thalamus and contains modality-specific sensory (presumably including gustatory) areas in reptiles, birds, and mammals (Ten Donkelaar, 1999).

If the ascending gustatory pathway is homologous from the CN fibers up to thalamic (forebrain) level in several classes of vertebrates (tetrapods), the possibility remains that mammal-specific gustatory cortices could be heterogeneously structured across different mammal species. There is, nonetheless, evidence to the contrary. That is, all mammals seem to have a primary somatosensory area and homologous adjoining fields (Kaas, 1980). In addition, homologous limbic, orbital, and lateral gustatory fields can also be found in different mammal species (Northcutt and Kaas, 1995; Preuss, 1995), unlike, for example, some prefrontal regions specific to primates such as the dorsolateral prefrontal cortex (Preuss, 1995). In particular, when comparing the connection patterns of the rat insular cortex with those on the insular cortex of cats and monkeys, Guldin and Markowitsch (1983) suggested that on the basis of thalamocortical connections, the insular cortex is a heterogeneous structure with homologous subdivisions in each of these species, including a separate gustatory (somatosensory) insular region. Likewise, basal mammals seem to possess structures supporting higher-order taste-related cortical areas homologous to higher mammals, such as the orbital fields of the hedgehog tenrec (*Echinops telfairi*; Radtke-Schuller and Künzle, 2000).

The strongest indication that the mammal central gustatory system is conserved across different species comes from molecular genetic studies performed on mice by Charles Zuker, Nicholas Ryba, and colleagues (Mueller *et al.*, 2005; Zhang *et al.*, 2003; Zhao *et al.*, 2003). As mentioned, mice and humans show different sensitivities for some sweet stimuli, such as aspartame, which cannot be recognized by mice. In this regard, mice engineered to express the human T2R homologous gene in place of the mice T2R gene develop a preference for aspartame, recognized as a sweet compound (Zhang *et al.*, 2003). This seems to indicate that when different mammal species are provided with receptors for the same class of ligands, then behavior (e.g., avoidance/approach) is controlled through an innate, homologous dedicated (Sugita and Shiba, 2005) neural circuitry.

In summary, based on the available neurophysiological data from mammals and especially humans, it seems that gustatory processing has also benefited from the mammal-specific development of cortical layers supporting higher-order, cross-modality associations. This would allow tastants and other biologically relevant intraoral stimuli to be represented in multisensory maps, whose processing is distributed across different regions of the cortex. Information about the physical properties of a given compound will be combined with information about the internal physiological state of the organism. Information about the physiological state of the organism is carried by visceral inputs to the taste cortices (as in the case of conditioned taste aversion; Garcia *et al*., 1955) and by indexes on the animal's current fluid and energy status (as provided by specific hypothalamic regions responsive to changes in levels of hormones – leptin, insulin, angiotensin; e.g., Niswender *et al*., 2004). These cortical sensory–visceral maps will then generate, through back-projections to the brainstem mediated by hypothalamic and amygdalar areas, a large repertoire of complex behaviors regulating food intake and body weight that is unique to mammals.

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3.26 Vestibular System

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Glossary

1° Primary afferent (vestibular nerve).

2° Second-order vestibular neuron (vestibular nucleus).

AC Anterior semicircular canal.

AIN Abducens internuclear neuron.

ampulla A bulb-like hollow structure housing a sensory organ.

analogous Similar in function, but without phyletic continuity (e.g., human hands and the tongue of a chameleon used for prey catching: they both do the same thing at one time, but their origins are completely different).

ATD Ascending tract of Deiters.

C_2 Second cervical vertebra.

C_7 Seventh cervical vertebra.

cilia Sensory processes of hair cells.

common crus Common leg (Latin), a portion of the semicircular canal system shared by two canals.

cupula Receptor system of the labyrinth in the semicircular canal ampullae detecting angular accelerations (rotations).

ectoderm One of the three germ layers formed during the gastrula stage in embryogenesis and giving rise to, among other organs, the nervous system. The other two germ layers are the mesoderm and the entoderm.

FP Frankfurt plane.

hair cell Sensory cells of the inner ear and some other sense organs whose name derived from their mechanically sensitive cilia, the so-called kinocilia and the stereocilium. During a stimulus, e.g., mechanical or auditory, these cilia undergo a bending and via a specific ion conductance perform a mechanoelectrical transduction.

halteres Fast-moving (rotating) clublike righting organs of flies, working much like gyroscopes.

HC Horizontal semicircular canal.

hemilabyrinthectomy Global extirpation of the labyrinth of one side, resulting in distinctive lesion symptoms.

homologous Inherited from a common ancestor (with phyletic continuity), but not necessarily similar in function (e.g., the limbs of horses and sea lions, used for walking in one case, and swimming in the other case; another example are the wings of birds and bats which

	are both homologous and analogous, since they were both derived from forelimbs and are used for flying).
Hor	Plane of horizontal semicircular canals.
HorC	Horizontal canal.
III	Oculomotor nucleus.
IN	Internuclear neuron.
invertebrates	Animals without a back bone (spinal column), e.g., insects, worms, spiders, crabs.
IO	Inferior oblique.
IR	Inferior rectus.
IV	Trochlear nucleus.
kinematics	Muscle actions upon a movable body part, e.g., a limb, an eye.
lateral line	A sensory system present in many aquatic vertebrates of mechanoreceptive (water current) or electroreceptive nature (electric fields).
LR	Lateral rectus.
MN	Motoneuron.
MR	Medial rectus.
neural crest	Inductive tissue to form sensory and neuronal elements appearing between the neural tube and the surface ectoderm.
neuromast cell	Hair cell of the lateral line system.
optokinetic reflex	Eye (or head) movements elicited by large moving visual scenes, e.g., when observing the passing landscape in a moving train.
otic placode	Thickening of the ectoderm and precursor of the otocyst.
otoconia	Ear stones consisting of calcium carbonate crystals embedded in the otolith membrane.
otocyst	Invagination of the otic placode forming a cyst at first that later subdivides and gives rise to the complex adult three-dimensional structure of the labyrinth.
otolith	Receptor system of the labyrinth, so-called graviceptor, detecting linear accelerations (translations).
Otx	Member of a gene family (orthodenticle).
PC	Posterior semicircular canal.
rhombomere	Elements of segmentation of the rhombencephalon formed during embryology and thought of as an expression of developmental organization.
semicircular canal	Tubelike structure of the labyrinth filled with endolymph to detect angular accelerations.
SO	Superior oblique.
SR	Superior rectus.
statocyst	The balance organ of an invertebrate.
VAPC	Vertical anterior parasagittal canal.
vertebrates	Animals with a spinal column (back bone), i.e., fish, amphibians, reptiles, birds, mammals.
vestibulo-ocular reflex	Eye movements elicited by stimulation of the labyrinth.
VI	Abducens nucleus.
VPPC	Vertical posterior parasagittal canal.
VTC	Vertical transverse canal.

3.26.1 Introduction

The sense of balance, the vestibular system, is our unknown sense. We recognize its existence only under pathological conditions, such as seasickness, dizziness, vertigo, etc. Among the classical five senses, i.e., vision, taste, smell, touch, and hearing, our sense of balance is not mentioned. Quite often, the sense of balance is just considered as an appendix of the auditory sense due to the anatomical unity of cochlea and vestibular apparatus, the so-called inner ear (Figures 1a and 1b). The inner ear is really a fabulous example of the engineering capabilities of nature and evolution, as it is one of the most complex anatomical structures in the vertebrate history: in humans, we find two hypersensitive hyperprecise sensory organs housed within the space equivalent to that of an M&M ball – the auditory sense and the sense of balance. Moreover, under normal life conditions, we are not even aware of the latter's existence. The sense of balance could thus be considered our sixth sense, and its functions are manifold. At least four different and vital functions should be mentioned:

1. postural control and postural stabilization;
2. reflex movements;
3. perception of self-movement; and
4. autonomous control.

3.26.2 Spatial Coordinates

A large portion of our daily activity requires moving between different positions. To that end, we use different means of locomotion or transport, e.g., individual locomotion (walking, running, swimming), or with the aid of mechanical devices (bicycle, car, train, escalator, etc). During all these dynamic events, we have a sensation of self-motion, which we will largely attribute to visual inputs. The

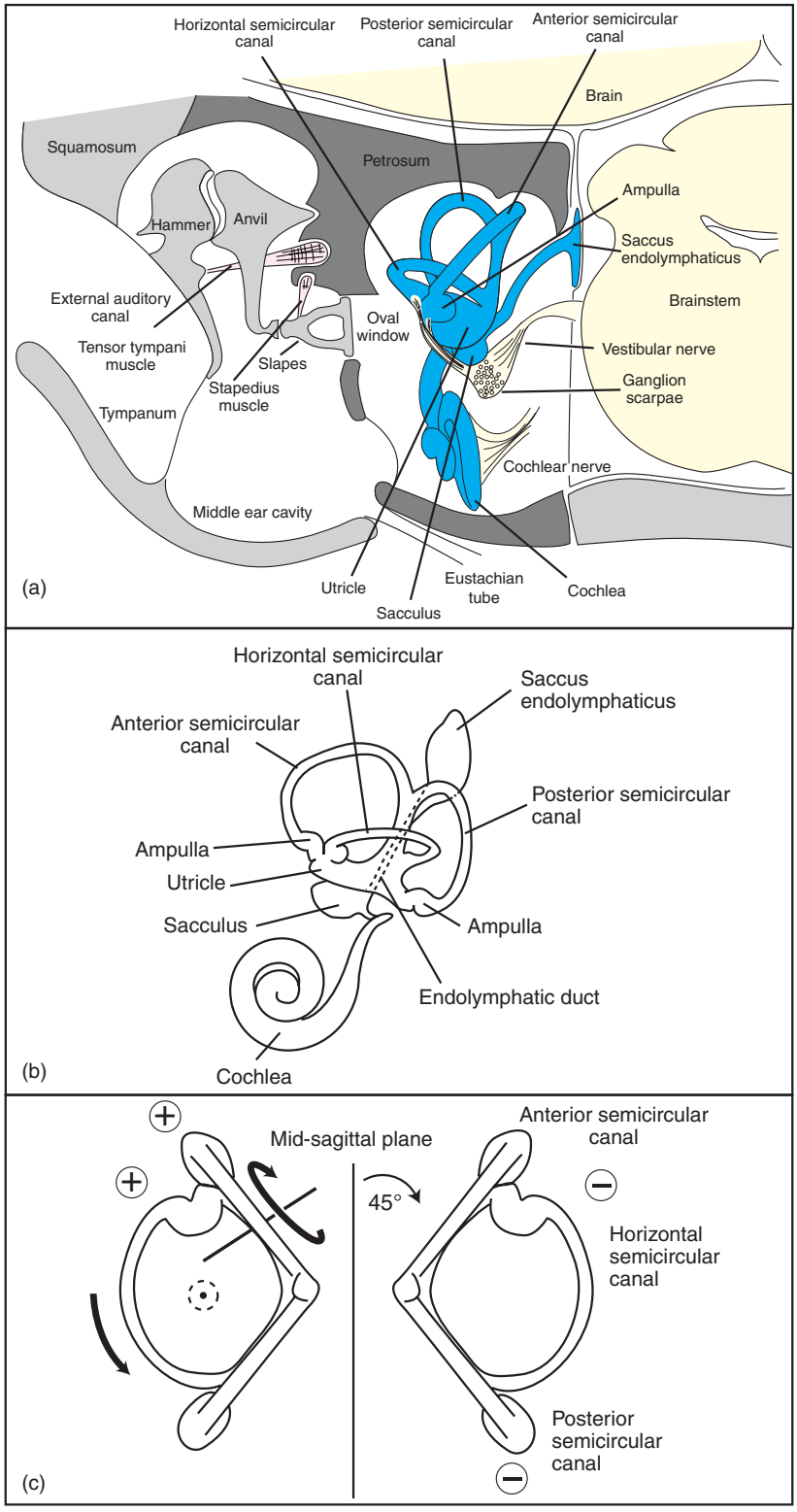

Figure 1 Anatomy of the organ of the sense of balance. a, Position of the labyrinth in the cranium. Cross section of the right Os petrosum with a frontal view of the right labyrinth and its topographical neighborhood. b, Lateral view of the left human labyrinth showing the portions of the sense of balance (semicircular canals and otoliths) and of the sense of hearing (cochlea). c, Spatial orientation of an idealized semicircular canal system (top view). Anterior and posterior canals are oriented vertically, horizontal canals are oriented horizontally. The vertical canals are oriented 45° off the midsagittal axis (diagonal orientation). Note bilateral symmetry, mutual orthogonality between canals, and the push–pull operational mode illustrated for the right posterior and the left anterior canals, and the right and left horizontal canals. When one canal becomes excited (+), its coplanar counterpart becomes inhibited (–). Canal on-directions are indicated by the directions of the arrows about the canal rotation axes. The combined excitatory and inhibitory responses of all canals during head movements produces a meaningful activity pattern in the afferent nerves and recipient brain nuclei to represent a movement vector in physical space (Werner, 1960).

role of the sense of balance in this function is often not realized, although fast reflex movements, such as certain eye movements or postural control adjustments are mediated by this sensory system. Triggering a fast eye movement via the sense of balance (the vestibulo-ocular reflex, VOR) requires only 16 ms, while eliciting the corresponding reflex via the visual system (optokinetic reflex) takes 80–150 ms.

Humans live in a three-dimensional environment; however, they rarely use the third dimension for every day transport in comparison to many bird and fish species, or even nonhuman primates. Nevertheless, our sense of balance uses a sensory organ to detect self-movements in three-dimensional space. Two fundamentally different movement categories have to be distinguished: rotations and translations. Each one of these has three degrees of freedom. Classically, these movements are described in a Cartesian coordinate system anchored to the head, which includes one vertical axis (along the gravity vector), and two earth horizontal axes, one naso-occipital (sagittal) axis, and one interaural (transverse) axis. All three axes intersect at one point in the middle of the head. It has to be mentioned, however, that the Cartesian coordinate system, as its name implies, is man-made (i.e., by the French philosopher and natural scientist René Descartes, 1596–1650) and bears no significance for the way biological systems developed movement detection systems during the course of evolution.

3.26.3 Receptors of Movement Input: The Labyrinth

3.26.3.1 Anatomy

The inner ear is a bilateral organ. It is located inside the petrosal part of the temporal bone of the cranium (Figure 1a). The balance organ is part of the inner ear and consists of the semicircular canals and the otoliths (Figure 1b). At first sight, the twisted and three-dimensional structure of the inner ear looks quite complicated and has earned the balance organ the name 'labyrinth'.

Semicircular canals and otoliths are sense organs, which detect accelerations. The semicircular canals detect angular accelerations (rotations), the otoliths linear accelerations. An example for a ubiquitous and permanent linear acceleration is earth gravity (gravity vector). Under normal living conditions, we rarely spend a thought about gravity, but when gravity becomes absent, the effects can be dramatic, as during space flight under microgravity conditions with resulting space motion sickness.

3.26.3.1.1 The semicircular canals The operational mode of the semicircular canals is independent of gravity. The canals are filled with a fluid, the so-called endolymph, which, during a given head movement, causes a so-called endolymph current, which displaces receptor cells inside a specialized area of the canal lumen, the so-called ampulla (Wilson and Melville Jones, 1979; Graf, 2003). An important characteristic of the macroscopic anatomy of the semicircular canals is their three-dimensional orientation. The ensemble of the six canals, three on each side, forms a physical coordinate system to detect angular accelerations in three-dimensional space. The semicircular canal system on each side of the head consists of a horizontal (lateral) canal, and two vertical canals (one anterior and one posterior canal) (Figure 1c). The horizontal canal is lightly tipped upward (about 30° in humans) at normal head resting posture (Figure 2a; see also Figure 2b). The vertical canals are oriented about 45° off the midsagittal plane of the head (Figure 1c).

The orientation of the semicircular canals in the head follows three interdependent functional principles:

1. Bilateral symmetry: both labyrinths are mirror symmetric.
2. Reciprocal operational mode: during head rotations receptors in a given canal will be excited, while the receptors in the contralateral coplanar canal will be inhibited; the so-called push-pull system.
3. Mutual orthogonality of canals: the functional planes of the canals enclose angles of 90°, or close to that value (Figure 1c).

The semicircular canal system thus constitutes an intrinsic sensory reference frame system, which provides a blueprint for the spatial coordination for a number of reflex functions and sensory interactions (Cohen *et al.*, 1965; Schaefer *et al.*, 1975; Simpson and Graf, 1981, 1985; Simpson *et al.*, 1981; Graf, 1988; Graf *et al.*, 1988; Leonard *et al.*, 1988).

3.26.3.1.2 The otoliths By contrast to the semicircular canals, the otoliths are receptors, which depend on the presence of gravity (graviceptors). They detect linear accelerations and do not function in microgravity. Most vertebrates, including humans, posses two otoliths on each side: the horizontal utriculus and the vertical sacculus. At normal resting posture of the head, the utriculus seems to be oriented earth horizontally (Figure 2b). The receptor cells of the otoliths are embedded in the so-called otolith membrane, which contains the

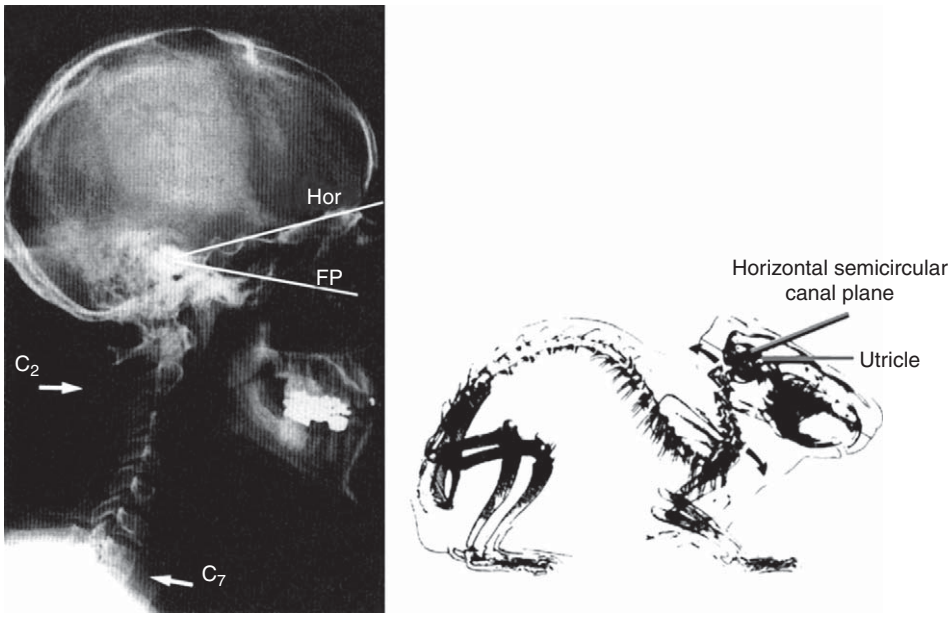

Figure 2 Lateral views of head and labyrinth orientation during normal head posture at rest in a biped and a quadruped. a, Radiograph of a human skull. C_2, C_7, second and seventh cervical vertebras; FP, Frankfurt plane; Hor, plane of horizontal semicircular canals. b, Artist's rendering of a lateral radiograph of an awake and unrestrained guinea pig. Note vertical orientation of the cervical vertebral column. Arrows indicate the direction of possible movement, i.e., at the upper cervical columns, only extension movements are possible, since the head is held in the extreme flexed position at the atlanto-occipital articulation, and at the cervicothoracic junction only flexion movements are possible, since here the vertebras are held at extreme extension. In both cases, the cervical vertebral columns are held vertically with the horizontal canals kept tipped upward by approximately 20°–30°. At this position, the utricles would be positioned about earth horizontally.

otoconia. During a displacement of the head from the normal upright position, the otoconia will slide across the otolith membrane and produce a shear force upon the receptor cells.

3.26.3.2 Evolutionary History of the Labyrinth

The phylogenetic origins of the vertebrate labyrinth are not known. The only living protochordate, *Amphioxus*, possesses sense organs, namely, a median eye, and bilateral balance organs (Lacalli, 2001; Lacalli *et al.*, 1994, 1999; Figure 3), but no functional–physiological data are available. Furthermore, only fragmentary fossil records exist that testify to the beginning of vertebrate life in Cambrian times, more than 500 Mya. However, there now seem to be indications from molecular biology data for a common ancestor regarding mechanoreceptor cell evolution between *Drosophila* and vertebrates, i.e., hair cells (Fritzsch *et al.*, 2000; see also Figure 6). Although for a long time, the balance organ had been thought to have evolved from the lateral line system, recent evidence based on multiple out-group comparison suggests that the inner ear of vertebrates evolved as a statolithic system before the lateral line system and before semicircular canals appeared.

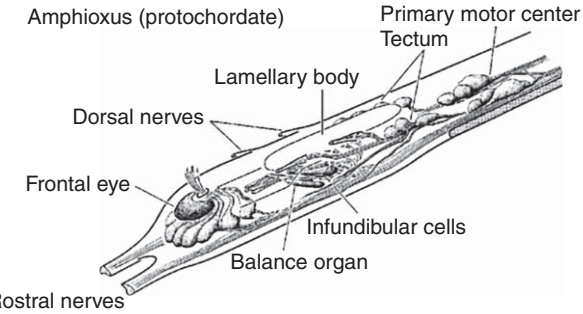

Figure 3 Amphioxus cerebral vesicle. Principal landmarks in the larval cerebral vesicle of amphioxus, showing the anterior pigment cup with the median eye, the ciliary bulb cells of the putative bilateral balance organ, and the lamellar body, which is assumed to be a pineal homologue (Th. Lacalli).

The fossil record becomes more complete only during the middle of the Paleozoic era, the Devonian period (350–400 Mya). The first record that demonstrates the existence of semicircular canals comes from jawless vertebrates, agnathan species of the Devonian and Silurian times, the ostracoderms. They possessed vertical, but not horizontal canals (Figure 4). Their vertical canals were oriented in the head as described before (Stensiö, 1927; Figure 1c). The ostracoderm labyrinth was

similar to the semicircular canal system of lampreys, the extant forms of their once-abundant ancestors. The Devonian period also marks the advent of jawed vertebrates (ganthostomes) and bony and cartilaginous fishes (osteichthyes and chondrichthyes, respectively; Figure 5). We know nothing about the labyrinth structure of the immediate ancestors of these newly appeared animals, but their modern successors display a new acquisition: horizontal semicircular canals. Thus, the vertebrate labyrinth now spans all three dimensions of physical space. The circumstances that led to the development of a horizontal semicircular canal system are unknown, but its presence most certainly introduced distinct advantages for the detection of three-dimensional space in comparison to the four canal system in the Agnatha. The acquisition of horizontal semicircular canals coincides with the expression of the vertebrate-specific gene *Otx1* (Fritzsch and Beisel, 2001, 2003; Figure 6). Knock-out mutants that do not express *Otx1* do not develop horizontal semicircular canals (Fekete, 1999). One could speculate that the appearance of horizontal semicircular canals, allowing an optimal solution, i.e., best and most economical (Gould, 1977), high signal-to-noise-ratio (Robinson, 1982; 1985; Graf, 1988) for movement detection in three-dimensional space constituted one prerequisite for the success of vertebrates later on in phylogeny. At any rate, it certainly provided one further advantage.

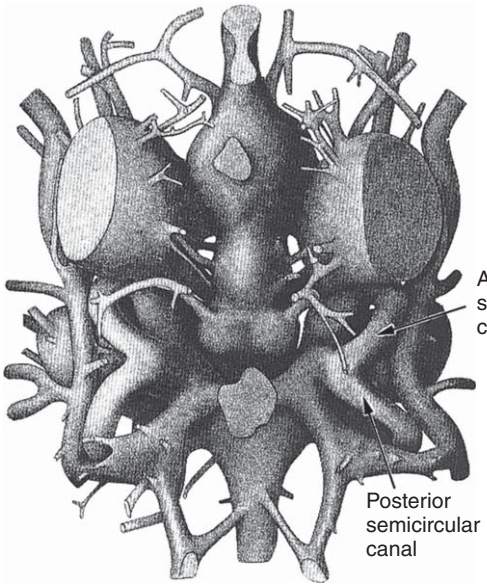

Figure 4 Photograph of a wax model of the cranial cavity of the fossil ostracoderm, *Kiaeraspis auchenaspidoides*, including labyrinths with anterior and posterior semicircular canals (Stensiö, 1927). Note diagonal orientation of the canals similar to the situation in extant vertebrates. Horizontal canals have not yet appeared.

Interestingly enough, there are two main lines of labyrinthine development in the surviving radiations, namely what we will refer to as the bony

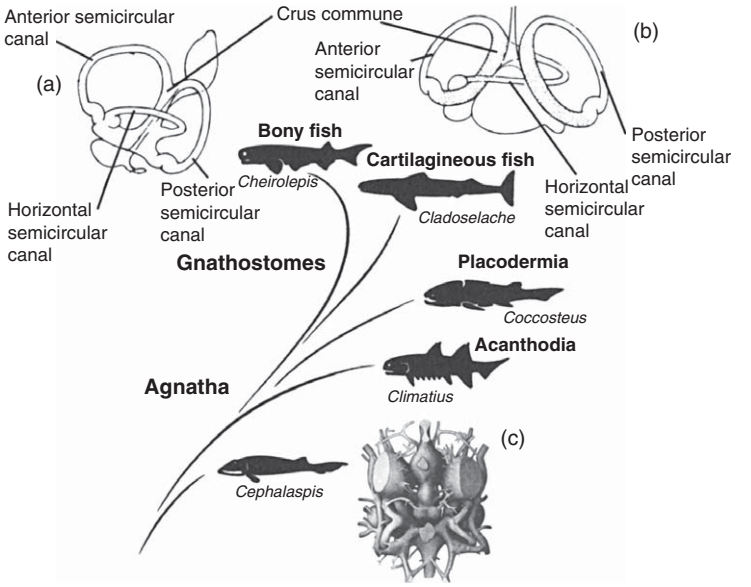

Figure 5 Phylogenetic relationship of early agnathans and gnathostomes (bony fishes, cartilaginous fishes, placoderms, and acanthodians) (Colbert, 1980), including prototypical vertebrate labyrinth characteristics. a, Human labyrinth (Werner, 1960). b, Shark labyrinth, *Chlamydoselachus* (Werner, 1930). c, Ostracoderm labyrinth without horizontal canals (Stensiö, 1927). Horizontal semicircular canals appear in bony fishes and cartilaginous fishes. In bony fishes through humans, the anterior and the posterior canal form a common crus. In cartilaginous fishes, there is no common crus between the anterior and the posterior canal. All labyrinths display a similar (diagonal) orientation of the vertical semicircular canals in the head.

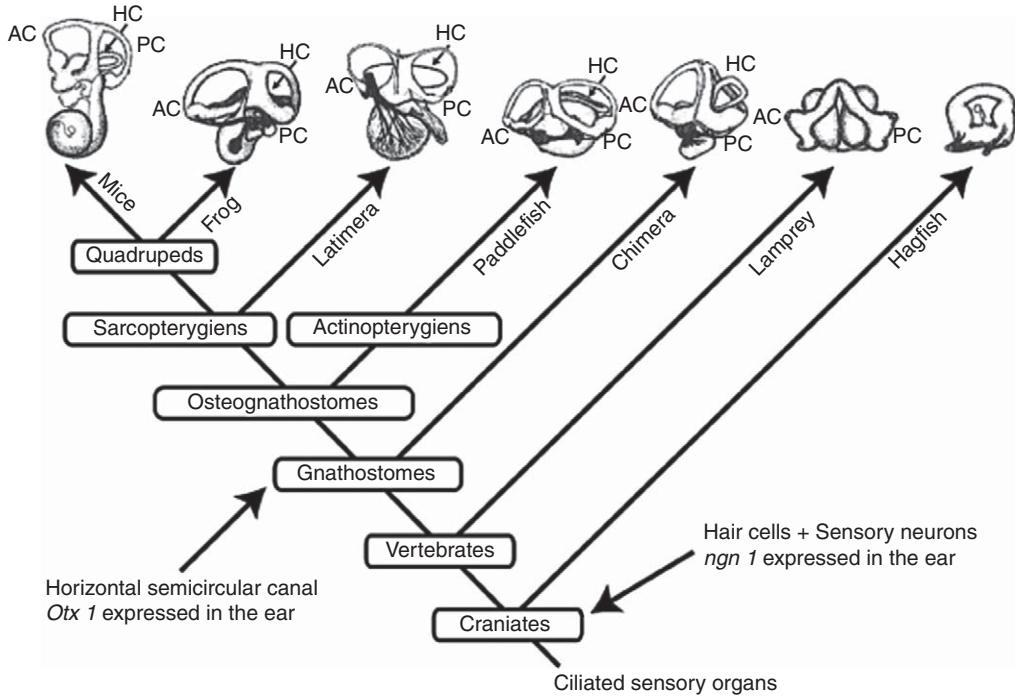

Figure 6 Morphogenic evolution of the vertebrate ear. Ciliated mechanosensory cells, so-called hair cells are now thought to be at the phylogenetic origin of the vertebrate inner ear. For the development of primary neurons, *ngn1* is necessary. One of the major morphogenetic events in vertebrate ear evolution was the appearance of horizontal semicircular canals in all gnathostomes. The development of horizontal semicircular canals coincides with the expression of the *Otx1* gene (Fritzsch and Beisel, 2001, 2003). Reproduced from Fritzsch, B. and Beisel, K. W. 2001. Evolution and development of the vertebrate ear. *Brain Res. Bull.* 55, 711–721, with permission from B. Fritzsch.

fish/tetrapod line and the cartilaginous fish line (Figure 5) (we are using the term 'bony fish/tetrapod' in the following to delineate vertebrate species between bony fish and mammals, quadrupedal and bipedal, i.e., including amphibians, reptiles, and birds). Unfortunately, no fossil record testifies to the labyrinth structures of earlier radiations that became extinct (e.g., acanthodians, placoderms).

In viewing a typical vertebrate labyrinth of the bony fish/tetrapod line, in this case a human labyrinth (Figure 5a), we observe that it consists of three canals, one anterior, one posterior, and one horizontal canal. Typically for this type of labyrinth, the anterior and the posterior canal form a so-called common crus; that is, they share a segment of their circular structure (Werner, 1960; Lewis *et al.* 1985). The typical cartilaginous fish labyrinth, in this case from a shark (Figure 5b) also possesses anterior, posterior, and horizontal canals that display the same orientation in the head as the bony fish/tetrapod labyrinth type. However, there is no common crus between the anterior and the posterior canals. The posterior canal is separate and has a communication with the sacculus, whereas a common crus-like structure is formed between the horizontal and the anterior canals (Daniel, 1928; Werner, 1930; Baird, 1974). This particular difference in labyrinth structure between bony fishes and cartilaginous fishes leads to the intriguing question of whether the phylogenesis of horizontal canals was monophyletic or polyphyletic in vertebrates.

3.26.3.3 Comparative Anatomy

A great variety of movement and position detectors, so-called statocysts, are found in invertebrates (Bullock and Horridge, 1965; Markl, 1974), and we will introduce only the most pertinent examples here (see Aggression in Invertebrates: The Emergence and Nature of Agonistic Behavioral Patterns, Evolution of Visceral Control in Invertebrates).

The statocysts of the fast-moving squid and cuttlefish (Figure 7a1) include grooves, which, similar to the vertebrate semicircular canals of vertebrates, direct endolymph flow toward a sensory crista with a cupula (Stephens and Young, 1978). These invertebrate canals, so-called tonoids, are oriented in space in a roughly orthogonal three-dimensional planar arrangement (Budelmann, 1977; Stephens and Young, 1978; Figure 7a2). Four canals on each side can be distinguished, which are oriented approximately in the main planes of the body, in contrast to the vertebrate

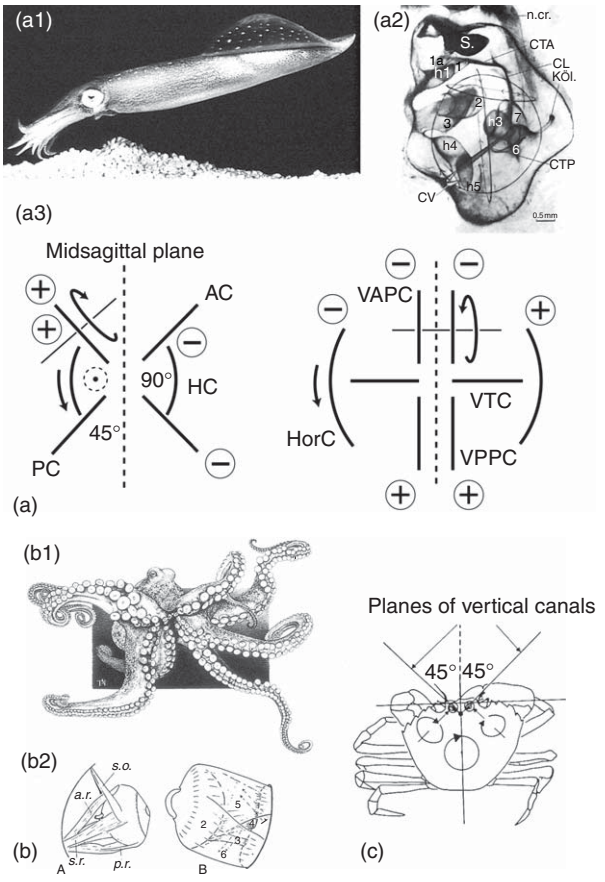

Figure 7 Convergent evolution of movement detection systems. a, Squid (a1), photo of a squid. The animal propulses itself rapidly backward by ejection of a jet of water. (a2), Retouched photograph of a squid statocyst indicating the orientations of the toroid planes (Stephens and Young, 1978). (a3), Comparison of the diagonal vertebrate semicircular canal system, and the principal axes system of squids. The squid system also fulfills the three criteria of orthogonality, bilateral symmetry, and push–pull operational mode. However, instead of six canals, it has eight toroid structures, and the on-directions of the sensory receptors are just about opposite that of vertebrates. Nevertheless, the squid movement detection system functions according to the same operational principles as the vertebrate semicircular canal system. b, Octopus (b1), drawing of an octopus. Despite its seemingly amorphous body structure, the octopus possesses a well-defined three-dimensional movement detection system similar to vertebrates. b2, Comparison of the extraocular muscles in a shark, left, and in the octopus, right. Note similar diagonal spatial arrangement in the two animals (Packard, 1972). c, Crab, spatial arrangement of the semicircular canal system in crabs. Although there are only four canals in crabs, the vertical canals are oriented just like the anterior canals of vertebrates, as are the horizontal canals (Fraser, 1981).

arrangement (Budelmann, 1977; Simpson and Graf, 1985; Figure 7a3). Sensory receptors detect movements in the transverse plane of the body, with excitation occurring during ipsilateral upward roll movements, in the longitudinal plane of the body, with receptors detecting pitch-up and pitch-down movements, and in the horizontal plane with receptors being excited during contraversive rotation (Figure 7a3, right). Some receptors also detect linear accelerations (Stephens and Young, 1978). The squid semicircular canal system can thus monitor three-dimensional angular accelerations just like the idealized vertebrate semicircular canal system with bilateral symmetry, orthogonality, and push–pull operational mode. Orthogonality of the semicircular canals would provide an optimal signal-to-noise ratio (Robinson, 1982, 1985), but in order to achieve paired orthogonality, the vertical semicircular canals need not necessarily be arranged in the familiar diagonal fashion. The one (and only) alternative arrangement is pairs in coronal, parasagittal, and horizontal planes as shown in Figure 7a3 (right). The squid/cuttlefish semicircular canal system could thus be termed a principal axes system, in contrast to the diagonal vertebrate arrangement.

In the octopus (Figure 7b1), the sensory receptor organ on each side consists of nine subsections, and is divided into three main planes, which are approximately orthogonal to each other (Young, 1960). The arrangement of the subsections suggests an angular acceleration detection system similar to that of vertebrates (Young, 1960; Budelmann, 1977). Interestingly, the extraocular muscle arrangement of the octopus resembles closely that of lateral-eyed vertebrates (Packard, 1972; Figure 7b2).

The third type of semicircular canal system introduced here in invertebrates of interest is found in crabs (Figure 7c), which possess one horizontal and one vertical toroid structure on each side. Depending on the species, these toroids can either be open or can form a closed canal system (Sanderman and Okajima, 1972; Sanderman, 1983; Fraser, 1981). In freely moving crabs, the horizontal canals are held earth horizontally, and since the horizontal and the vertical canals are close to orthogonal, the vertical canals are nearly vertical. Each vertical canal lies at an angle of 45° to the midsagittal plane in a configuration comparable to that of the anterior semicircular canals in vertebrates. Although there is only one vertical canal on each side, each one responds preferentially to movements about orthogonal axes and thus the canals of crabs are collectively capable of accurately transducing three-dimensional angular accelerations (Fraser, 1981).

Comparison of vertebrate and invertebrate solutions about how to build movement-detection systems shows a remarkable uniformity to an idealized three-dimensional geometry of optimal decomposition of all given rotation vectors. The semicircular canal systems of vertebrates and invertebrates are thus prime examples for convergent evolution.

3.26.3.4 Ontogeny and Phylogeny of the Labyrinth

The labyrinth develops from an enlargement of the ectoderm, the otic placode, which invaginates to form the so-called otocyst (for details see Rinkwitz *et al.*, 2001; Romand and Varela-Nieto, 2003). A number of genes and induction molecules play a role for the complicated morphogenesis of the labyrinth. There are genes that are necessary for the differentiation of various organ and system developments and some that are labyrinth-specific. Many genes work in parallel or are redundant. Gene duplication, or multiplication of genes during the progress of evolution has to be taken into consideration as well (Fritzsch *et al.*, 2000). The differentiation of the main structures of the labyrinth is guided by independent genes, which will be introduced in the following.

Although many vertebrate genes are homologous with *Drosophila* genes, the vertebrate labyrinth is a development of chordates and without precedent in other animal groups. Flies do not possess balance organs *per se*, but rely on relative movement of body parts (halteres) to orient in gravity. Homologies with other animal groups seem to be restricted to the development of receptor cells, which transform mechanical stimuli into electrical impulses (mechano electrical transduction). The receptors of the labyrinth are important examples for the general question of the origin of mechanoelectrical transduction at the level of receptor cells. For many years, evolutionary biologists believed that the labyrinth was derived from the neuromast cells of the lateral line organ of aquatic vertebrates. Meanwhile, however, functional interrelations between the pressure receptors of the nematode *C. elegans* and the sensory bristle receptors and proprioceptors of the fruit fly *Drosophila*, on one hand, and vertebrate hair cells, on the other hand, have been described (Fritzsch *et al.*, 2000; Fritzsch and Piatigorsky, 2005). The description of a mechanoelectrical transduction channel in *Drosophila* and *C. elegans* points to an early development of a mechanoelectrical receptor in evolution. The original receptors might have consisted of a cilia-like structure, including support cells. Thus, receptor cells seem to have been an important evolutionary component for the development of the sense of balance of vertebrates, but it was not the structure *per se* that led to the macroscopic expression of analogous sense organs. Interestingly, inner ear hair cells develop without involvement and influence of the neural crest, which normally guides the development of most of the sensory neurons of the peripheral nervous system of chordates.

The actual morphogenesis of the ear is governed by numerous genes, which also play a role in the development of lungs, kidneys, and extremities. Embryogenesis and morphogenesis occur during particular periods in ontogenesis, when certain genes are switched on or off, and when certain organs and characteristics are being developed. The development of sense organs is embedded into the general process of structurization and position specification. In this process, proneural genes will be activated, which are determining the precursors of the elements of sensory organs, such as support cells, glia, and portions of the actual sensory cells. Two mechanosensory basic helix-loop-helix (*bHLH*) genes are expressed in the ear, *Neurogenin 1* (*ngn1*) and *mammalian atonal homologue 1* (*Math1*). In insects, *atonal* (*ato*) is important, whose vertebrate homologue *Math1* is indispensable for the development of hair cells. Knock-out mutants without *Math1* develop support cells and primary neurons, but no hair cells. For the development of primary neurons, another *bHLH* homologue is required, i.e., *ngn1*, one of three so-called neurogenin genes.

For further labyrinth development, the so-called *FGF* and *FGFR* genes play an indispensable role (FGF, fibroblast growth factor; FGFR, tyrosine kinase receptor family). In particular, *FGF19* seems to be a fundamental element for the induction of ear development in chickens, which is activated together with *Wnt8c*. The interplay between FGFs and FGF receptors in vertebrates seems to induce the budding out of the growth zones of lungs, extremities, and the ear placode. With regard to inner ear development, the receptor FGFR-2(IIIb) is essential for the development of the semicircular canals, the endolymphatic duct, and the cochlea. Besides the *FGF* genes, *BMP*, *Pax*, *POU*, and zinc-finger genes were shown to be present in the ear. *POU4f3* knock-out mutants form a labyrinth with hair cells that are later lost. Missing the *Pax2* gene results in an ear without cochlea; however, with semicircular canals and otoliths (Fekete, 1999; Fekete and Wu, 2002).

While all genes described above have been shown to exist in insects, some vertebrate-specific genes are noteworthy, such as the above-mentioned *Otx1*, which regulates the development of the horizontal semicircular canals (Figure 6). Vertebrates without horizontal canals do not express this gene in the ear. Finally, the gene *mindbomb* should be mentioned, which plays a role during the development of the inner ear and the central nervous system. This gene is important in the context of the regeneration of inner ear hair cells, which has been demonstrated in

fishes and birds. The *mindbomb* gene seems to induce the transformation of the precursors of hair cells into support cells. Knock-out mutants produce an abundance of hair cells, but no support cells (Fekete, 1999).

Some homeotic genes also play a role for inner ear differentiation. Inactivation of *Hoxa1* produces various malformations of the inner ear and results in only the development of an epithelial cyst (Fekete, 1999).

3.26.4 Effectors of Sensory Input: Extraocular Muscles

3.26.4.1 The Extraocular Muscle Apparatus

The extraocular muscle apparatus can be considered as a prime example for the efficiency of biological systems. The spatial orientation of the extraocular muscles, in particular, illustrates in an almost ideal fashion how evolution solved a complicated problem of sensorimotor transformation.

The six extraocular muscles move the eye in a reference frame that corresponds to the spatial geometry of the vestibular semicircular canals, i.e., the typical diagonal, 45° off the midsagittal plane orientation of vertical canals, is reflected in the pulling direction of the vertical eye muscles (Helmholtz, 1910; Alpern, 1962; Figure 8). The vertical eye muscles are superior rectus (SR), inferior rectus (IR), superior oblique (SO), and inferior oblique (IO); the horizontal eye muscles are lateral rectus (LR) and medial rectus (MR). These anatomical designations give the impression of a distinct separation between straight and oblique eye muscles. In the true sense of the word, only LR and MR are straight eye muscles, whereas all vertical eye muscles, including SR and IR, are in reality oblique muscles. The illustrated example of Helmholtz's drawing demonstrates this fact very clearly (Figure 8). Unfortunately, an idealized, but erroneous figure by Bell (1823) has dominated the literature and clouded the understanding of spatial coordination of eye movements.

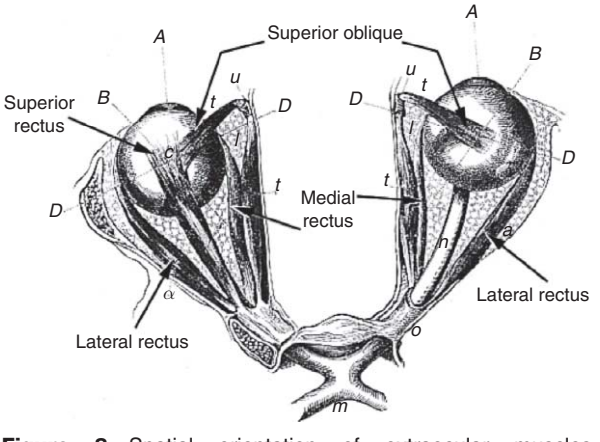

Figure 8 Spatial orientation of extraocular muscles (Helmholtz, 1910). Note diagonal orientation of vertical eye muscles, e.g., SR and SO.

3.26.4.2 Innervation of Extraocular Muscles

At the peripheral level of the six extraocular muscles, there are no gross differences between any of the vertebrate species, except that hagfishes do not possess eye muscles. However, at the central organization, distinct differences become evident. Across species, three patterns of eye muscle innervations can be distinguished:

1. The lamprey pattern where two eye muscles are innervated by ipsilateral (IO, IR), and one by contralateral (SR) oculomotor neurons, one by trochlear motoneurons (contralateral, SO), and one by the abducens nucleus (ipsilateral, LR).
2. The elasmobranch pattern with two ipsilaterally (IR, IO) and two contralaterally projecting (SR, MR) oculomotor motoneuron populations, one trochlear motoneuron population (contralateral, SO), and one abducens motoneuron population (ipsilateral, LR).
3. The bony fish/tetrapod pattern with three ipsilaterally (IR, IO, MR) and one contralaterally projecting (SR) oculomotor nucleus neuron population, one trochlear motoneuron population (contralateral, SO), and one abducens motoneuron population (ipsilateral, LR) (Figure 9; see also Fritzsch, 1998).

The lamprey pattern thus has only five extraocular eye movers, lacking the equivalent of a MR muscle (Fritzsch *et al.*, 1990). The main difference between the bony fish/tetrapod and the elasmobranch pattern is the positioning of the MR motoneurons, with MR motoneurons addressing a contralateral eye muscle in elasmobranchs, just as SR and SO motoneurons do (Figure 9b), and an ipsilateral placement with respect to the muscles it innervates in animals of the bony fish/tetrapod line (Figure 9a). This difference in motoneuron placement needs to be further investigated and reflected upon regarding vestibulo-oculomotor reflex connections (see below).

In evolutionary history, the MR muscle in elasmobranchs is thought to have evolved from a split of the dorsal rectus (SO) of ancestral agnathans, whereas it was derived from a split of the rostral rectus (IR) in the ancestors of the

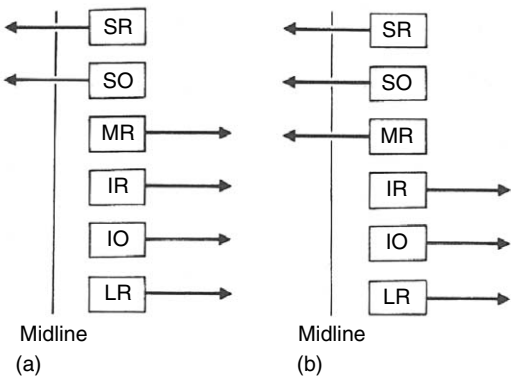

Figure 9 Oculomotor neuron projections in vertebrates of the bony fish/tetrapod line (a) and in elasmobranches (b). Note difference in MR motoneuron placement projecting ipsilaterally in bony fish/tetrapods and contralaterally in elasmobranchs. IO, inferior oblique; IR, inferior rectus; LR, lateral rectus; MR, medial rectus; SO, superior oblique; SR, superior rectus.

osteognathostomes (Nishi, 1938). Such a scenario would also explain the different motoneuron placements in the two vertebrate radiations.

There was also an idea that lungfishes actually possessed an elasmobranch innervation pattern, which would bring them taxonomically close to elasmobranchs (von Bartheld, 1992). This has now been shown not to be the case (Puzdrowski and Morshedi, 2003; Graf, unpublished observation). Clearly, lungfishes, at least the examined species, the African lungfish, *Protopterus dolloi*, shows a clear bony fish/tetrapod innervation pattern, with an ipsilaterally projecting MR subpopulation.

3.26.4.3 Ontogeny and Phylogeny of the Extraocular Muscles and Their Innervation

Although the geometric arrangement of the extraocular muscles is basically identical in all vertebrates that possess eyes, the horizontal eye muscles seem to have followed slightly different evolutionary paths in elasmobranchs and the bony fish/tetrapod line. Embryonically, the MR muscle seems to arise from the dorsal part of the premandibular head cavity in elasmobranchs; in other vertebrates it comes from its ventral part (see Graf *et al.*, 2002). This difference in embryonic origin may be a concomitant explanation for the contralateral versus ipsilateral placement of MR motoneurons in elasmobranchs when compared to bony fish/tetrapods. Other differences exist regarding abducens motoneurons. Abducens motoneurons originate in embryonic rhombomeres 5 and 6 in most vertebrates (lamprey, teleosts, birds, reptiles) (Gilland and Baker, 1993), exclusively from rhombomere 5 in frogs (Straka *et al.*, 1998) and mammals (Gilland and Baker, 1993), but only from rhombomere 6 in elasmobranchs (Gilland and Baker, 1992).

Abducens motoneurons are surmised to be somatic, originally being part of a series of homologous spinal-like nerves. Thus, the abducens nerve would have simply invaded a position once foreign to it (for a review of the pertinent literature, see Baker, 1992). According to this argument, the abducens would therefore not belong to the branchiomotor category. The special role of abducens motoneurons (and abducens internuclear neurons) is also underlined by the inhibitory transmitter employed by afferent vestibular and reticular neurons, i.e., glycin. In oculomotor and trochlear motoneurons, the inhibitory transmitter is GABA. Oculomotor myoblasts are thought to be derived from the premandibular region, trochlear myoblasts from the mandibular region.

The abducens nucleus is the only extraocular motor nucleus inside the *Hox* gene-expressing region, its expression being under the control of *Hoxb3*. The other extraocular motor nuclei are found around the brainstem–midbrain isthmus, with the trochlear nucleus originating in rhombomere 1. Development of the trochlear and the oculomotor nuclei seems to be primarily governed by two molecules, i.e., wingless (*wnt*) and engrailed (*en*).

3.26.4.4 Vestibulo-Ocular Connectivity

We described the three-dimensional geometry of the sensory periphery, the semicircular canals, and its related motor effectors earlier in this article. Clearly, there is a similarity between these geometries. The pulling directions of the horizontal eye muscles correspond with the orientation of the horizontal semicircular canals, that of the vertical recti with the orientation of the ipsilateral anterior semicircular canal, and the pulling directions of the oblique eye muscles are in line with the orientation of the ipsilateral posterior canal (Figures 10 and 12). We find this orientation principle from fish to humans.

The conservation of the coincidence of the spatial geometries of semicircular canals and eye muscles during vertebrate evolution is also accompanied by a conservation of the principal neuronal connections for the production of compensatory eye movements (VOR) from fish to humans. Within this framework, excitatory connections are formed between the anterior canal and the ipsilateral SR and the contralateral IO muscles, between the posterior canal and the ipsilateral SR and the contralateral IR muscles, and between the

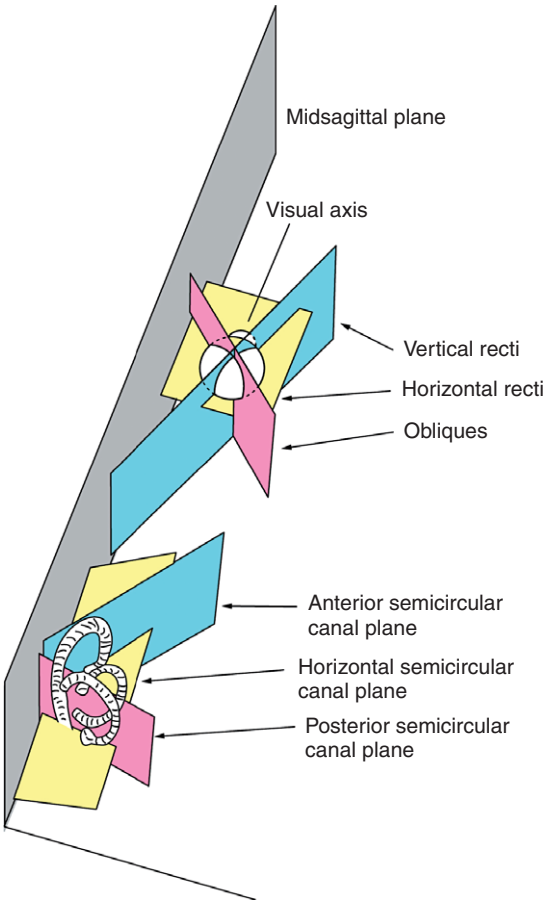

Figure 10 Three-dimensional orientation of semicircular canal planes and extraocular muscle pulling directions in humans. Note alignment of certain eye muscle pulling directions with particular canal planes, forming an intrinsic reference frame system.

horizontal canal and the ipsilateral MR and the contralateral LR muscles. Since the antagonists to these muscles will have to relax at the same time, we observe the existence of inhibitory connections to these antagonists arriving from the same semicircular canals (Figure 11). This innervation scheme has been termed the elementary VOR arc (Lorente de Nó, 1933) or the three-neuron arc (Szentágothai 1943, 1950) by the pioneers working in this field of research. The three neurons involved in this reflex arc are the primary vestibular neurons, the second-order vestibular neurons, and the respective extraocular motoneurons (Figure 11).

The development of the brainstem vestibular nuclei is under the control of a number of *Hox* genes, whose interactions are not yet completely understood (for details see Baker, 1998).

Compensatory eye movements following labyrinth stimulation in lampreys can be induced in any direction, although these animals do not possess horizontal semicircular canals or an equivalent of a MR muscle (Rovainen, 1976). The details of the neuronal connectivities underlying this behavior still need to be worked out.

While vestibulo-oculomotor connectivities have been elaborated in detail in the bony fish/tetrapod line (Figure 11), we are still lacking a definite answer as to the exact nature of the horizontal canal connections in elasmobranchs. Of particular interest are the special horizontal eye movement pathways in light of the contralaterally placed MR motoneurons in these animals.

Horizontal conjugate eye movements are produced by the simultaneous contraction of the LR muscle in one eye and the MR muscle in the other eye. In animals of the bony fish/tetrapod line, the decussating internuclear pathway from the abducens nucleus to the MR subdivision provides the necessary neuronal link between the two motoneuron populations (Figures 11c and 12a; Highstein and Baker, 1978; Carpenter and Baton, 1980; Highstein *et al.*, 1982). Since MR motoneurons in elasmobranchs are located contralateral to their respective muscles, they are found on the same side as the co-activated LR motoneurons. Therefore, we hypothesized that in these animals the organization of the horizontal VOR circuitry may be similar to that of the vertical systems, where one second-order vestibular neuron class links either the anterior or the posterior canal to two co-activated extraocular motoneuron populations (so-called yoke muscles) (Uchino *et al.*, 1980, 1982; Graf *et al.*, 1983; Graf and Ezure, 1986). In such a scenario, one horizontal second-order neuron would contact both LR and MR motoneurons to mediate conjugate eye movements in the horizontal plane (Graf and Brunken, 1984; Figure 12b). However, recent evidence suggests the existence of a contralaterally projecting internuclear pathway, besides other connectivities (Graf *et al.*, 2002; Figure 12b).

3.26.4.5 Lateral- and Frontal-Eyed Animals

Head movements in animals with different interocular angles, e.g., the extreme examples of rabbits and humans, seemingly require different compensatory eye movements. For instance, a head movement about the naso-occipital axis in a rabbit results in vertical eye movements, in a human, in torsional eye movements. In fact, if the reference frame is tied to the optic axis, such a difference is observed. However, if the reference frame is linked to the head, no difference occurs.

We had in fact elaborated the requirements necessary for compensatory eye movements in lateral, and frontal-eyed animals (Simpson and Graf,

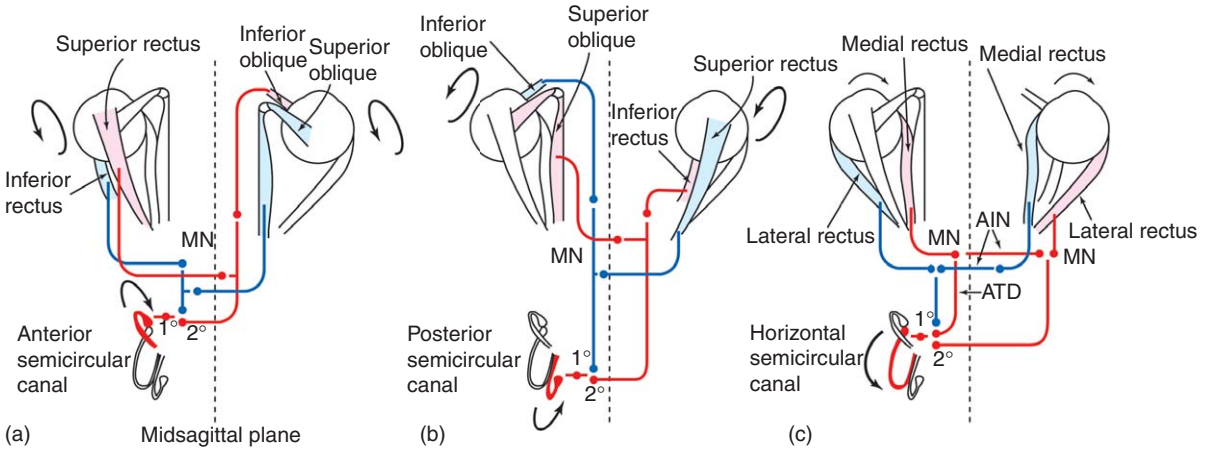

Figure 11 Spatial coordination of compensatory eye movements. Corresponding elements are illustrated in the same colors (red and blue). Semicircular canals and extraocular muscles form a three-dimensional intrinsic reference frame system for the production of VORs. The reflex arc consists of three neurons, the primary neuron (1°, vestibular nerve), the second-order vestibular neuron (2°, vestibular nucleus neurons), and the oculomotor neuron (MN, in oculomotor, trochlear, and abducens nuclei). Excitatory connections are shown in red, inhibitory connections are shown in blue. Contralaterally projecting vestibular neurons are in general excitatory, ipsilaterally projecting ones inhibitory. The respective semicircular canals (a, anterior canal; b, posterior canal; c, horizontal canal) and their efferent nerve pathways are marked in red. The on-directions of the semicircular canals are illustrated by thick black arrows. The connectivity of the horizontal system has a few peculiarities, such as an ipsilaterally projecting excitatory connection, the ascending tract of Deiters (ATD), and the abducens internuclear neuron pathway (AIN).

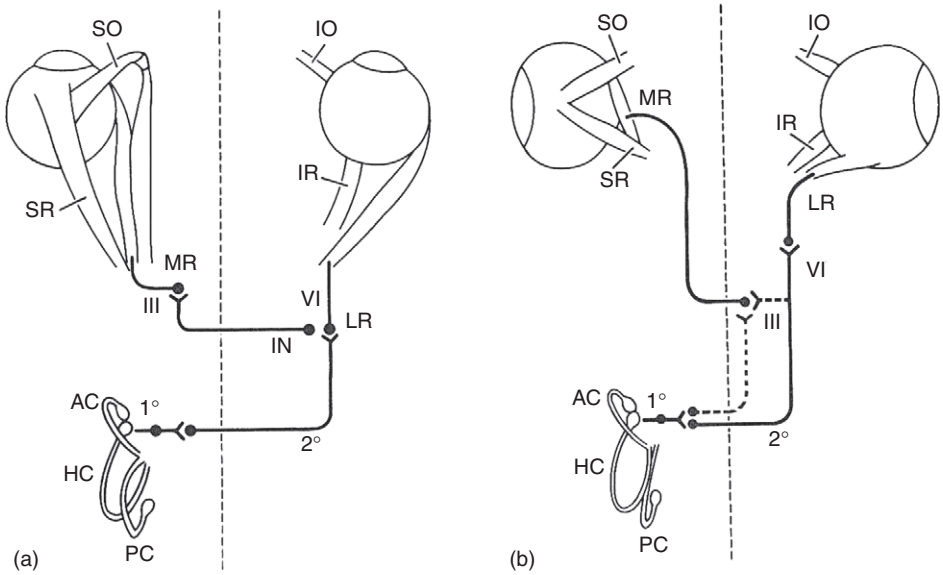

Figure 12 Schematic representation of vestibulo-ocular organization in vertebrates of the bony fish/tetrapod line (a) and elasmobranchs (b), including horizontal canal pathways. Note alignment of canals and related yoke muscles (left anterior canal, AC, with left SR, and right IO; left posterior canal, PC, with left SO, and right IR; left horizontal canal (HC) with left MR and right LR. The difference between the two prototypical vertebrate systems occurs in the horizontal reflex pathways. In vertebrates of the bony fish/tetrapod line (a), the connectivity to the lateral rectus muscle is of a three-neuron arc nature (vestibular afferent, 1°, second-order vestibular neuron, 2°, LR motoneurons in the abducens nucleus, VI), while an additional neuron, the abducens internuclear neuron (AIN) is inserted into the link to the co-activated MR muscle (MR motoneurons in the oculomotor nucleus, III). The three-neuron arc nature of the horizontal canal pathway in elasmobranchs (b), in particular the second-order vestibular neuron connectivity to LR motoneurons and MR motoneurons (2°) in the oculomotor nucleus, is hypothetical (Graf and Brunken, 1984). This fact is symbolized by the indication of the pathway in broken lines. Similarly, the nature of the contralaterally projecting internuclear pathway is not yet clear (Graf *et al.*, 2002).

1981, 1985; see also Ohm, 1919). There is no difference in the principal central nervous reflex connectivity, but subtle changes in eye muscle kinematics resulting from small changes in the insertion of vertical eye muscles during the course of evolution and the process of frontalization of the eyes.

3.26.5 Effectors of Sensory Input: Head–Neck Muscles

3.26.5.1 The Head–Neck Movement Reference Frame

Naturally, the system of the head–neck muscles used to perform head movements is more complex than that of the eye muscles, not only because of the far greater number of muscles involved (approximately 20 muscle pairs), but also because of the additional postural control functions these muscles have to fulfill. By contrast to the extraocular muscles, which do not have any postural function, one major task of head–neck muscles is to assure an upright head posture. Without the support function of the head–neck muscles, the head could not be balanced at labile equilibrium on top of the cervical vertebral column. Although the cervical vertebral column *per se* is relatively rigid, it has to be held upright, nevertheless, together with the head.

We were able to demonstrate the existence of a vestibular-based reference frame system also within the head–neck muscle system (Schaefer and Meyer, 1992; Graf *et al.*, 1997). However, the kinematic characteristics of the head–neck muscles are complex, and several muscle groups may cooperate and co-contract to perform a particular movement. Thus, the intrinsic geometry of the head–neck reference frame may not have become immediately obvious, although the very first systematic experiments by Flourens in the first half of the nineteenth century (Flourens, 1825, 1828) already pointed out its existence. These experiments involved selective transsections of semicircular canals in pigeons who subsequently performed movements in the plane of the lesioned canal. These could be eye–head, or even whole body movements (see also Suzuki and Cohen, 1964).

3.26.5.2 Vestibular Output and Postural Control

Some of the earliest motor control systems of vertebrates are the tectospinal and the vestibulospinal pathways. Tectospinal connections underlie visually based orienting and control mechanisms. Vestibulospinal pathways essentially provide tonic postural and balance control. This function can be impressively demonstrated following ablation of one entire labyrinth (hemilabyrinthectomy; see Schaefer and Meyer, 1974) or components thereof (de Waele *et al.*, 1989; Graf *et al.*, 1992). In essence, the horizontal semicircular canals provide the straight-ahead direction of the head, whereas the utricles assure the upright posture of the entire head–neck ensemble in the midsagittal plane, at least in birds and mammals. The sacculi seem to play a similar role regarding lateral tilt displacements of the head (Graf *et al.*, 1992). In this context, we have to mention that mammals in general possess a vertical cervical vertebral column, regardless of bipedal or quadrupedal locomotion (Vidal *et al.*, 1986; Graf *et al.*, 1995; Figure 2). The transition to bipedalism from quadrupedalism in mammals thus requires bringing the thoracic vertebral column into an upright position and modifications at the cervicothoracic junction and the lumbar level, but not within the cervical vertebral column or the atlanto-occipital articulation.

When considering the intrinsic geometry of the head–neck apparatus in the midsagittal plane, we observe that at resting position, mammals keep the articulations of the head–neck ensemble at the atlanto-occipital articulation and the upper cervical vertebral column in extreme flexion, and at the lower cervical and the upper thoracic vertebral column at extreme extension (Graf *et al.*, 1995; Figure 2b). At these endpoints, the head–neck ensemble takes on an intrinsic geometry that only has to be oriented into the correct direction by the vestibular system (Vidal *et al.*, 1993).

Since the cervical column is quite rigid, it also cannot be bent easily laterally. Lateral tilt of the entire head–neck ensemble in quadrupeds happens via rotation of vertebras at the cervicothoracic junction. Again, vestibular input provides the correct upright orientation (Graf *et al.*, 1992, 1995).

The intrinsic and semi-self-supporting architecture of the cervical column is a conserved feature in evolution, as some pertinent dinosaur findings have shown (dal Sasso and Signore, 1998). With regard to a general organization of postural mechanisms, vestibular circuits and their intrinsic three-dimensional coordinates are thought to provide a blueprint for a number of sensory and motor systems, and sensorimotor transformations (Cohen *et al.*, 1965; Schaefer *et al.*, 1975; Simpson *et al.*, 1981; Simpson and Graf, 1981, 1985; Graf, 1988; Graf *et al.*, 1988; Leonard *et al.*, 1988).

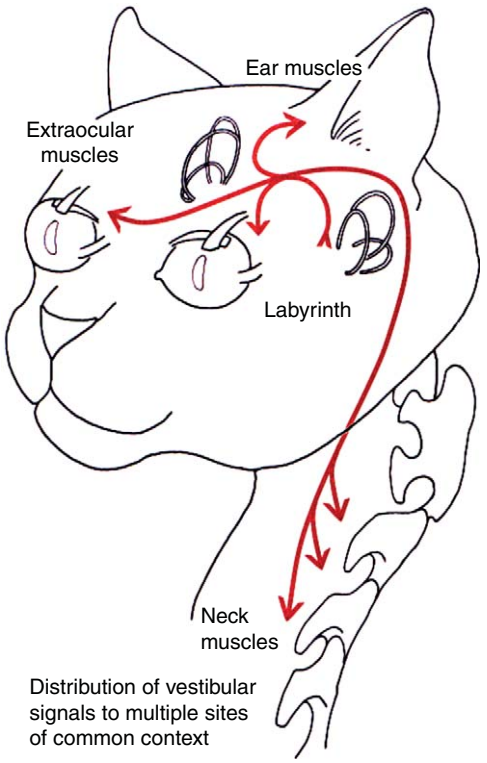

Figure 13 Schematic of vestibular neuron connectivity to motor centers of related architecture and common behavioral context. The shared neuronal pathway to eye, ear, and neck muscles would provide an economical distribution of an identical motor control signal to motoneuron pools involved in orienting behavior.

3.26.5.3 Vestibulocollic Connectivity

Within the vestibulocollic reflex connectivities, we find equally stereotypic innervation patterns as in the vestibulo-ocular circuitry (Wilson and Maeda, 1974; Shinoda *et al.*, 1994, 1996, 1997; Graf *et al.*, 1997). The only difference is that we are dealing with more muscles. Another indication for the similarity of reference frame systems for eye and head movements was indicated by the existence of vestibulo-ocular-spinal neurons (Graf and Ezure, 1986). These neurons would transmit their signals to oculomotor and spinal-motor centers at the same time (Figure 13). Thus, the same spatial information meaningful for the oculomotor system must carry a meaningful message for the spinal-motor system as well.

3.26.6 The New Wave of Vestibular Interest

The intriguing geometry and three-dimensionality of the vertebrate labyrinth has fascinated scientists since the beginning of modern science, i.e., Scarpa (1789), and numerous comparative studies have dealt with the expression of labyrinthine structures in basically almost all known vertebrates (Gray, 1907; Retzius, 1872, 1881, 1884; Werner, 1960; Lewis *et al.*, 1985). While all these studies used invasive methods to visualize ear structures, modern imaging methods have now opened a way to study them noninvasively in living tissue (Archer *et al.*, 1988; Spoor and Zonneveld, 1995); fossilized heads have also become accessible to large-scale investigations (Spoor *et al.*, 1994, 2002, 2003; Wittmer *et al.*, 2003; Clarke, 2005). These possibilities led to a number of interesting morphological discoveries that added to the vast data set already available.

In general, there were no surprises regarding the spatial orientation of the semicircular canals. These followed the familiar pattern (see Figure 1c), although some researchers seemed to be surprised by it (Spoor *et al.*, 1994). A number of authors also sought to make use of their new investigative tool to reinterpret the functional context of the vestibular system by putting it into the sole context of locomotion (Spoor *et al.*, 1994, 2002, 2003; Wittmer *et al.*, 2003). These authors argued that the dimensional morphology of the semicircular canals gave an indication about the locomotor capabilities of their owners. Thus, conclusions were drawn as to the point of effective bipedalism in certain hominids (Spoor *et al.*, 1994), or the agility of Neanderthal man (Spoor *et al.*, 2003). We have argued against such interpretations based on a number of known facts and characteristics of the vestibular system (Graf and Vidal, 1995). In essence, the former authors had based their arguments largely on the size differences in the circumference of semicircular canals within one species and across different species. However, canal fluid dynamics affecting sensitivity are also largely governed by the lumen of the canal, i.e., its cross section. Furthermore, to base locomotor activities solely on peripheral morphology means ignoring any well-known adaptive mechanisms at the receptor level, ion channel dynamics, and above all, the vast apparatus of the neuronal processing machinery that make use of vestibular signals from the brainstem and cerebellum to the cortex. Focusing on locomotion alone also ignores all the other important and vital functions subserved by the vestibular system, notably compensatory eye movements and perceptual mechanisms. Without compensatory eye movements, in particular, we would not be able to have unblurred vision during any movement. In addition, during active movements, a number of postural reflexes become suppressed, which is reflected in

elimination or attenuation of vestibular movement signals in the vestibular nuclei (McCrea *et al.*, 1999; Roy and Cullen, 2001). The arguments of Spoor *et al.* (2002) and Wittmer *et al.* (2003) have been forwarded to explain the behavior of cetaceans and pterosaurs, proposing a link between apparent extreme aquatic and aerial acrobatic capabilities of these animals, respectively. Although these findings received wide acclaim in the popularizing science literature (Stokstad, 2003; Unwin, 2003), the vestibular argument again did not take into consideration all aspects of vestibular function or the entirety of a biological system. Against the aerial capabilities of pterosaurs could be brought forward, for instance, the size and shape of their cerebellum, given that the cerebellum plays an eminent role in motor coordination. Pterosaur cerebella resemble closely that of certain bats (Baron *et al.*, 1996), and bats are not the very best flyers. As we have seen, postural control, locomotion, and eye movements are closely related to vestibular output, and there is a lot more to consider than meets the eye at first glance.

3.26.7 Conclusions

The evolution of the sense of balance of vertebrates and analogous systems in invertebrates suggests a number of important features of brain operations. We also observe conserved vestibulomotor organizations and circuitry in vertebrates after the development of one optimal solution, when arrangements have been preserved throughout subsequent vertebrate history. Compared to the many developments of eyes, for instance, the estimate is that eyes have been invented 40–65 times in evolution, only two basic types of three-dimensional movement detectors have been retained, the diagonal ones of vertebrates, octopus, and crabs, and the principal axes ones of squids. Each one of the two possibilities constitutes an ideal physical solution, with an optimal signal-to-noise ratio.

An additional important characteristic of central nervous operation seems to be that peripheral mechanisms are employed to simplify central operations. Such an operational principle has been ideally demonstrated in the common reference frames of the vestibulo-oculomotor system, including the central nervous connectivity. Thus, the workload of the brain is decreased in favor of animal economy and presumably higher-order operations (learning, perceptive functions, etc). When considering how the brain works, we have to look into similarities among apparent differences of expressions or behaviors. Disregarding obvious similarities of sensorimotor operations across species would mean disregarding one significant aspect of brain operation.

When viewing the particular example of the vestibulo-oculomotor systems across species, the conserved nature of the arrangement in its geometry, and to a large extent, its embryologic development is quite striking. Modern methods will hopefully enlighten us in the future, where traditional methods have failed and fossil records are absent. However, the early appearance of a viable and up-to-date conserved vertebrate vestibulo-oculomotor system, in tandem with systems of similar geometry in certain invertebrates may suggest that close to ideal physical solutions developed early in vertebrate history, onto which more advanced functions were added as a result of environmental pressure, or whatever circumstance, such as smooth pursuit or vergence eye movements. Finally, the initial function of the vestibulo-oculomotor system may well not have been to move the eyes, but to hold them still with respect to the environment in order to stabilize the visual world (Walls, 1962).

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3.27 Neuropeptide Systems and Social Behavior: Noncoding Repeats as a Genetic Mechanism for Rapid Evolution of Social Behavior

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Glossary

avpr1a	The gene encoding the vasopressin 1a receptor (V1aR).
compound microsatellite	A microsatellite locus that has more than one kind of repeat motif.
evolvability	The potential to mutate imparted by a flexible genome.
luciferase reporter assays	A genetic technique that allows visualization of the ability of a regulatory sequence to promote gene expression.
microsatellite	DNA comprised of repetitive sequences, usually with the smallest repeated unit in the 1–6 bp range; this kind of DNA is usually polymorphic and is often used in genetic fingerprinting; also known as SSR and VNTR.
monogamy	A social structure characterized by a suite of traits including high levels of selective affiliation, territorial aggression, separation distress, and biparental care; sexual monogamy is not implied in this definition.
nested deletions	A method of manipulating a sequence of DNA into a set of smaller and smaller fragments of the original sequence.
paralogous divergence	The evolutionary divergence of two genes within a species after a gene duplication event; oxytocin and vasopressin are paralogues.
psuedogene	A DNA sequence that has hallmarks of a protein coding region, but does not appear to produce a protein product.
slipped-strand mispairing	A DNA replication error where the two strands of DNA do not align properly.
SSR	Simple sequence repeat.
viral vector gene transfer	A genetic technique wherein artificial or foreign DNA sequences can be introduced into a population of cells and the foreign DNA will integrate into the genome of the host cell and stably express the protein encoded within it.
VNTR	Variable number of tandem repeats, a locus that is polymorphic for the number of repeats.

3.27.1 Social Behavior Evolves Rapidly

Diversity in social behavior is evident both between and within species. There are both species-typical behavioral tendencies as well as personality traits that distinguish among individuals within a

population. For example, humans are without question a highly social species, but there are clearly individuals who prefer to isolate themselves. This behavioral diversity within species could lead to the differences between species as extreme behavioral phenotypes diverge. The underlying genetics and molecular neurobiology producing individual and species differences in behavior are relatively unknown. The rich behavioral diversity of closely related species, domestic dog breeds, inbred strains of mice and rats, as well as the rapid domestication of foxes, indicates that behavior is a rapidly evolving phenotypic trait.

3.27.2 Genetic Mechanisms Generating Diversity

This idea of rapid evolution of social behavior presupposes (1) rich genetic polymorphism and/or (2) some genetic mechanism that can generate meaningful behavioral diversity *de novo*. From a gene-centric perspective, mechanisms of generating heritable behavioral diversity include gene variation that alters protein function, timing, and/or location of gene expression.

3.27.2.1 Single Nucleotide Polymorphisms

There are several well-established genetic mechanisms that could produce such changes. Single nucleotide polymorphisms (SNPs) can change a protein or its regulation depending on where the SNP occurs. For example, an SNP in the coding region of the neuropeptide Y receptor-like protein gene (*npr-1*) of the roundworm, *Caenorhabditis elegans*, results in an amino acid sequence change from a valine residue to a phenylalanine. This allelic variation in *npr-1* results in altered protein function and altered social feeding behavior (de Bono and Bargmann, 1998). In contrast, an SNP that occurs outside of a coding region might alter the level of expression by changing the quality of protein–DNA interactions of transcriptional regulators. Recently, individual variation in an SNP in the promoter of the vasopressin gene in rats has been implicated in individual differences in anxiety-like behavior that appear to be related to differences in levels of vasopressin gene expression in the paraventricular nucleus of the hypothalamus (Murgatroyd *et al.*, 2004; Wigger *et al.*, 2004).

3.27.2.2 Gene Duplication and Deletion

In addition to genetic changes in the sequence or regulation of genes, gene duplications as well as deletions must also play a role in the evolution of brain substrates for behavior. One infers from the complexity of the mammalian brain and genome that gene duplication with paralogous divergence creates new neural substrates, perhaps by adding layers of sensitive regulatory mechanisms as suggested in duplicated genes for circadian rhythmicity (reviewed in Looby and Loudon, 2005). Deletion of gene families also changes the capacity for behavior. In a recent example, demonstration of the pseudogenization of sweet taste receptors in feline lineages illustrates a mechanism whereby functional gene deletion can change an animal's perception of and behavior toward the outside world (Li *et al.*, 2005; see The Evolution of the Sweetness Receptor in Primates).

3.27.2.3 Microsatellites

In addition to SNPs and the duplication and deletion of genes, there is increasing interest in sequences in the genome that have higher rates of mutation as a consequence of their base pair content. Not all regions of the genome share equal probabilities of mutation. In particular, DNA comprised of repetitive sequences, such as microsatellites, has an estimated four orders of magnitude higher rate of mutation than the rate of SNP occurrence in nonrepetitive DNA (Radman *et al.*, 1995). The nature of mutation at repeat loci involves expansion or contraction of the number of repeats by slipped-strand mispairing during DNA replication. Therefore, a repeat locus not only has a higher rate of mutation than nonrepeat DNA, but it also has the possibility of generating more alleles than a single base pair mutation. Instead of being limited to one of four base pairs, the repeat DNA can expand and contract any number of times to any number of repeat units.

3.27.2.3.1 Microsatellites as trash or treasure Repeat DNA, or microsatellite DNA, can be found in both coding and noncoding regions of genes. Therefore, their expansion and contraction qualities can affect protein function as well as location, timing, or levels of expression. Repeat DNA has been appreciated in neuropathology since several neurological disorders appear to be a result of grossly expanded repeat number. Expanded repeats in coding regions as in Huntington's disease (The Huntington's Disease Collaborative Research Group, 1993) as well as in regulatory regions of genes like the repeat found in Fragile X (Yu *et al.*, 1991) have been determined to play a causal role in those disorders. Initially, these discoveries raised questions about the reason for the perpetuation of these repeats, or junk DNA, since they appeared to

carry such a potentially high cost, with no apparent benefits. In light of this puzzle, King (1994) proposed that perhaps this kind of DNA, when not grossly expanded, allowed for the generation of quantitative phenotypic traits. King and colleagues (Kashi *et al.*, 1997) have since suggested that, like a tuning knob, microsatellite expansion and contraction within some normal range may dial new heritable traits.

To date, there are several examples providing support for this very exciting hypothesis. There are both coding and noncoding repeat polymorphisms that appear to affect phenotypic changes within the normal range. For example, in the coding region of the period gene of the fruit fly *Drosophila*, there are differences in repeat number in threonine–glycine residues. The repeat number appears to influence the timing of the mating dance during courtship behavior of male flies (Yu *et al.*, 1987; Petersen *et al.*, 1988). Another more visually apparent example comes from Fondon and Garner's (2004) analysis of genetic correlates of dog snout morphology. In this analysis, coding region repeats in several developmental genes were very highly correlated with dog snout morphology (Fondon and Garner, 2004). This suggests a role for microsatellite expansion and contraction in the robust morphological variation of domestic dogs.

Microsatellite or repeat expansion and contraction in regulatory regions of genes have also been implicated in variation in behavioral traits as well as psychiatric disease risk in humans. For example, simple sequence repeats (SSRs) in the 5′ noncoding region of the serotonin transporter have been associated with risk for anxiety and depression (Lesch *et al.*, 1996; Pezawas *et al.*, 2005). The number of repeats in the 5′ noncoding region of the monoamine oxidase A gene has been associated with aggression (Manuck *et al.*, 2000), especially when paired with early adverse experience (Caspi *et al.*, 2002). In both examples, the numbers of repeats have been demonstrated to modify gene expression in *in vitro* cell culture assays (Lesch *et al.*, 1996; Sabol *et al.*, 1998).

3.27.3 Vole Species as a Model for the Evolution of Social Behavior

The following data derived from laboratory investigations of vole species provides support for the tuning knob hypothesis. In sum, the following data will implicate a microsatellite locus in the regulatory region of the gene encoding the vasopressin 1a receptor (*avpr1a*) in the evolvability of social behavior. By comparing this locus both between and within vole species, and even primates, the emerging picture is that this locus has an evolvability that allows its brain distribution pattern to be easily altered. This alteration in brain distribution changes the modulatory effects of the neuropeptide vasopressin, and therefore the behavioral response to external stimuli that cause the release of vasopressin within the brain. Furthermore, increasing evidence suggests that any genetic mechanism that alters the distribution patterns of neuromodulatory receptors may be an important general mechanism generating individual and species differences in complex behaviors.

3.27.3.1 Vole Social Behavior

Voles (*Microtus* spp.) comprise a large and diverse genus of rodent. Voles are quite behaviorally diverse and therefore comprise a tractable genus for questions regarding evolution of brain substrates underlying diverse behavior. Some species of voles, such as prairie (*M. ochrogaster*) and pine voles (*M. pinetorum*), are socially monogamous: males and females form long-term pair bonds, the males contribute to parental care, and the pair defends a shared nest site. In contrast, nonmonogamous montane and meadow voles (*M. montanus*, *M. pennsylvanicus*) do not pair bond, the males do not contribute to parental care, and none but the nursing mother and her offspring share a nest site (Thomas and Birney, 1979; Getz *et al.*, 1981; Jannett, 1982; Gruder-Adams and Getz, 1985; Shapiro and Dewsbury, 1990). Additionally, monogamous species show selective aggression toward strangers after becoming sexually experienced (Winslow *et al.*, 1993; Insel *et al.*, 1995) and the young of monogamous species show separation distress vocalizations and increased serum levels of the stress hormone, corticosterone (Shapiro and Insel, 1990).

3.27.3.2 Measuring Social Behavior in the Laboratory

Laboratory tests revealed that as in the wild, prairie and pine voles are socially monogamous and montane and meadow voles are not. The main laboratory assay used to investigate pair bonding is the partner preference test. In this test, a male and female are paired for a period of 6–24 h co-habitation with or without mating. After co-habitation, the animals are briefly separated as they are prepared for the 3 h behavioral assay. A three-chambered apparatus is used for this assay. The partner animal is tethered in one chamber, and a stranger

animal is tethered in another chamber. These two chambers are connected to each other with short Plexiglas tubes and a third neutral chamber that does not contain an animal. The dependent measure of this test is the amount of time the animal spends in side-by-side contact with the partner animal compared to the stranger, as well as the time spent alone in the neutral cage. Both the male and female of the pair can be tested in this apparatus. In this assay, prairie voles spend very little time in the neutral chamber and spend more time in side-by-side contact with their partner. In contrast, montane and meadow voles spend equal amounts of time with the partner and stranger but mostly spend their time in the neutral cage. The amount of time spent in side-by-side contact with the partner animal can be predictably altered in prairie voles by prolonging or truncating the duration of co-habitation and allowing or preventing mating during the co-habitation period. Mating and longer periods of co-habitation increase the amount of time that animals spend in side-by-side contact with their partner, indicating that these are important variables in the formation of a pair bond (Insel *et al.*, 1995; Williams *et al.*, 1992b).

3.27.4 Social Bonding and Oxytocin

This laboratory assay of social bonding has allowed for determination of molecular players involved in the formation of pair bonds. In female prairie voles, the neuropeptide oxytocin contributes to the formation of partner preferences (Williams *et al.*, 1992a, 1994). Oxytocin was originally identified as one of two neurohormones secreted by the posterior pituitary, or neurohypophysis, and was first appreciated as a critical player in parturition and lactation. Due to its flooding presence from the posterior pituitary during these early stages of the onset of maternal behavior, it was further hypothesized and confirmed to play a role in the transition to maternal behavioral states and in the formation of the mother–infant bond (Kendrick *et al.*, 1987; Pedersen and Prange, 1979). This action of oxytocin is a result of oxytocin released into the brain rather than by oxytocin released into blood circulation by the posterior pituitary. By extension, it was hypothesized that this neuropeptide acting in the brain might also play a role in the development of adult social bonds such as that found in monogamous social structures. Indeed, injections of oxytocin into the cerebral ventricles of the prairie vole brain prior to the co-habitation period facilitate the formation of partner preferences (Williams *et al.*, 1994). Additionally, delivery of oxytocin receptor antagonists to the brain blocks the formation of partner preferences (Williams *et al.*, 1994; Cho *et al.*, 1999). It appears that oxytocin and its receptor are necessary for the formation of social preferences in females, a prerequisite for the social bonds of monogamous species.

3.27.5 Social Bonding and Vasopressin

Vasopressin is the complementary neurohormone of the posterior pituitary and appears to contribute to partner preference formation in male prairie voles (Winslow *et al.*, 1993). This neurohormone was pursued as a candidate for partner preference formation because of its previously established role in male species-typical social behavior, such as aggression in hamsters (Ferris *et al.*, 1997). Studies in prairie voles indicated that vasopressin injected into the ventricles promotes partner preference formation (Winslow *et al.*, 1993). Furthermore, central infusion of vasopressin 1a receptor (V1aR) antagonists inhibit the formation of partner preferences (Cho *et al.*, 1999; Winslow *et al.*, 1993). Therefore, as with oxytocin, vasopressin and the V1aR appear to be necessary for the formation of social preferences.

All species studied to date have oxytocin and its receptor, as well as vasopressin and the V1aR. Selection pressures are very strong to keep these neuropeptides and their signaling mechanisms intact. Loss of oxytocin results in the inability for the female to lactate (Nishimori *et al.*, 1996). Loss of vasopressin results in diabetes insipidus (Bohus *et al.*, 1975). Only very artificial situations allow for survival or reproduction when these players are disrupted. If these players are so highly conserved and laboratory experiments in prairie voles indicate their role in partner preference formation, why are monogamous social structures rare among mammals?

3.27.5.1 Evolutionary Lability of Receptor Distribution Patterns

Behavioral pharmacology evidence from closely related bird species indicates that not all species respond in the same way to treatment with the same neuropeptide. Vasotocin, the avian orthologue of vasopressin, administered to the septum of zebra finches results in increased aggression (Goodson and Adkins-Regan, 1999), whereas the same treatment in closely related field sparrows or violet-eared waxbills inhibited aggression (Goodson, 1998a, 1998b). Similarly, in vole species, oxytocin and/or vasopressin injection into the brain increases

affiliative behavior in monogamous prairie voles, but not in nonmonogamous montane or meadow voles (Young *et al.*, 1999). The effect of any pharmacological agent depends, of course, on the presence of the pharmacological target, in this case the receptors for oxytocin and vasopressin. It turns out that while the brain distribution of the neuropeptides oxytocin and vasopressin are very highly conserved across species (Wang *et al.*, 1996), the location of their receptors is not conserved (Figure 1) (Young, 1999). This alteration in the location of receptors in the brain across species underlies some of the behavioral differences. The altered location of receptors must change behavior because it alters the neural circuits that can be modulated by oxytocin or vasopressin signaling. For example, in monogamous prairie voles, V1aR binding is very dense in the ventral forebrain, in particular the ventral pallidum, a brain region recognized for its role in reward and motivation (Lim *et al.*, 2004a; see Forebrain Size and Social Intelligence in Birds). Interestingly, montane and meadow voles have very low densities of receptors in this region (Insel *et al.*, 1994). This particularly dense receptor binding in monogamous prairie voles appears to be a key factor in the capacity for monogamous social structure in these species. Specifically, site-specific injections of a V1aR antagonist into the ventral pallidum blocks partner preference formation (Lim and Young, 2004). Additionally, experimentally increasing the levels of V1aR in the ventral pallidum using viral vector gene transfer increased partner preference in prairie voles (Pitkow *et al.*, 2001). In the definitive experiment, the nonmonogamous meadow vole was induced to form pair bonds by viral vector gene transfer of V1aR to the ventral pallidum (Lim *et al.*, 2004b). These series of experiments clearly demonstrate the importance of species-specific patterns of V1aR in the evolution of social behavior.

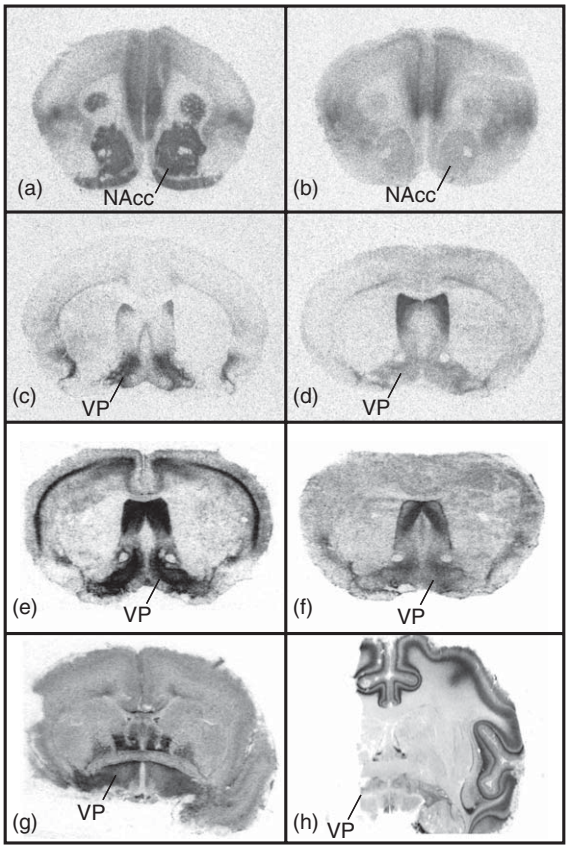

Figure 1 Neuropeptide receptor distribution patterns are associated with social structure. Monogamous prairie voles (a) and nonmonogamous montane voles (b) show dramatic differences in brain distribution of oxytocin receptor (Insel and Shapiro, 1992). V1aR distribution patterns are also associated with social structure. Monogamous prairie voles (c), California mice (e), and marmosets (g) all show high levels of V1aR in the ventral pallidum. In contrast, nonmonogamous montane voles (d), leucopus mice (f), and rhesus macaques (h) all have very little apparent V1aR in this brain region.

3.27.5.2 Genetic Mechanism of Diversity in V1aR Distribution Patterns

3.27.5.2.1 Comparative evidence for microsatellites as a genetic mechanism As one would expect, the pharmacokinetics of receptor–ligand interaction are identical among the vole species tested thus far (Insel *et al.*, 1994). Therefore, species differences in receptor patterns are not likely due to differences in the quality of ligand–receptor interaction. Additionally, analysis of mRNA distribution of the V1aR also reveals similar species differences in distribution (Young *et al.*, 1997). Theses results point to differences in gene regulation mechanisms. In support of this idea, a mouse transgenic for the prairie vole V1aR gene (*avpr1a*), including the coding region and flanking noncoding regulatory domains, displayed an altered receptor distribution pattern and an altered behavioral response to the central administration of vasopressin (Young *et al.*, 1999). Comparison of the *avpr1a* gene sequences from monogamous and nonmonogamous vole species revealed high homology across species in the coding regions. The noncoding elements were also very highly homologous, with the exception of an expansion of a compound microsatellite in the 5′ noncoding region in the monogamous prairie vole compared to the nonmonogamous montane vole (Figure 2). In the prairie vole, this expanded element consists of several dimer and tetramer repeat blocks interspersed with nonrepetitive sequences (Young *et al.*, 1999). Additionally, there appears to have

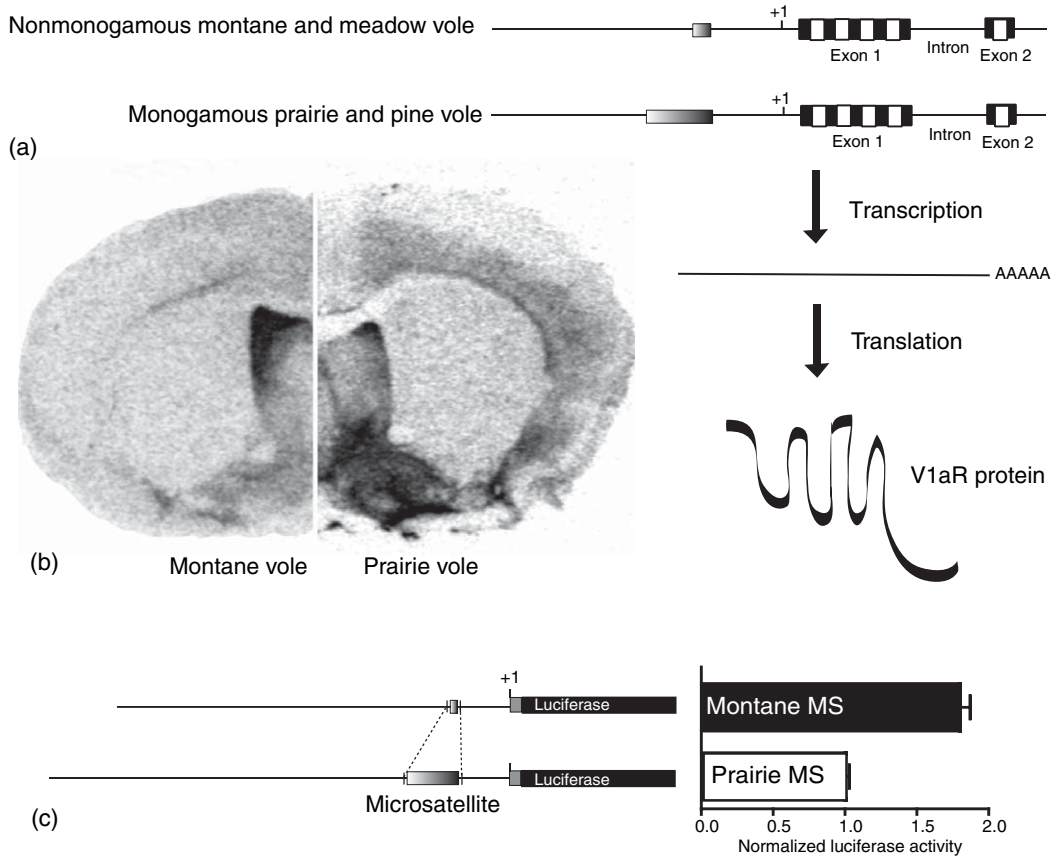

Figure 2 Species differences in receptor distribution pattern are associated with functional species differences in the length of a microsatellite in the 5′ regulatory region of the gene encoding V1aR (*avpr1a*). The microsatellite, indicated by shaded boxes, is highly expanded in *avpr1a* in prairie and pine voles compared to montane and meadow voles (a). This divergence in sequence length could lead to differences in cell-type-dependent gene transcription, which could explain the differences in protein distribution measured by autoradiography (b). The species differences in microsatellite length do modify gene expression. In cultured rat A7r5 cells, the montane microsatellite drives reporter gene expression more than the prairie microsatellite (c). Reproduced from Hammock, E. A. D. and Young, L. J. 2004. Functional microsatellite polymorphism associated with divergent social structure in vole species. *Mol. Biol. Evol.* 21, 1057–1063, by permission of Oxford University Press.

been a gene duplication event in the prairie vole species that if expressed would result in a truncated form of the receptor. The potential function of a truncated V1aR has not been addressed. Monogamous pine voles have the compound microsatellite similar in length to the prairie vole, whereas the nonmonogamous meadow vole has a short element similar to the montane vole. Because there were very few differences between species elsewhere in the *avpr1a* gene and because the differences appeared to be at the level of gene regulation, we investigated the role of 5′ noncoding elements, including the microsatellite, in the regulation of gene expression.

3.27.5.2.2 *In vitro* evidence for microsatellites as a genetic mechanism In a series of *in vitro* experiments, we determined that the species differences in microsatellite length were sufficient to change gene expression (Hammock and Young, 2004). First, we created a series of nested deletions of 3.5 kb of the prairie vole *avpr1a* 5′ region, which included the microsatellite locus. Using luciferase reporter assays in several rat cell lines, we observed that the effects of deleting sequences in the microsatellite altered reporter gene expression in a cell-type-dependent manner. In other words, deletions of microsatellite sequence altered the reporter gene activity in some but not all cell lines. We further probed the function of the microsatellite locus by deleting only the microsatellite locus out of the surrounding 3.5 kb of prairie vole *avpr1a* 5′ region. We tested this construct (no cassette) against the full-length prairie vole *avpr1a* in several rat cell lines and also observed a cell-type-dependent effect on reporter gene activity, in a manner similar to the results from the nested deletion series of experiments. Finally, to establish that species differences in microsatellite length at this locus could alter gene expression, we inserted either the prairie vole microsatellite or the montane vole microsatellite into the microsatellite position in

the no-cassette vector. The prairie and montane microsatellites were tested against each other in one of the rat cell lines that had previously demonstrated an effect of alterations at this locus. In this particular rat cell line, species differences in the microsatellite resulted in robust differences in reporter gene expression. Specifically, the montane vole microsatellite resulted in higher reporter activity than the prairie vole microsatellite. Because the previous reporter experiments demonstrated a cell-type-specific effect of changes at the microsatellite locus, it is possible that there would be no species differences in some other cell lines and there is likely to be a cell line wherein the prairie vole microsatellite drives gene expression to a greater extent. Regardless, these cell culture data have confirmed that the species differences in this locus can alter gene expression levels. Furthermore, the cell-type-specific effect of changes at the microsatellite locus indicate that the microsatellite would alter the expression of *avpr1a* in some but not all cells, and could therefore alter the pattern of expression rather than overall levels of expression.

Taken together, these data indicate that expansion or contraction mutation in a microsatellite locus in the regulatory region of the vole *avpr1a* gene likely resulted in altered distribution patterns of V1aR in the brains of voles. This altered distribution pattern modifies the behavioral response to external stimuli that cause the central release of vasopressin into the brain, such as mating or stress. Therefore, the instability of this microsatellite locus likely contributed to the evolution of divergent social behavior strategies.

3.27.5.2.3 *In vivo* evidence for microsatellites as a genetic mechanism If this hypothesis is correct, that microsatellite instability at this locus causes behavioral diversity across species, then a relationship should still exist between microsatellite length and behavior within a species. Like most repeat loci, there is ample intraspecific variation in this locus in the prairie vole species (Hammock and Young, 2002). The range of variation within the prairie vole species is much narrower than between prairie and montane voles. Likewise, there is intraspecific variation in V1aR distribution patterns (Phelps and Young, 2003) as well as behavior in wild-caught and captive populations of prairie voles. First, we established that like the species differences in microsatellite length, intraspecific variation in microsatellite length could modify gene expression in cell culture (Hammock and Young, 2005; Figure 3a). To investigate potential relationships between the microsatellite, V1aR distribution patterns and behavior, we first screened 20 males selected at random from our laboratory colony. Using a high throughput screening method, we determined that there were more significant relationships among the variables than could be due to chance alone (Hammock *et al.*, 2005), suggesting that there is likely a causal relationship between microsatellite length, V1aR distribution patterns, and behavior. To clearly address these relationships, we then set up a genetic selection breeding strategy (Hammock and Young, 2005). We genotyped our laboratory colony of prairie voles and created breeding pairs that were homozygous for either longer or shorter than the average length. Three litter cohorts were obtained from the 25 breeder pairs and two out of three cohorts were randomly cross-fostered on the day of birth to reduce potential nongenomic transmission of brain and behavioral traits. We measured the parental care of the breeding pairs and the social and anxiety-like behavior as well as the V1aR distribution patterns of the F1 male offspring. This analysis revealed that microsatellite length likely gives rise to altered V1aR binding patterns (Figure 3). Males with longer microsatellites displayed higher levels of V1aR in the olfactory bulb and lateral septum and lower levels in the hypothalamus, basolateral amygdala, and posterior cingulate cortex, for example. Additionally, breeder males with longer microsatellite alleles showed higher rates of pup licking and grooming, an important aspect of rodent parental care. F1 male offspring with longer microsatellites were quicker to approach a novel social odor and a novel juvenile animal and were also more likely to form pair bonds under unfavorable conditions (truncated co-habitation period). There was less of an effect on anxiety-like behavior, suggesting that the observed genotype differences in social behavior were not due to underlying differences in trait anxiety. This breeding strategy demonstrated a role for the microsatellite in generating diverse brain substrates that are associated with diverse behavioral traits. Furthermore, the identification of such relationships within the prairie vole species provides further support for the idea that instability in the microsatellite locus can produce sufficient change in brain and behavior upon which natural selection may act.

3.27.6 Comparative Evidence in Primates for Microsatellites as a General Mechanism

Microsatellite instability may have played a role in the evolution of human social behavior as well (Figure 4) (Hammock and Young, 2005). The human *avpr1a* locus has three polymorphic microsatellites in the 5′ flanking region (Thibonnier *et al.*,

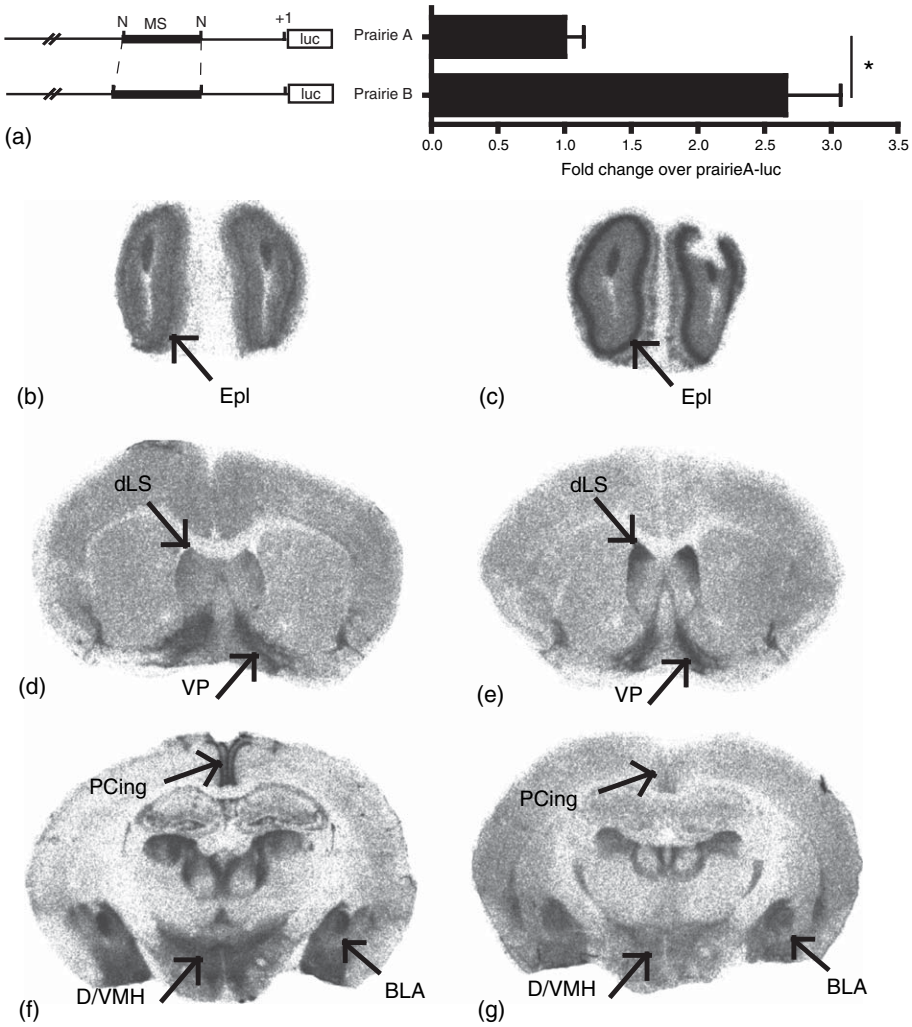

Figure 3 Intraspecific variation in V1aR distribution appears to be regulated in part by individual variation in the length of a compound microsatellite in the 5′ regulatory region of the prairie vole *avpr1a* gene. The gene encoding V1aR (*avpr1a*) contains a compound microsatellite in the 5′ noncoding region. The length of this microsatellite varies among individual prairie voles (a). Two representative microsatellites were tested against each other for their ability to regulate gene expression in luciferase reporter assays in rat A7r5 cells (a). In this cell line, the longer microsatellite allele drives luciferase expression to a greater extent than the shorter allele. Selectively breeding prairie voles for the microsatellite length reveals a genotype effect on the distribution of V1aR in the brain. In short-alleled males (b, d, f), compared to long-alleled males (c, e, g), there was reduced V1aR density in the external plexiform layer (Epl) of the olfactory bulb and dorsal lateral septum (dLS). In contrast, the short-alleled males had higher V1aR density in the posterior cingulate cortex (PCing), dorsal and ventral hypothalamus (D/VMH), as well as the basolateral amygdala (BLA). There was no apparent genotype effect in the ventral pallidum (VP). Reprinted with permission from Hammock, E. A. D. and Young, L. J. 2005. Microsatellite instability generates diversity in brain and sociobehavioral traits. *Science* 308, 1630–1634. Copyright 2005 AAAS.

2000). One of these, a compound microsatellite comprised of CT and GT dinucleotide repeats, located 3625 bp upstream of the transcription start site, has been reported in two independent studies to be associated with autism, suggesting that variations in this sequence may indeed result in variations in human social behavior (Kim *et al.*, 2002; Wassink *et al.*, 2004). Sixteen alleles of the microsatellite at –3625 kb have been found in the human population (Kim *et al.*, 2002). More recent studies have reported associations between alleles at this locus and normal social behavior, specifically levels of sibling conflict (Bachner-Melman *et al.*, 2005b). One recent study has even reported an association between *avpr1a* microsatellite polymorphisms, in combination with a serotonin transporter promoter polymorphism, and creative dance in humans, arguably a form of social communication (Bachner-Melman *et al.*, 2005a). Interestingly, the –3625 bp microsatellite is absent in the common chimpanzee *avpr1a* gene, a finding that parallels the species differences in the vole gene. In contrast, bonobos, known for high levels of social reciprocity, empathy, and sociosexual bonding (De Waal, 1988), have a microsatellite nearly

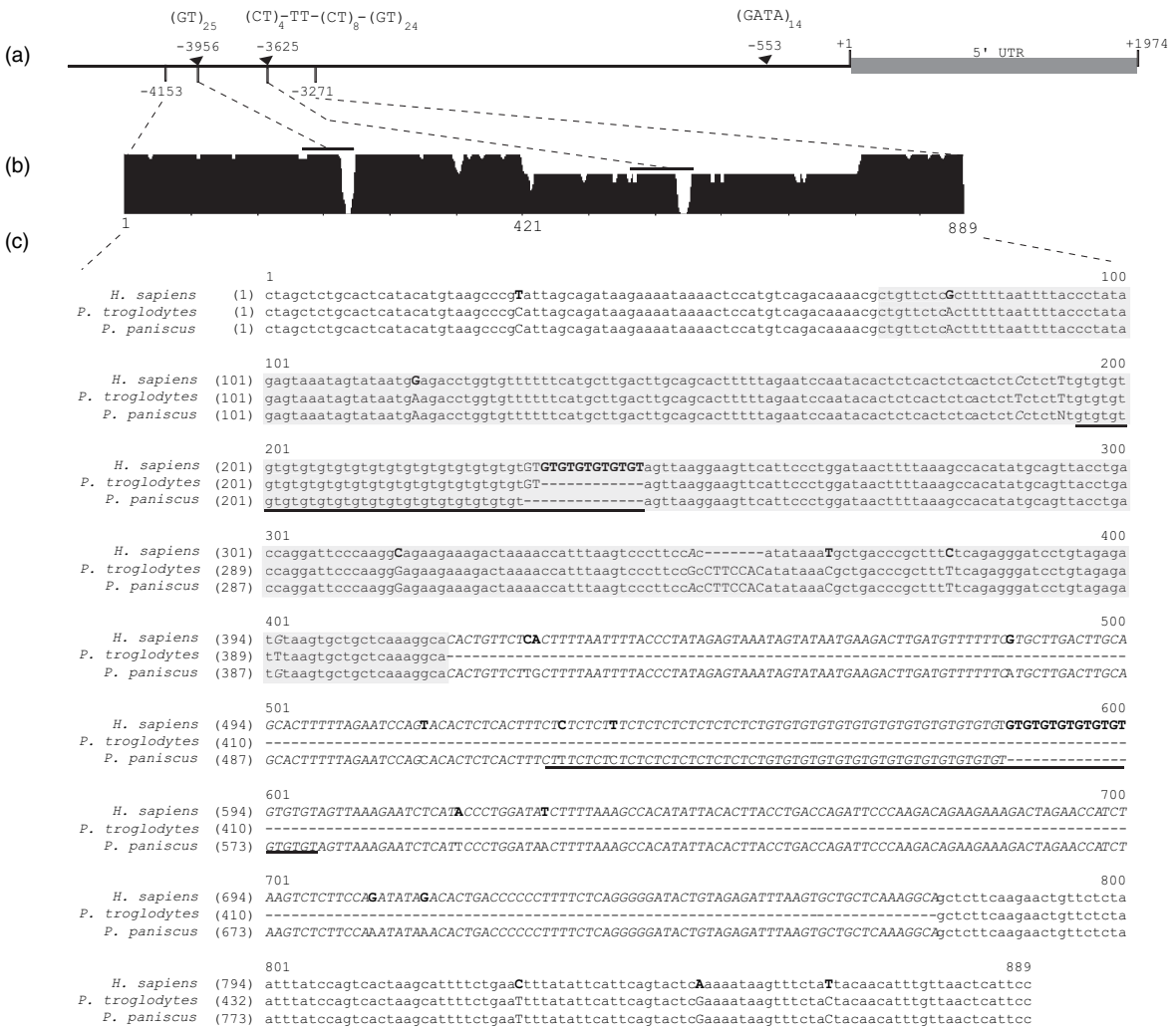

Figure 4 Humans (*H. sapiens*) and bonobos (*P. paniscus*) share a compound microsatellite element in the 5′ regulatory region of *avpr1a,* which is absent in the chimpanzee (*P. troglodytes*). The schematic (a) illustrates the position of 5′ microsatellite elements in relation to the annotated transcription start site (+1) of the human *avpr1a* gene (start codon at +1974). Human, chimpanzee, and bonobo similarity plot (b) at this region reveals high levels of homology around the –3956 bp microsatellite, whereas the microsatellite at –3625 bp is not as conserved. The reduction in similarity around the –3625 bp microsatellite is due to the complete absence of approximately 360 bp in chimpanzees (c). Underscores indicate the positions of the two repeat regions. Lower case letters indicate a sequence that is conserved across all three species. Upper case sequence represents conservation in two out of three species, while italicized upper case sequence represents sequence that is shared between human and bonobo, but not chimpanzee. Bolded upper case letters highlight sequence that is unique to humans. The sequence that is shared between bonobo and humans, but not chimpanzees, appears to be a duplication of the preceding sequence, indicated by the shaded grey box. Reprinted with permission from Hammock, E. A. D. and Young, L. J. 2005. Microsatellite instability generates diversity in brain and sociobehavioral traits. *Science* 308, 1630–1634. Copyright 2005 AAAS.

identical to that of the human (Hammock and Young, 2005). Studies are currently underway to survey *avpr1a* microsatellite structure in a diverse group of apes and monkeys with varying social structure.

3.27.7 Caveat Regarding the Importance of Development

The simple V1aR model only highlights the differences in receptor expression and its behavioral effects in the adult animal, thereby completely ignoring the role of development and developmental plasticity in the life of the organism. It is highly probable that changes in gene expression patterns very early in development would affect the developmental trajectory of brain substrates for behavior by changing the fine-tuning effects of experience. For example, the presence of V1aR in the ventral pallidum of neonatal and juvenile prairie voles likely modifies their behavior during those important developmental windows and contributes to the creation of a social

environment that would drive further development and maturation of the social brain.

3.27.8 Conclusions

The evolutionary lability of expression patterns of neuromodulatory receptors such as V1aR and the oxytocin receptor may be a general mechanism producing diverse neural substrates. The presence of V1aR and oxytocin receptor in reward areas in the prairie vole gives vasopressin and oxytocin release a much different meaning than if these receptors were present in aggression-related areas. Furthermore, microsatellite instability in the regulatory region of *avpr1a* appears to contribute to the intra- and interspecies differences in V1aR distribution patterns. The high rates of expansion and contraction mutation in microsatellites might give genomes a useful tool for rapid generation of diverse phenotypic traits for "rapid exploration of the fitness landscape" (Radman *et al.*, 1999).

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3.28 The Evolution of Motor Cortex and Motor Systems

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Glossary

autoradiograph (or autoradiogram) An image produced on photographic emulsion or film by the radiation emitted from a radioactive isotope that has been absorbed by tissue; e.g., radiolabeled amino acids can be used to visualize neuronal tracts via axoplasmic transport.

Betz cells The largest layer V pyramidal neurons. They are believed to comprise a large proportion of corticomotoneuronal cells.

cladogram A branching, treelike diagram in which the endpoints of the branches represent specific species of organisms. It is used to illustrate phylogenetic relationships and show points at which various species have diverged from common ancestral forms.

corticomotoneuronal Referring to corticospinal neurons that form monosynaptic contact with spinal cord motoneurons.

corticospinal tract Collective term for neurons with somata located in cerebral cortex that send axons to the spinal cord via the cerebral peduncles and medullary pyramids.

cytoarchitecture The cellular composition of a bodily structure; e.g., used to define a structurally distinctive cortical area.

eutherian Belonging to the infraclass Eutheria, a division of mammals to which all placental mammals belong.

funiculus One of the major white matter tracts in the spinal cord consisting of large bundles of ascending and descending fibers.

intracortical microstimulation A technique for stimulating a small neuronal cell population

	by introduction of a microelectrode and delivery of current pulses; used to stimulate corticospinal neurons to define functional motor representations in cerebral cortex.
lissencephalic	Characterized by a smooth cerebral cortex.
microelectrode	A very small insulated wire or fluid-filled micropipette used to study functional characteristics of living cells and tissues; commonly used for recording action potentials from one or more neurons, or for stimulating a small group of neurons.
prototherian	Neurologically primitive, egg-laying mammals found only in Australia, Tasmania, and New Guinea; the monotremes.
striatum	The phylogenetically more recent part of the corpus striatum (neostriatum) consisting of the caudate nucleus and the putamen.
supraspinal	Origin located above the spinal cord.
tetrapod	A vertebrate animal with four feet, legs, or leglike appendages.

3.28.1 Introduction

The varied and complex motor behaviors exhibited by vertebrates are the result of extraordinary alterations and enhancements over hundreds of millions of years of nervous system evolution. While this brief article cannot convey a complete picture of all of the selective pressures that likely gave rise to the differentiation of specialized motor structures, we will try to highlight some of the salient features that have marked the evolution of vertebrate motor systems and the uniquely mammalian motor cortex (see Evolution of the Somatosensory System – Clues from Specialized Species).

3.28.2 The Phylogenetic History of Descending Control of Spinal Cord Motoneurons

3.28.2.1 The Basic Vertebrate Plan

Based upon a large number of tract-tracing studies conducted over the past few decades, it is possible to survey extant mammals and surmise the probable evolution of descending control of spinal cord motoneurons in vertebrate species. This allows us to determine what features might be unique in specific lineages (e.g., the anthropoid lineage leading to humans), or specific ecological niches, and to appreciate the parallel contribution of common selective pressures regardless of lineage. To put the mammalian motor system in perspective, and to derive general principles for the evolution of motor systems, it is first necessary to review briefly descending control of spinal cord motoneurons prior to the divergence of mammals. With the addition of new evidence, this review represents an update of an earlier survey (Nudo and Masterton, 1988).

Even in the most primitive vertebrate forms still extant, neurons in the spinal cord are influenced by axons originating at supraspinal levels. Some of these descending pathways emerged in the most primitive vertebrate forms. The application of retrograde transport techniques has demonstrated supraspinal origins of spinal fibers in a wide variety of vertebrates (ten Donkelaar, 1976; Kokoros and Northcutt, 1977; ten Donkelaar *et al.*, 1980; Smeets and Timerick, 1981; Wolters *et al.*, 1982; Forehand and Farel, 1982; Kimmel *et al.*, 1982; Kunzle and Woodson, 1983; Oka *et al.*, 1986; Prasada Rao *et al.*, 1987; Ronan, 1989; Lee and Eaton, 1991; Cruce, *et al.*, 1999; Zhang *et al.*, 2002). Table 1 summarizes these results in various vertebrate classes for some of the major descending pathways that are found in mammals. Although these data do not provide a perfectly complete picture of all descending spinal afferents, and the species that have been studied cannot be considered to be either perfect representatives or random samples of their entire class, the many cases already available display several clear similarities that shed light on the very early, premammalian history of those descending pathways now found in mammals.

As shown in Table 1, at least three major supraspinal cell groups have projections to the spinal cord in every species of vertebrate studied to date: the reticular formation (a collective term for a diverse set of neurons that reside in the midbrain and hindbrain (Lee and Eaton, 1991) (reticulospinal tract), the vestibular nuclei (vestibulospinal tract), and the interstitial nucleus of the medial longitudinal fasciculus (interstitiospinal tract). Further, in all vertebrates yet studied, the spinal projections of each of these three cell groups travel down the cord in the ventral funiculus and each terminates in the ventromedial spinal gray (ten Donkelaar, 1976). Based upon their similar origins, trajectories, and terminations, it is probably safe to conclude that each of these three pathways is a true homologue of its respective counterpart throughout the vertebrate subphylum. If so, they can be considered to constitute a vertebrate common plan of descending spinal pathways.

In addition to these three pathways, tract-tracing studies in the most neurologically primitive

Table 1 Supraspinal cell groups with descending projections to the spinal cord in various vertebrate classes

	Agnatha	*Chondrichthyes*	*Osteichthyes*	*Amphibia*	*Reptilia*	*Mammalia*	*Aves*
Reticular formation	+	+	+	+	+	+	+
Vestibular nuclei	+	+	+	+	+	+	+
Interstitial nucleus	+	+	+	+	+	+	+
N. descending trigeminal tract	?	+	+	+	+	+	+
Raphe complex	?	+	+	+	+	+	+
N. solitary tract	?	+	+	+	+	+	+
Hypothalamus	+	+	+	+	+	+	+
Red nucleus	−	±	±	±	±	+	+
Cerebellar nuclei	−	−	−	+	+	+	+
Telencephalon	−	−	−	+	?	+	−

+, Pathway has been positively identified in all species examined in the class; −, pathway has been sought, but has not been found in any species examined in the class; ±, pathway has been identified in some, but not all species examined in the class; ?, differentiation of these structures is still unsettled in class Agnatha.

vertebrates (lampreys and hagfish; class Agnatha) have revealed spinal cord projections from brainstem neurons that may be homologous to those identified in gnathostomes, or jawed vertebrates. Despite their seemingly simple neurological organization, agnathans may possess spinal pathways originating in the nucleus of the descending trigeminal tract, the Raphe complex (many investigators include the cells identified here as the Raphe complex to be part of the reticular formation), the nucleus of the solitary tract, and the hypothalamus (Ronan, 1989). Thus, many of the cell groups that form spinal pathways in mammals were probably already in place early in vertebrate evolution. While these various cell groups became more specialized in different vertebrate classes, once established, this basic vertebrate plan was maintained throughout hundreds of millions of years of evolution, and still exists in all known living vertebrates.

3.28.2.2 Augmentation in Jawed Vertebrates (Gnathostomes): The Curious Case of the Rubrospinal Tract

Table 1 shows that in all vertebrate classes except Agnatha, descending spinal fibers have been identified that originate from the red nucleus and project to the contralateral spinal cord (rubrospinal tract). Although descending fibers originate from cells in the mesencephalic tegmentum in lampreys, the red nucleus is not differentiated (Ronan, 1989). It appears that all mammals and birds studied to date possess rubrospinal neurons. In contrast, in cartilaginous fish, bony fish, amphibians, and reptiles, rubrospinal neurons are present in some species and absent in others (see Somatosensory Adaptations of Flying Mammals, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?).

Variability in the presence of the rubrospinal tract in vertebrate species has generated considerable discussion regarding its functional significance. At least in mammals, the rubrospinal tract is thought to play a major role in the control of limb movements (Massion, 1988). In this regard, it is interesting that the rubrospinal tract is absent in boid snakes, caecilians (wormlike amphibians), and sharks, but present in limbed amphibians, limbed reptiles, and in rays (ten Donkelaar, 1988). Curiously, a crossed rubrospinal tract has also been described in lungfishes (Ronan and Northcutt, 1985) and goldfish (Prasada Rao *et al.*, 1987). This raises the possibility that the rubrospinal tract functions to control fins in aquatic vertebrates as well as limbs in terrestrial vertebrates.

If the rubrospinal tract emerged early in the gnathostome radiation, then it apparently became vestigial in many limbless amphibians and reptiles. If so, the disappearance of the rubrospinal tract would represent a rare exception to the rule that, once established, descending spinal pathways are maintained throughout subsequent evolution.

3.28.2.3 The Tetrapod Augmentation

Table 1 shows that the cerebellum (i.e., the deep cerebellar nuclei) projects to the spinal cord in amphibians, reptiles, mammals, and birds. Again, since this cerebellospinal tract has a similar origin, trajectory, and termination pattern, this tract is probably homologous across the tetrapod classes. If so, the basic vertebrate plan appears to have undergone a second augmentation with the appearance of tetrapodal vertebrates, the true amphibians. Because the cerebellum provides a major input to the red nucleus via the crossed cerebellorubral pathway, it is likely that much of the evolution of the cerebellum and the emergence of cerebellospinal fibers occurred in concert with

the emergence of a differentiated red nucleus and rubrospinal tract.

3.28.2.4 The Mammalian Augmentation

Table 1 shows that telencephalic cell groups project to the spinal cord in amphibians, mammals, and possibly reptiles (Bruce *et al.*, 1980; ten Donkelaar *et al.*, 1981; Nudo and Masterton, 1988). However, the telencephalospinal neurons identified in amphibians and reptiles are located in the basal telencephalon, possibly homologues of amygdalospinal neurons of mammals (Nudo and Masterton, 1988). No spinal cord projections have been found in pallial (cortical gray matter) structures in any vertebrate species except for mammals. Therefore, in the lineage leading to mammals, it appears that the vertebrate common plan again was augmented with the addition of fibers originating in neocortex. Of course, this unique and possibly sole major addition to the mammalian lineage, a true corticospinal tract (CST), became more massive than any other descending pathway in some mammals.

In parallel with the enlargement of the CST, it has been suggested that the rubrospinal tract became reduced in size in the anthropoid lineage (Nathan and Smith, 1955, 1982). This would seem to contradict the consistent finding of rubrospinal neurons except in certain limbless jawed vertebrates. Though it has been rather widely accepted that the rubrospinal tract assumed a more modest role in the human lineage, comparative analysis suggests otherwise. When subjected to strict statistical tests for a variety of old and new hypotheses regarding the evolution of the rubrospinal tract and its adaptation to particular ecological niches, the number of rubrospinal neurons appears to be related to both body size and brain size of the host, with the relationship with brain size the dominant feature. Furthermore, the size of the tract was probably increased radically over the evolution of the carnivores (lineage leading to raccoons and cats) but not over the evolution of primates or rodents (lineage leading to tree squirrels), whether or not concomitant changes in brain size are held constant. Despite previous conclusions to the contrary, the number of rubrospinal neurons is positively, not negatively related to the number of corticospinal (CS) neurons (Masterton *et al.*, 1989).

3.28.2.5 Summary

Comparison of the major descending spinal pathways in vertebrate classes suggests that many of the pathways found in mammals can be traced to even more distant, premammalian ancestry, with several possibly as old as the entire vertebrate subphylum. The phylogenetic history of direct descending projections from brain to spinal cord along the ancestral lineage leading to mammals seems to have been marked by a series of widely spaced, steplike augmentations of the previous complement of descending tracts. The appearance of rubrospinal neurons coincides with the radiation of jawed vertebrates, but its presence is variable and is probably related to the presence of appendages. The appearance of cerebellospinal neurons coincides with the radiation of tetrapods, and seems to have been maintained in subsequent lineages. Finally, the appearance of CS neurons coincides with the radiation of mammals and the emergence of neocortex. The further differentiation of the CST in the mammalian lineage leading to humans is reviewed in a later section.

3.28.3 Emergence and Differentiation of Motor Cortex

3.28.3.1 Forebrain Motor Systems of Reptiles

Because a six-layered neocortex emerged in early mammals, there is no true homologue for mammalian motor cortex in nonmammalian species. Reptiles possess a telencephalic structure that is part of a neural circuit involved in the modulation of movement by sensory information. This structure, the anterior dorsal ventricular ridge (ADVR), receives information from the visual, auditory, and somatosensory systems and projects to the striatum, which sends efferents to descending pathways that modulate activity of motoneurons (Ulinski, 1983).

Early stimulation studies reported a motor area in dorsal cortex of turtles (*Chelydra serpentina*) in the rostrolateral subdivision or pallial thickening (Johnston, 1916). Due to its topographic position, the investigator concluded that this area represents a motor cortical area corresponding to motor cortex in the mammalian brain. Others reported a similar motor area in dorsal cortex of alligators (Bagley and Langworthy, 1926). Still others claimed to elicit motor responses in reptiles only when the striatum (most likely ADVR) was stimulated (Goldby, 1937; Koppanyi and Pearcy, 1925; Schapiro and Goodman, 1969). These early stimulation studies of the telencephalon in reptiles most likely evoked motor responses due to current spread to subcortical areas and the meninges (Peterson, 1980). Thus, to date, studies of the telencephalon in reptiles suggest

that there is no motor area in the dorsal cortex comparable to motor cortex in mammals.

3.28.3.2 Motor Cortex in Neurologically Primitive Mammals and the Concept of the Sensorimotor Amalgam

The somatosensory-motor cortex of most mammals (e.g., primates) is not an undifferentiated mass, but is comprised of at least two distinguishable somatotopically organized areas (Woolsey, 1958). According to Woolsey, although each of these areas displays both sensory and motor characteristics, one modality predominates. Woolsey introduced the term 'somatic sensory-motor area I', or SmI, to describe the postcentral, predominantly somatosensory area of primates (and its homologues in nonprimates), and the term 'somatic motor-sensory area I', or MsI, to describe the precentral, predominantly motor area of primates (and its homologues). During the evolutionary development of somatosensory-motor cortex, clear-cut changes seem to have taken place in the cytoarchitecture, input, and output of MsI, resulting in a separate motor area. But did this separation emerge by progressive differentiation of SmI, or did a true motor cortex emerge *de novo*, adding yet another augmentation to the previous complement of descending spinal cord pathways? Studies of neurologically primitive mammals still extant may hold clues to the emergence of early motor cortex.

Endocasts of skulls in the fossil record reveal that the brains of early mammals had a small volume of lissencephalic neocortex, similar to a number of small-brained species of extant Didelphid marsupials (Jerison, 1990). The family of Didelphid opossums has long been thought to have retained the basic brain structure approximating a form that can serve as a starting point for tracing the evolution of modern mammalian neural organization, whether marsupial or placental (Smith, 1910; Loo, 1930; Edinger, 1948; Simpson, 1949, 1959; Olsen, 1959; Frost and Masterton, 1992; Frost *et al.*, 2000).

The basic scheme of a progressively differentiating somatosensory and motor cortex in mammals was proposed at least by the 1940s. In 1945, von Bonin stated: "It is of the essence of cortical organization that sensory and motor areas become divorced more and more from each other – pulled further apart as it were – as evolution proceeds" (von Bonin, 1945).

Studies using cortical surface recording and stimulation in Didelphid opossums (*Didelphis virginiana*) conducted in the early 1960s by Richard Lende seemed to provide support for this notion. Lende referred to the undifferentiated cortical area as a sensorimotor amalgam (Lende, 1963a, 1963b, 1964) based on a complete motor map of the body superimposed on a complete somatosensory map. Similar results were found in *Didelphis azarae*. In both studies, the amalgam was quite large (perhaps due to the use of surface recording and stimulation techniques), encompassing the complete length of the parietal region.

Based strictly on electrophysiological grounds, however, it is now clear that the opossum's sensorimotor amalgam is not motor cortex in the same sense as it is in other mammals, such as primates. To begin with, the somatosensory cortex of primates is not without some motor properties. Evoked motor responses from electrical stimulation in primary somatosensory cortex (S1) of eutherian mammals resemble those evoked in primary motor cortex (M1), with the exception that stimulation thresholds are significantly higher (Welker *et al.*, 1957; Woolsey, 1958; Doetsch and Gardner, 1972; Sessle and Wiesendanger, 1982). In stimulation studies of cortex in opossums (Lende, 1963a; Beck *et al.*, 1996; Frost *et al.*, 2000), movements were most often evoked using relatively high stimulating currents compared to those typically observed using microelectrode stimulation in layer V of M1 in primates (Sessle and Wiesendanger, 1982; Nudo *et al.*, 1992; Stepniewska *et al.*, 1993; Nudo and Milliken, 1996; see figures 12 and 14 in Frost *et al.*, 2000, and figure 9 in Nudo *et al.*, 1996). This disparity in current thresholds for evoked movements suggests that the electrically evoked motor responses observed in opossums are representative of the motor component of somatosensory cortex found in eutherian mammals.

Further, even after M1 is extirpated in primates, electrical stimulation of somatosensory cortex produces movements in skeletal musculature. Therefore, a motor component in the opossum's somatosensory-motor area is not sufficient grounds to consider it motor cortex. Finally, the cytoarchitecture and myeloarchitecture of the motor response area in Sm1 of Didelphids also suggests that this is a sensory area rather than a true motor cortex. This area has a notable granular layer (layer IV) and does not have the agranular appearance characteristic of true motor cortex (Walsh and Ebner, 1970; Frost *et al.*, 2000). The sensorimotor amalgam of opossum cortex is probably no more motor than the somatosensory cortex of, say, monkey. It is probably safe to suggest that in opossum cortex specifically, and primitive cortex generally, there is no true motor cortex. If this is the case, and if opossum provides a reasonable representation of early neocortical organization, then motor cortex must have arisen anew sometime later in evolution.

3.28.3.3 Emergence of a True Motor Cortex

In eutherian species, M1 (i.e., MsI) is situated rostral to S1 (i.e., SmI) and has a parallel somatotopic organization, though modern microstimulation studies (e.g., Gould *et al.*, 1986) have revealed that the motor map is organized in a fractionated mosaic distribution with respect to evoked joint movements, in contrast to the relatively precise topography of receptive field organization in somatosensory cortex. The neocortex of two extant marsupials, the North American opossum (*D. virginiana*) and the South American gray short-tailed opossum (*Monodelphis domestica*) have been studied extensively in order to determine if any evidence of a primordial motor cortex could be found rostral to S1. These more recent studies employed microelectrode stimulation and recording techniques, allowing much greater spatial resolution of somatosensory and motor maps than afforded by the surface stimulation and recording techniques used in the early 1960s. Examination of *Didelphis* cortex using microelectrode techniques revealed that movements can be evoked from several sites within, and some sites just rostral to S1 (Beck *et al.*, 1996). Stimulation most often resulted in movements of the tongue, though evoked movements of body parts, including several forelimb movements, were also seen. Together with the evidence that most CS fibers originate from S1 in *Didelphis*, and very few originate from areas rostral to S1, the results so far seem to favor the idea that *Didelphis* cortex contains S1, but not a true motor cortex.

The gray, short-tailed opossum (*M. domestica)* may possibly represent an even earlier stage of somatosensory-motor differentiation. In this species, movements can be evoked from a large area coextensive with the somatosensory representation, as was found for *Didelphis* (Frost *et al.*, 2000). However, unlike *Didelphis*, in which orofacial as well as proximal and distal forelimb movements were evoked, electrical stimulation in *Monodelphis* evoked movements restricted almost exclusively to the vibrissae, and to a lesser extent, jaw (see Somatosensory Specializations in the Nervous Systems of Manatees). In this study, three modes of stimulation were used:

1. intracortical microstimulation (750 kΩ electrode impedance) in layer V;
2. low-impedance depth stimulation (100–200 kΩ electrode impedance) in layer V; and
3. bipolar surface stimulation.

Results were qualitatively similar using the different techniques, though the excitable area increased progressively with methods 2 and 3 (Frost *et al.*, 2000). No motor representation of body parts below the level of the head was found in S1 or in more rostral areas.

These results suggest that evolutionary pressures for development of motor components in cerebral cortex may have begun long before the emergence of a true, differentiated motor cortex, possibly due to a need for more refined movements of the face (Frost *et al.*, 2000). This putative selective pressure may have resulted in an early substrate for mediating movements of vibrissae, long before sufficient CS pathways were in place to mediate direct control of the forelimbs and hindlimbs, and long before a separate motor representation rostral to S1 was established (Figure 1). In this regard, it is important to note that *Monodelphis* contains almost 20 times fewer CS neurons than *Didelphis*, and nearly 40 times fewer than rat (Nudo *et al.*, 1995). Thus, S1 of *Monodelphis* may represent a primordial condition in which a complete somatosensory map has been achieved and retained, but the motor components of S1 have not yet fully developed. In this primitive condition, the anatomical substrate required for cortical control of movements below the level of the face is not yet present.

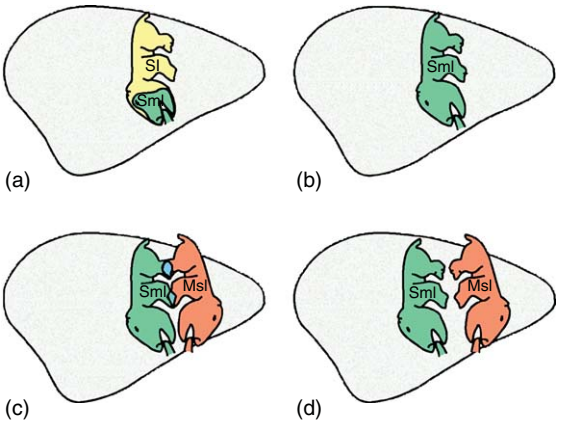

Figure 1 Hypothetical model of evolutionary divergence in sensorimotor cortex. a, Somatomotor representation in the earliest mammals, represented by *Monodelphis*. A complete somatosensory (SI) representation exists. An incomplete motor representation is congruent with the sensory representation of the face (SmI). b, A complete motor representation exists and overlaps the somatosensory representation (SmI). As noted by Lende, this sensorimotor amalgam is not unlike S1 of placental mammals. A reversed map characteristic of true motor cortex is absent. c, True motor cortex has emerged, as evident from a reversed motor representation (MsI) rostral to the somatosensory representation (SmI). MsI is largely segregated from SmI, though a partial overlap exists in the hindlimb and forelimb areas. d, In primates, MsI is completely segregated from SmI. Somatosensory cortex retains some motor properties, and motor cortex retains some somatosensory properties.

Based on these and other studies, it is thought that in opossums, motor functions at the level of the cerebral cortex, if they exist, are mediated by motor components of somatosensory cortex. Further, the motor map in S1 may have developed in stages, as it is restricted to the face, or to the face and upper extremity in *Monodelphis* and *Didelphis*, respectively. The cortical organization in monotremes, however, suggests that sensorimotor differentiation may not have followed a simple linear progression. For example, using surface stimulation techniques in the prototherian echidna (*Tachyglossus aculeatus*), Lende (1964) found a complete motor representation overlapping the somatosensory representation. Using surface stimulation techniques in the platypus (*Ornithorhynchus anatinus*), Bohringer and Rowe (1977) found a partial overlap of the motor and somatosensory representations. Electrical stimulation evoked movements of the bill and forelimb, but not the hindlimb. Thus, it is possible that the motor component of S1 is more complete in monotremes than in *Monodelphis* opossums.

Although motor components in S1 have been found in all mammals studied to date, the issue of whether a separate primordial motor cortex rostral to S1 exists in neurologically primitive mammals is not yet clear. In early surface stimulation studies in echidna (Abbie, 1938; Goldby, 1939) evoked movements were observed in an area rostral to S1. Surface stimulation studies in platypus also resulted in evoked movements rostral to S1, including representations of the forelimb. But the area immediately rostral to S1 in monotremes (rostral field or field R) contains neurons responsive to stimulation of deep receptors (Krubitzer *et al.*, 1995), similar to neurons in area 3a of eutherian mammals. Area 3a is considered to be a transition zone between area 3b of S1 and area 4 (M1). In eutherian mammals, movements can be evoked by microelectrode stimulation in area 3a using relatively low current levels. This raises the possibility that area 3a became differentiated from S1 prior to the emergence of a true motor cortex. Furthermore, based on electrophysiological and cytoarchitectonic criteria, a separate cortical area (the manipulation field, or field M) has been identified in both echidna and platypus (Krubitzer *et al.*, 1995). As field M shares some cytoarchitectonic similarities with M1, it is possible that this area is a homologue of M1 in eutherian mammals (Krubitzer *et al.*, 1995). However, at this point it is also possible that field M is a unique specialization in extant monotremes. Clearly, additional studies of its physiology and anatomy are needed to resolve this issue.

These findings of a possible M1 homologue rostral to S1 in monotremes suggest that the antecedents to a true motor cortex may have existed very early after the emergence of mammals. As evolutionary pressures for specialized motor functions (e.g., manual dexterity) grew, augmentations of the CST from a primitive to a more advanced pathway seem to have paralleled the origin of a true motor cortex.

It is not entirely clear when true motor cortex might have emerged. But it seems that somatosensory-motor differentiation occurred independently in several orders, and not just in primates. For example, a true motor cortex, as evidenced by a reversed motor representation rostral to the somatosensory representation and by movements evoked at relatively low microelectrode stimulating currents, is apparent in rats (Donoghue and Wise, 1982). Motor cortical fields have been localized to frontal cortex in other rodent species as well (Woolsey *et al.*, 1952; Hall and Lindholm, 1974). A separate and distinct topographic pattern comprising M1 in the rat forms a rough mirror image of the S1 representation, with the hindlimb caudomedial, the face rostrolateral, and the proximal and axial representations rostromedial (Neafsey *et al.*, 1986; Neafsey and Sievert, 1982). In rats, the separation of S1 and M1 is not complete, since some overlap has been demonstrated over most of the hindlimb representation and part of the forelimb representation (Hall and Lindholm, 1974; Donoghue and Wise, 1982; Sanderson *et al.*, 1983). This area of overlap has features similar to both S1 and M1 cortex. The overlapping hindlimb area receives a convergence of thalamic projections from the ventrolateral nucleus and the ventrobasal complex, whereas in nonoverlapping areas these projections are segregated (Donoghue *et al.*, 1979). Regardless of the partial overlap in rats, it appears that a true, separate motor area, distinct and separate from the S1 cortex exists in rodents, primates, and carnivores.

3.28.4 Evolution of the CST

The CST places the neocortex in direct neural contact with the spinal cord. The existence of direct synaptic connections between cortical neurons and spinal motoneurons in some eutherian mammals (including many primates) has been the structural basis for implicating the CST in a variety of motor functions, such as control of digital dexterity, conditioned movements, flexor activity, muscle tonus, and volitional movements (Lawrence and Kuypers, 1968; Beck and Chambers, 1970).

Since rather strong structure–function relationships for the CST can be advanced in primates, including humans, it has been tempting to presume that its function is the same in all mammals. However, it is now becoming apparent that because the morphology of the CST is so varied across mammals (in size, spinal course, manner of termination, etc.), the motor functions ascribed to the CST of primates probably cannot be extended *in toto* to other orders. Likewise, it cannot be assumed that the CST subserves only a motor function. It is also thought to be involved in descending control of afferent inputs, modulation of spinal reflexes, and trophic functions, to name a few (Lemon and Griffiths, 2005).

Furthermore, since its morphology is so varied, it often has been assumed that the CST arose more than once and possibly many times during the phylogeny of mammals (e.g., see Goldby, 1939; Noback and Shriver, 1969). More specifically, it has been suggested that the tract emerged independently within each mammalian order. In this view, any similarity in the tract across orders (such as its common origins in layer V of somatosensory-motor cortex) would be regarded as a consequence of parallel or convergent evolution and any similarity in function would be regarded as coincidental.

The present review attempts to reconstruct the morphological changes that have occurred in the CST during the course of mammalian evolution. Briefly, it is argued that the tract arose initially as collaterals of a phylogenetically older, corticobulbar tract (CBT), which itself includes part of the descending somatosensory system. Further, it is argued that these corticobulbar collaterals penetrated the cord after the major mammalian orders had diverged. Therefore, CSTs cannot, in principle, be traced to a single, common mammalian ancestor, but they can be traced to common corticofugal pathways that had their own origins early in mammalian evolution.

3.28.4.1 Variation in the Trajectory of CS Fibers in the Medullary Pyramids

Many pyramidal tract fibers terminate in the medulla before reaching the cord. However, many aspects of the pyramidal tract's morphology have been examined in a great number of species. Thus, to the extent that the CS fibers constitute a significant fraction of pyramidal fibers, a brief comparative analysis of the morphology of the pyramidal tract is not without interest.

It was first noted by Clarke in 1858 (Wiesendanger, 1981) and Spitzka in 1879 (Spitzka, 1879, 1886) that the pyramidal tract varies in size across mammals and that the anthropoid apes possess some of the largest pyramidal tracts. It is clear that absolute size of the tract, the number of fibers, the size of the largest fibers, and the average fiber size each increases in a phyletic series leading to humans. However, when body weight is held constant, all but one of these correlations collapses. The phyletic level remains significantly correlated only with the cross-sectional area of the pyramidal tract (Heffner and Masterton, 1983).

It is generally acknowledged that in most mammals, 90–95% of the CS fibers cross in the pyramidal decussation before entering the cord. But the exact location of the pyramidal decussation is not invariant. In the vast majority of mammals studied so far, the pyramidal decussation occurs just above the medulla–spinal junction. However, in at least one species of bat, and possibly in the anteater, the pyramidal fibers cross earlier, in the rostral medulla (Fuse, 1926; Broere, 1966); in at least two other species (pangolin, echidna), the pyramidal fibers seem to cross in the pons (Goldby, 1939; Chang, 1944); and in three other species (hyrax, mole, and hedgehog), no decussation has been observed at all (Linowiecki, 1914; Verhaart, 1967; Kunzle and Lotter, 1996). Finally, variation in the pyramidal decussation in humans has been frequently reported. Clearly, more information is needed on the consistencies and variation in this important aspect of pyramidal tract morphology.

3.28.4.2 Variation in the Trajectory of CS Fibers in the Spinal Cord

If the CST were homologous across mammalian orders, one might expect it to descend through the spinal cord in the same funicular pathway in each order. In contrast, CS fibers descend through the spinal cord in any of six different funiculi in mammalian species. These include the dorsolateral (sometimes called the lateral tract), dorsomedial (sometimes called the dorsal tract), and ventral funiculi (Armand, 1982). Further, CS fibers can travel in either side of the cord, i.e., contralateral or ipsilateral to the cells of origin in layer V of the cerebral cortex (Figure 2).

In an individual species, there is usually more than one CST. The principal tract (sometimes called the main tract) is usually defined as the one larger in area, containing more and larger fibers, and terminating in more caudal levels than the others. When the principal CSTs are arranged according to their funicular trajectories on a cladogram of mammalian

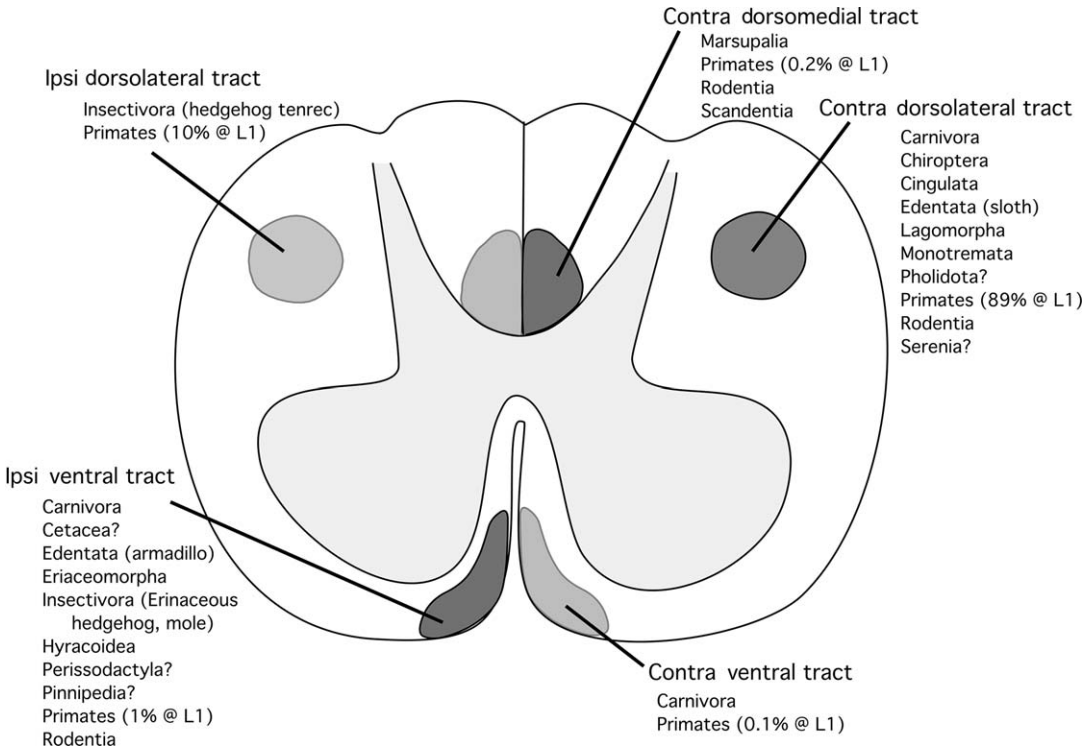

Figure 2 Location of CST in the spinal cord of mammals. All mammals studied to date possess CS neurons. The CST can travel in any one of six spinal cord funiculi: The dorsolateral, dorsomedial, or ventral funiculi, and either on the ipsilateral or contralateral side with respect to the cells of origin in layer V of cerebral cortex.

orders (based on other paleontological and comparative data), they do not appear to group into any other obvious subsets (Figure 3). This marked variation in the spinal location of the CST among orders of mammals has been the major point of reference for establishing the tract's early phylogeny. Based entirely on this variation in funicular trajectory among orders and the equally marked lack of variation within orders, it has been suggested that the CST may have penetrated the cord (and thus, arisen independently) within each mammalian order (Goldby, 1939; Noback and Shriver, 1966b).

Based upon the funicular trajectory of its principal tract then, the CST can be traced to a common ancestry within each mammalian order, but not to any higher-level taxa or more remote ancestry. Consequently, up to this point one may tentatively conclude that the majority of CS fibers entered the cord only after the various orders of mammals had diverged. This consistency within orders has not always been apparent because of changes in the taxonomy of the mammalian orders. For example, tree shrews, which possess a dorsomedial principal tract, were once classified as primates that possess a dorsolateral principal tract. Also, rabbits, now classified as lagomorphs, possess a dorsolateral principal tract, but were once classified as rodents, which possess a dorsomedial principal tract. It is of interest here that in the continuing debate concerning the taxonomy and evolutionary origin of tree shrews, the funicular location of the CST has been proposed as a criterion of classification. For more information, see review by Haines and Swindler (1972). This idea gains some further support by the observation that in some neurologically primitive mammals, relatively few CS fibers extend past upper cervical levels of the spinal cord. For example, in the short-tailed opossum, only 500 CS neurons have been identified (Nudo *et al.*, 1995); in the hedgehog tenrec, only 600 CS neurons have been identified (Kunzle and Rehkamper, 1992).

Until the late 1970s, the majority of studies examining the course and termination of the CST relied on either degeneration or autoradiographic techniques that have been superceded by more sensitive tract-tracing methods such as injection of wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) or more recently, biotinylated dextran amine (BDA). The older techniques were quite adequate for identifying the principal CST, and thus, the early findings have largely been replicated numerous times. However,

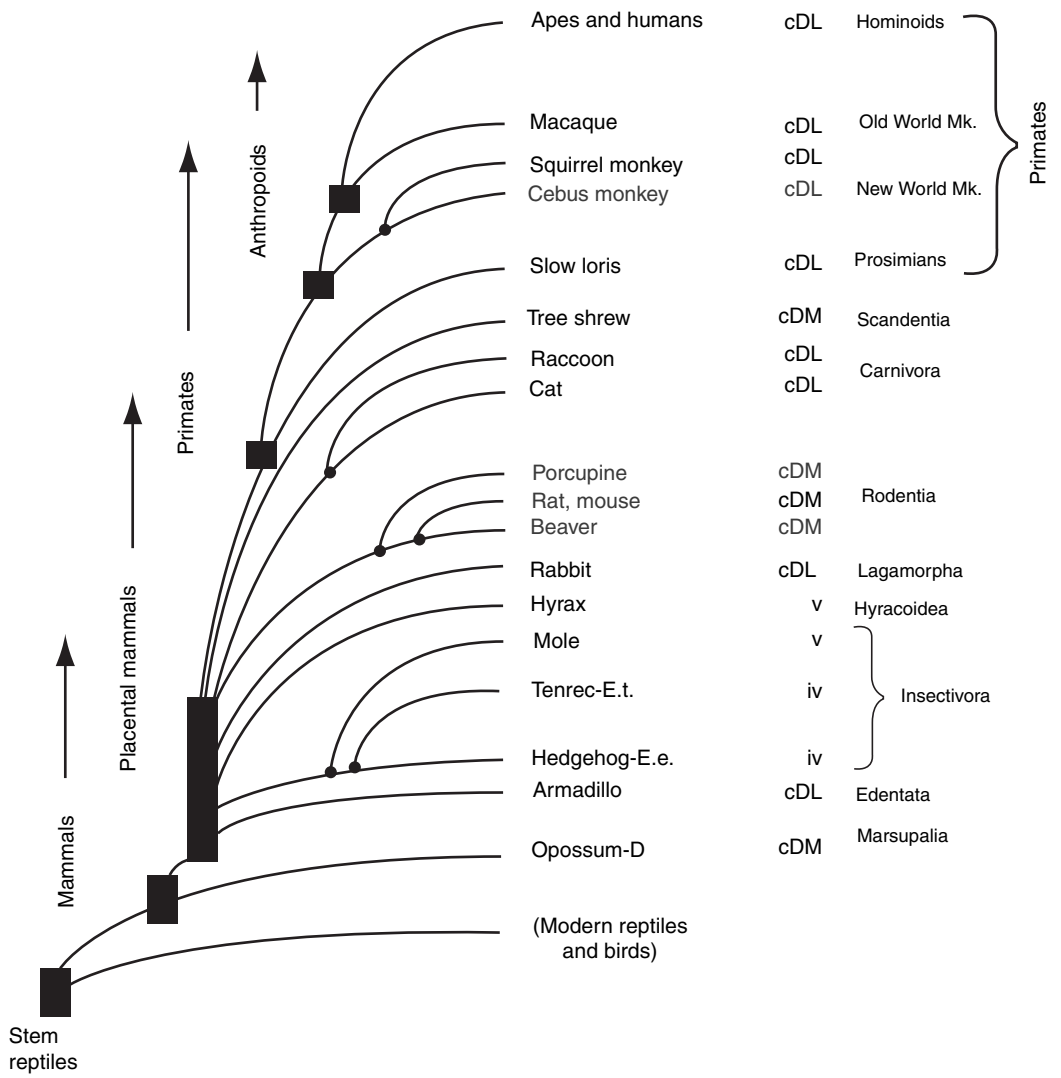

Figure 3 Principal CSTs in mammalian orders. Species are arranged according to progressively more recent common ancestry with apes and humans. Whereas the trajectory of the principal CSTs are the same within mammalian orders, they differ between orders. This has historically been the primary evidence to suggest that the CST emerged independently within each mammalian order. DL, dorsolateral funiculus; DM, dorsomedial funiculus; V, ventral funiculus; c, contralateral (to cell bodies of origin); I, ipsilateral (to cell bodies of origin).

as the more sensitive tracers have been employed, it has become evident that CS fibers travel in multiple funiculi in the same species. To date, while CS fibers have been examined using the modern, sensitive tract-tracers in only a few species (cat, macaque monkey, mouse, rat, hedgehog tenrec), at least some CS fibers travel in the dorsolateral funiculus (contralateral and ipsilateral), the ventral funiculus (ipsilateral), and the dorsomedial funiculus (contralateral) in each of these species (Table 2) (Satomi *et al.*, 1989; Kunzle and Lotter, 1996; Brosamle and Schwab, 1997; Lacroix *et al.*, 2004; Steward *et al.*, 2004). In cat, CS fibers have been described in all six spinal funiculi (Satomi *et al.*, 1989).

These recent results demonstrating common trajectories across orders of secondary CSTs suggest that at least some CS fibers in one or more spinal funiculi may be homologous. If so, the number of such fibers is likely to be very low indeed, based on results in *Monodelphis* opossum and the hedgehog tenrec. Thus, what we now call the principal CST very likely is a more recent specialization within each mammalian order that resulted from augmentation of the primordial CST. Because the bulk of the CST probably emerged after the radiation of mammalian orders, its funicular trajectory is probably of little value in determining its early origins in primordial mammals. However, the variation in the location of the principal CST in the various orders,

Table 2 Location of CS fibers in mammalian orders

		Funicular trajectory[a]		
Order	*Species (common name)*	*DM(c/i)*	*DL(c/i)*	*V(c/i)*
Artiodactyla	Goat		+	
Carnivora	Cat; raccoon	+/+	++/+	+/+
Chiroptera	Bat		++	
Edentata	Armadillo		++	
Hyracoidea	Hyrax			++
Insectivora	Mole, hedgehog	–/–	?/?	?/+
Lagamorpha	Rabbit		++	+
Marsupalia	Opossum, wallaby, potorou, kangaroo	++	+	
Monotremata	Echidna		++	
Pholidota	Pangolin		++	
Primates	Slow loris, bush baby, macaque, Cebus monkey, squirrel monkey	+/–	++/+	+/+
Rodentia	Porcupine, guinea pig, gopher, hamster, beaver, mouse, woodchuck, coypu rat, common rat	++/+	+/+	–/+
Scandentia	Tree shrew	++	+	+

[a]A fourth location of CS fibers, the intracommissural bundle, has been identified, though not with modern tract-tracing techniques. They will not be described further here.
++, Substantial number of fibers; +, relatively few fibers; ?, current data equivocal; DM, dorsomedial funiculus; DL, dorsolateral funiculus; V, ventral funiculus; c, contralateral; i, ipsilateral.

and the consistency within orders remains an intriguing clue that may yet provide a unique perspective with regard to the function of the CST.

3.28.4.3 Evolutionary Origins of CS Fibers: The Caudal Extension of the CBT

Although CS fibers appear to have entered the cord independently in each mammalian order, it is unlikely that they appeared, throughout their extent from cortex to cord, *de novo*. In seeking a more likely, more gradual origin, the most reasonable first guess is the CBT. This tract has a similar cortical origin and a similar trajectory within the brainstem as the CST. It is dissimilar only in its extent down the neuraxis. That is, regardless of order, both the CST and CBT descend in the internal capsule, cerebral peduncle, and pyramid before entering the cord (as the CST) or arborizing in the pons/medulla (as the CBT). Further, the CST and several of the CBTs originate in some of the same cortical areas (areas 4, 3, 1, and 2) (Wiesendanger and Wiesendanger, 1982).

Therefore, it is not unreasonable to suppose that CS fibers made their way into the spinal cord via the already established capsular, peduncular, and pyramidal tracts previously ending in the medulla, and it is not unlikely that they emerged first as collaterals of this phylogenetically older pathway to the medulla. This notion, shared by other investigators (Noback and Shriver, 1966a), has received support from the demonstration that many CS neurons send collaterals to dorsal column nuclei (Rustioni and Hayes, 1981). From an evolutionary viewpoint, however, the CS fibers are probably the collaterals of the older corticobulbar axons ending in the dorsal column nuclei, not vice versa.

3.28.4.4 Termination of CS Neurons

As would be expected from its multiple origins, the termination pattern of CS fibers (that is, where the CS fibers end within the spinal grey) varies greatly among mammalian orders. But perhaps unexpectedly, it also often varies within orders. This variation in termination pattern, along with the relationship to variations in function, have been discussed by Heffner and Masterton (1975) and will only be reviewed briefly here.

Two major points can be made concerning the termination of CS fibers. First, the caudal extent of the tract varies from cervical levels in goat and hedgehog to sacral (and even coccygeal) levels in primates, rodents, and some carnivores. Second, the fibers terminate in many spinal grey laminae. On one hand, regardless of order, all mammals are alike in that most CS fibers terminate at the base of the dorsal horn (lamina V–VI) at each spinal level. On the other hand, both among orders and among families within orders, the ventral (or anterior) limit of the CS terminations within the spinal cord grey varies widely. Although in many mammals, CS terminals reach no more ventral than lamina VI (e.g., goat, rabbit, marmoset, wallaby), in others (raccoon and most primates), they extend to both medial and lateral motoneurons in lamina IX.

Even though it appears that CS fibers penetrated the cord only after the orders diverged, certain trends in the termination patterns of the CST can be observed in a phyletic series across orders known to have successive (as opposed to sequential) origin (Heffner and Masterton, 1983). These trends are especially evident in a phyletic series of extant mammals having successive propinquity with humans. If CS termination parameters are plotted in such a series (with body weight constant), it is clear that with successive grades, first the CST reaches more caudal levels of the spinal cord, and second, at each cord level, the CST innervates more ventral laminae, eventually establishing direct contact with spinal motoneurons.

3.28.4.5 Cells of Origin of the CST

The search for the cortical cells originating the CST has a long history. Over the past century, as soon as a new neuroanatomical technique had been developed, it had been applied to this tract. Early in the history of its study, around 1830, Gall and Spatzheim recognized (by gross dissection) that the pyramidal fibers had their origins in cortex, but it was not until 1851 that Turck realized that many of these same fibers enter the spinal cord (Lassek, 1954; Clark and Dewhurst, 1974). When the Marchi technique for staining degenerating myelin was developed, electrical stimulation of the cortex guided investigators to the most probable sites for extirpation. For the next century, until the advent of modern neuronal tract-tracing techniques, this general procedure of electrical stimulation, extirpation, and the subsequent study of degenerated myelin, axons, or terminals (i.e., the Nauta–Gygax and Fink–Heimer techniques) yielded most of the knowledge concerning the origins of the CST.

However, the anterograde degeneration method can only reveal the general areas of cortex-originating pyramidal or CS axons. The exact cells of origin were still unknown by the turn of the century. At the time, it was suggested that large pyramidal cells (Betz cells) in the area gigantopyramidalis give rise to the CST. Although this idea has proved to be true, at the time it was erroneously thought that these were the only cells originating CS neurons. This incorrect notion was reinforced by retrograde degeneration after spinal hemisection (Holmes and May, 1909). Chromatolytic changes in Betz cells were observed but, once more, it was concluded erroneously that the CST originated exclusively from them.

Despite the powerful influence of Holmes and Page May's study over the next quarter century, some doubts were beginning to be cast on the notion that CS fibers arise strictly from Betz cells. By the 1930s, the anterograde degeneration method had revealed that the cortical origins of the CST were much more widespread than was previously thought (Kennard, 1935). The advent of precise electroanatomical techniques in the 1940s enabled investigators to locate pyramidal tract cells by electrically stimulating the tract on the ventral surface of the medulla and recording from antidromically stimulated cells in the cortex (Lance and Manning, 1954). Later, antidromically identified pyramidal tract neurons were injected with dye so that they could be visualized in cortical layer V of area 4 (Batuev and Lenkov, 1973).

However, the electroanatomical techniques hold one problem in common with the retrograde chromatolytic technique: large neurons are overrepresented. Nevertheless, it is clear that the CS area defined by either antidromic activation or by retrograde chromotolysis is coextensive with the area defined by anterograde degeneration techniques. But by the mid-1970s a detailed picture of the distribution of the cells originating CS axons had yet to be realized.

Precise information concerning the cells originating CS axons was obtained beginning in the mid-1970s based on the retrograde transport of horseradish peroxidase and other materials. This technique was first applied to the CST by Catsman-Berrevoets and Kuypers in 1975 (Catsman-Berrevoets and Kuypers, 1976). Based on retrograde tract-tracing studies over the past few decades, considerable anatomical information is now available regarding the morphology of neurons originating the CST in a wide variety of mammalian species (Nudo and Masterton, 1990a, 1990b; Nudo *et al.*, 1995). Several general features have emerged:

1. All CS neurons reside in cortical layer V. No exception to this sweeping generalization has yet been seen.
2. CS neurons are most concentrated in, but not confined to area 4.
3. Though less heavily concentrated, a very large number of CS neurons reside outside of area 4, especially in the somatosensory areas 3, 1, and 2 and in areas 5 and 6. This extent represents a far wider distribution than shown by earlier techniques, especially electrical stimulation, antidromic activation, or retrograde chromatolysis.
4. The giant Betz cells are neither the sole nor predominant originators of CS axons. In fact, Betz cells probably account for no more than about 3% of all pyramidal tract fibers. Pyramidal cells of many sizes contribute axons to the CST.

Aside from these sweeping generalizations, the morphology and distribution of CS neurons varies considerably. With regard to their distribution, there does appear to be some consistency across mammalian orders in that either two or three separate broad regions of neocortex originating CS fibers in all mammalian species studied. These regions are large but relatively well demarcated and are stable both in absolute and relative location on the cortical surface in all orders examined.

One broad region (region A in Nudo and Masterton, 1990a) contains nearly 90% of CS neurons. This region comprises primary somatosensory and motor cortex, as well as CS neurons in the dorsal premotor cortex, supplementary motor area, cingulate motor areas (at least in primates), and scattered cells in the parietal and frontal cortex. A second broad region (region B) comprises CS neurons in the second somatosensory area and related somatosensory areas of the parietal cortex. These two broad regions of CS neurons have been identified in every mammalian species studied to date, and thus can theoretically be traced to a common mammalian ancestor.

A third region of CS neurons is clustered in an area most likely corresponding to the primate ventral premotor cortex (region C). This group of cells appears to be uniquely present in all primate species studied to date, but in representatives of no other mammalian order. Another region (region C′) is present in all rodents studied as well as rabbit, but in no other mammalian species. This region, corresponding to the rostral forelimb area (RFA) of rats, will be discussed in a later section.

The total number of CS neurons varies considerably across mammalian species, with the lowest numbers in many insectivores and *Monodelphis* opossum, and the highest numbers in most primates and carnivores (Nudo *et al.*, 1995). As expected, body size and brain size are significant co-variates in the total number of CS neurons and in the amount of cortex devoted to the CST. However, even when body weight is held statistically constant, the number of CS neurons increases in the anthropoid ancestral lineage. Other characteristics of CS neurons also appear to have changed significantly in the anthropoid lineage, including an increase in average soma diameter and a decrease in volume density and concentration. Thus, with more recent common ancestry with humans, it appears that CS neurons became larger, more numerous, and less concentrated. This latter finding is interesting as it implies that the region of cortex-originating CS fibers added more non-CS neurons and more neuropil. Perhaps one of the unique attributes of primate motor cortex is the expansion of intracortical connectivity of regions originating CS neurons.

3.28.5 Specialization of Motor Areas in Primates

3.28.5.1 Criteria for Differentiation

It is generally accepted that no single feature is sufficient for characterizing an area as a distinct region. Features used to define cortical motor fields include its cytoarchitectonics, pattern of afferent and efferent connections, features of intrinsic connectivity, chemoarchitectonics, behavioral effects of ablation, and, particularly for motor cortical areas, the ability to elicit movements upon electrical stimulation.

A differentiated motor field has a unique cytoarchitecture, traditionally defined by stains for Nissl bodies and myelin. Additionally, areas have been examined and characterized based on cytochrome oxidase staining, acetylcholinesterase staining, neurofilament antibody staining, and receptor binding. Unique characteristics of cell types, laminar organization, cell density, fiber density, and various staining densities are all used for characterization. Extensive tract-tracing studies have been used to identify subareas of motor cortex based on differential afferent and efferent connections with the thalamus, basal ganglia, and other cortical areas, as well as their projections to the spinal cord. Intracortical microstimulation mapping procedures have been used to define somatotopic organization within each secondary motor area, with attention paid to minimal threshold requirements for initiation of movements, as well as the characterization of movements themselves. Finally, secondary motor areas have been characterized based on functional differences in ablation-behavior studies in nonhuman primates and functional imaging studies in humans.

3.28.5.2 Secondary Motor Areas in Primates

In addition to a primary motor area (M1 or area 4), there are several secondary motor areas recognized in the primate cortex (Kaas, 2004). These areas have been defined as having direct connections to both M1 and to the spinal cord. The premotor cortex, the supplementary motor area and the cingulate motor cortex have been identified in all primate species examined, including prosimian primates. Each of these secondary areas has been divided into subareas based on differences in cortical architecture that are related to hodological and functional differences. The lateral premotor area is divided into ventral and dorsal areas (PMv

and PMd, respectively), the supplementary motor area (SMA) into SMA-proper and pre-SMA, and the cingulate motor area has been subdivided into rostral (CMAr) and caudal (CMAc) divisions.

Neurologically more primitive primates represented by the prosimian bush baby (*Galago garnetti*) have a well-differentiated M1, with representation of the trunk, hindlimb, and face and a large forelimb representation, although there is little control of individual digit movements (Kanagasuntherum and Leong, 1966; see figures 1–5 in Wu *et al.*, 2000). In addition to M1, galagos possess most of the secondary motor areas that have been recognized in simian primates based on architectonic features, multiple somatotopic organization, and patterns of cortical and subcortical connections. These include the premotor areas, the supplementary motor areas, and the cingulate motor areas. These results suggest that as many as 10 motor fields emerged early in primate evolution (Wu *et al.*, 2000).

3.28.5.3 Is There a Primate Homologue to the Rodent RFA?

Intracortical microstimulation studies of sensorimotor cortex in the rat have shown a complete motor representation that is cytoarchitectonically defined as agranular cortex (Hall and Lindholm, 1974; Donoghue and Wise, 1982). The portion of this motor area in caudal portions of frontal cortex that is devoted to forelimb movements is referred to as the caudal forelimb area (CFA). In addition, a second motor representation of the forelimb has been identified in more rostral portions of the frontal cortex. This second forelimb representation, referred to as the RFA, is smaller than the CFA (Neafsey *et al.*, 1986). The RFA is separated from the CFA by a zone where intracortical microstimulation (ICMS) elicits vibrissa or neck muscle movements.

Because the presence of a secondary motor area in rats would appear to parallel the differentiation of motor areas in primates, suggestions have been made that the RFA is a homologue of one of the primate secondary motor areas. Based solely on the topographic location of CS neurons that originate in the RFA, Nudo and Masterton (1990a) concluded that secondary motor areas emerged independently in primates and rodents, and that there was no obvious homologue of RFA in primates.

However, it is important to consider additional details regarding the structure and function of the RFA in order to draw more firm conclusions. Tract-tracing studies of motor cortical connections in rat have shown differences in the thalamic, striatal, and cortical connections of CFA and RFA (Rouiller *et al.*, 1993). Comparison of these connections to the pattern of connections of primate motor areas suggests that RFA has some similarities to primate premotor areas. Roullier and colleagues found that the origins of thalamic inputs to the two areas are largely segregated, similar to the thalamic inputs to M1 and premotor areas in monkeys (Schell and Strick, 1984; Matelli *et al.*, 1989). Additionally, CFA has predominantly ipsilateral projections to the striatum, similar to M1 in primates (Leichnetz, 1986; Whitworth *et al.*, 1991). (The caudate and putamen are not differentiated in rodents. Thus, the collective term 'striatum' is used throughout this discussion for both rodents and primates.) RFA has diffuse bilateral corticostriatal projections equally dense to both hemispheres, similar to SMA and premotor cortex in primates (McGuire *et al.*, 1991).

In contrast, evidence suggests that lesions of RFA in rat result in more severe behavioral deficits than lesions of SMA in primates (Barth *et al.*, 1990; Passingham, 1993). Furthermore, the predominant layers of origin of intracortical connections between the RFA and CFA differ from those of M1 and PM/SMA in primates (Dum and Strick, 2005; Figure 4). In addition, the predominant thalamic connections of the RFA are with ventromedial thalamus, with few connections with the ventrolateral thalamus, rather than the predominantly ventrolateral and ventroanterior thalamic connections of primate PM and SMA (Figure 4).

Although corticofugal projections from CFA and RFA are similar, the RFA has interconnections with insular cortex similar to SMA and premotor cortex in primates. Additionally, the RFA does not appear to have cutaneous receptive fields, similar to supplementary motor cortex in primates (Neafsey *et al.*, 1986). Overall, based on its connections, CFA is more similar to the M1 forelimb area in primates and RFA in some ways appears to be more similar to nonprimary motor cortex in primates. It is not currently possible to decide whether RFA is a homologue of primate premotor cortex, supplementary motor areas, or a combination of secondary motor areas in primates (Rouiller *et al.*, 1993).

3.28.5.4 Further Differentiation of Primate Motor Areas

3.28.5.4.1 Nomenclature The nomenclature used for subdivisions of primate motor areas based on the study of macaques has varied across laboratories. The generalized current scheme includes M1 (or Brodmann's area 4); four subdivisions of the lateral

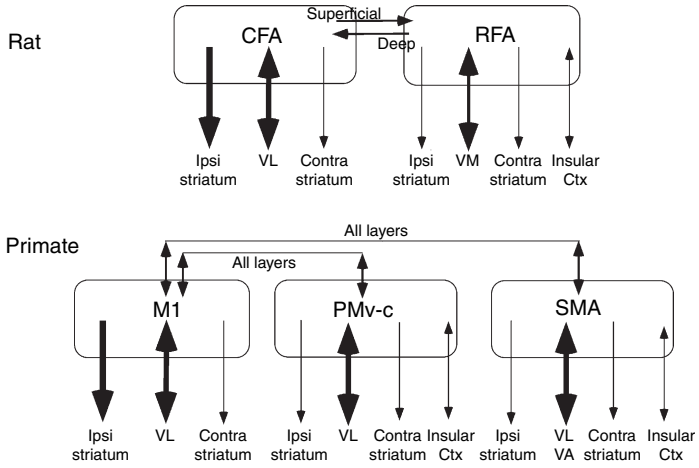

Figure 4 Comparison of secondary motor areas in rats and primates. Schematic diagram of the major (predominant) connections of the CFA and RFA in rat (top) compared to similar connections in M1, PMv-c, and SMA-proper in primates (bottom). CFA is similar to M1 based on its connections with ventrolateral thalamus (VL) and its strong projection to the ipsilateral lateral striatum (putamen in primates). RFA is similar to premotor areas of primates by its diffuse projections to ipsilateral and contralateral striatum (caudate and putamen in primates), and its interconnections with insular cortex. RFA differs from primate premotor cortex in its major connections with the thalamus (ventromedial thalamus in RFA vs. VL in PMd-c; VL and ventroanterior thalamus (VA) in primates) and its laminar differences in intracortial connectivity (deep layers of RFA project to CFA and superficial layers of CFA project to RFA, whereas all layers contribute projections between M1 and PMd or SMA).

premotor cortex (area 6) (PMd-c, PMd-r, PMv-c, PMv-r, or F2, F7, F4, and F5, respectively); two premotor subdivisions on the mesial surface of the hemisphere (SMA and pre-SMA, or F3 and F6), and three subdivisions of the cingulate motor area within regions lining the cingulate sulcus (CMAr, CMAd, CMAv, or area 24c, area 6c, and area 23c). SMA has also been referred to as M2 (Figure 5).

3.28.5.4.2 Differentiation of lateral premotor cortex Studies suggest that a premotor area first appeared with the divergence of prosimian primates, as evidenced by lateral premotor representations coincident with distinct cytoarchitectonics rostral to the M1 representation. Two major subdivisions, PMd and PMv, have been identified in the prosimian Galago (Wu *et al.*, 2000). PMd has been further subdivided in Galago into rostral and caudal components (PMd-r and PMd-c, respectively; see figures 4 and 5 in Wu *et al.*, 2000). To the extent that Galagos represent a primordial state of primate motor cortex, it is likely that PMd-r, PMd-c, and PMv are homologues in all extant primates. Intracortical microstimulation studies have also identified PMv and PMd in New World and Old World monkeys (Gould *et al.*, 1986; Stepniewska *et al.*, 1993; Preuss *et al.*, 1996; Frost *et al.*, 2003; Hoshi and Tanji, 2004; see Figure 5). The PMd has been shown to consist of representations of both hindlimb and forelimb (He *et al.*, 1993; Ghosh and Gattera, 1995; Preuss *et al.*, 1996; Raos *et al.*, 2003), while PMv contains representations of the forelimb and orofacial muscles (Stepniewska *et al.*, 1993; Preuss *et al.*, 1996).

In addition to the areas noted above that have been identified in prosimian primates and New World monkeys, the PMv is further differentiated in Old World monkeys. A total of four subareas of lateral premotor cortex are identifiable in macaques: PMd-c, PMd-r, PMv-c, and PMv-r (or F2, F7, F4, and F5, respectively) based on cytoarchitectonics, intracortical microstimulation results, and connections (Rizzolatti and Fadiga, 1998; Rouiller *et al.*, 1998; Morel *et al.*, 2005).

Studies implicate PMv in the initiation and control of limb movements based on visual cues and other sensory information (Kurata and Tanji, 1986; Gentilucci *et al.*, 1988; Rizzolatti *et al.*, 1988; Mushiake *et al.*, 1991; Mushiake *et al.*, 1997), whereas PMd is involved with movement parameters (Fu *et al.*, 1993; Kurata, 1993; Crammond and Kalaska, 2000). Thus, PMv is important for the integration of visual information derived from extrapersonal three-dimensional space and involved in the spatial guidance of limb movements (Kakei *et al.*, 2001), whereas PMd is involved in the integration of internal body representation and target information for the preparation of motor actions (Kurata, 1994; Hoshi and Tanji, 2004).

Unlike macaque monkeys, there have been no delineations of subareas F4 and F5 (PMv-c and PMv-r) in PMv of prosimian primates or New World monkeys.

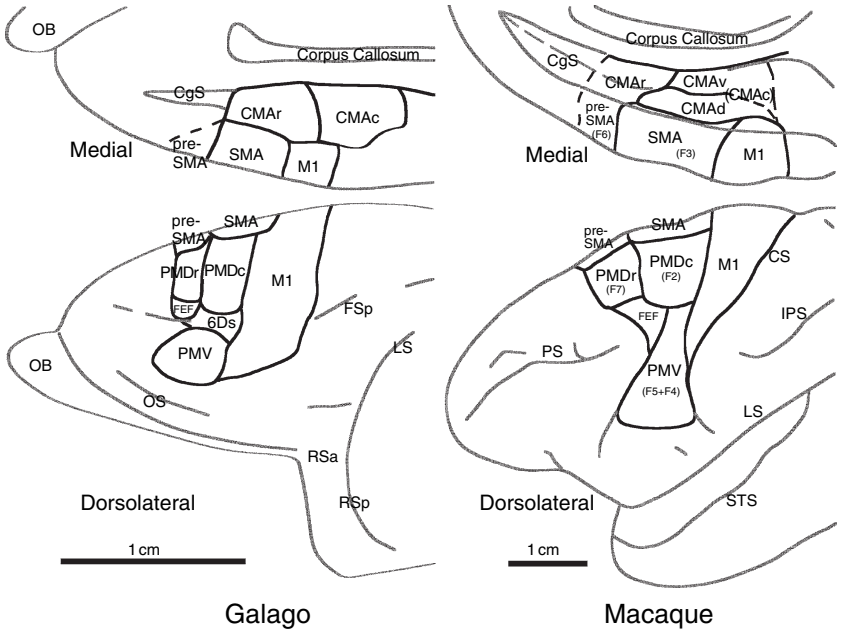

Figure 5 Multiple motor areas in galagos and macaques. Neurologically more primitive primates represented by the prosimian bush baby (*Galago garnetti*; left) have a well-differentiated M1, with representation of the trunk, hindlimb, face, and a large forelimb representation. Galagos possess most of the secondary motor areas that have been recognized in simian primates (macaque shown on right). These results suggest that as many as 10 motor fields emerged early in primate evolution.

In macaques, PMv is subdivided into caudal and rostral divisions based on cytoarchitectonic, connectional, histochemical, and physiological distinctions (Matelli *et al.*, 1985, 1991; Luppino *et al.*, 1999; Rizzolatti and Luppino, 2001; Rizzolatti *et al.*, 2002; Morel *et al.*, 2005).

In macaques, F4 appears to code goal-directed actions mediated by spatial locations (Rizzolatti *et al.*, 2002), and F5 has been shown to be involved in motor-action recognition (Umilta *et al.*, 2001) as well as in hand shaping in visuomotor transformations for grasping and manipulation (Fogassi *et al.*, 2001). Human area 44 has been shown to be involved in sensorimotor transformations for grasping and manipulation (Binkofski *et al.*, 1999) and is thought to be homologous to F5 in macaques (Rizzolatti *et al.*, 2002).

The special role of vision and visuomotor control in primates has led some to suggest that the remarkable increase in the size of the frontal lobes in this order, and perhaps the differentiation of frontal motor areas was driven by the increase in direct and indirect connections of the visual cortex with the frontal, especially motor cortex (Whishaw, 2003). The selective pressures for visually guided forelimb movements may have led to the specialized motor and sensorimotor areas in primates to integrate the somatosensory and visual frames of reference required for skilled movement.

3.28.5.4.3 Differentiation of the supplementary motor area Studies suggest that a supplementary motor area first appeared with prosimian primates, as evidenced by two distinct SMA motor representations corresponding to distinct cytoarchitecture located on the medial surface of the hemisphere in Galago. These two areas are referred to as SMA-proper and the pre-SMA, situated more rostrally (see figures 4 and 5 in Wu *et al.*, 2000). A supplementary motor area has also been identified in New World monkeys (Gould *et al.*, 1986).

Both SMA and pre-SMA (or areas F3 and F4) have been identified in macaques based on cytoarchitecture, intracortical microstimulation, and connections (Matelli *et al.*, 1991; Matsuzaka *et al.*, 1992; Luppino *et al.*, 1993; Rouiller *et al.*, 1999; Liu *et al.*, 2002; Morel *et al.*, 2005). Compared to SMA, pre-SMA has only sparse spinal projections, and high thresholds for evoking movement, and likely plays less of a direct role in the execution of movement (Luppino *et al.*, 1993; He *et al.*, 1995; Dum and Strick, 1996; Liu *et al.*, 2002). Pre-SMA is thought to have greater involvement in cognitive aspects of motor processing.

3.28.5.4.4 The cingulate motor areas Studies suggest that a cingulate motor area first appeared with prosimian primates, as evidenced by two cingulate motor representations and distinct cytoarchitecture in CMAr and CMAc in Galago (Wu *et al.*, 2000). A

third area that also has dense connections with M1 and the spinal cord in Galago was identified posteriorly in cingulate cortex and referred to as the cingulate somatomotor area (CSMA), although this may correspond to a supplementary sensory area (Wu *et al.*, 2000).

The primate cingulate cortex has traditionally been divided into rostral and caudal architectonic subdivisions (areas 24 and 23). More recently, in the macaque, the caudal CMA has been further differentiated into two distinct areas (CMAd and CMAv) based on distinct cytoarchitectonics and intracortical microstimulation studies identifying a third forelimb representation (Walsh and Ebner, 1970; Vogt *et al.*, 1987; Takada *et al.*, 2001; Hatanaka *et al.*, 2003).

Most medially, the rostral, dorsal, and ventral cingulate areas are buried in the cingulate sulcus (CMAr, CMAd, and CMAv, respectively). As with other secondary motor areas, the CMA areas send somatotopic projections directly to M1 and the spinal cord (Muakkassa and Strick, 1979; Dum and Strick, 1991, 1996; Luppino *et al.*, 1993; He *et al.*, 1995; Wang *et al.*, 2001). The somatotopy of CMA has been examined using intracortical microstimulation, demonstrating at least a forelimb representation in each of the subareas of CMA (Mitz and Wise, 1987; Luppino *et al.*, 1991, 1994; Takada *et al.*, 2001; Wang *et al.*, 2001; Hatanaka *et al.*, 2003). Tract-tracing studies in the macaque have shown that the CMAr and the caudal cingulate motor area (involving both CMAd and CMAv) are characterized by distinct patterns of intracortical and thalamocortical connections (Hatanaka, *et al.*, 2003). Functional studies examining cingulate motor areas suggest that CMAr plays a role in the cognitive control of voluntary movements, whereas the caudal CMA (CMAd and CMAv) is directly involved in the execution of voluntary movement (Devinsky *et al.*, 1995; Picard and Strick, 1996, 2001; Carter *et al.*, 1999; Tanji *et al.*, 2002).

3.28.6 Functional Significance of the Evolution of Motor Cortex

Because of the evolutionary changes that occurred in sensorimotor cortex and in the CST, especially in the human lineage, the functional contribution of the motor cortex and its descending outflow to the spinal cord is of natural interest. There is no question that evolutionary trends in motor control occurred in the human lineage resulting in increased dexterity of hand, especially of the digits. Investigators have long sought a morphological basis for the special motor skills of primates, including humans. It has been assumed that major changes must have taken place in the neural control of spinal cord motoneurons, since the peripheral anatomy of primates is remarkably similar (Napier, 1962).

In 1869, Spitzka suggested for the first time that the relative size of the pyramids might be related to the fine control of distal musculature. But even then, Spitzka realized that some species (e.g., seals and sea lions) possess large pyramidal tracts, but poor dexterity. In their review, Heffner and Masterton (1975) argue that among mammals, pyramidal tract morphology (size, number of fibers, fiber size) corresponds more closely to body size than to digital dexterity. However, the role of total number of fibers and fiber size should not be dismissed outright. Somewhat different results are obtained when correlations of pyramidal/CST parameters with digital dexterity are restricted to the human lineage (Heffner and Masterton, 1983). In this analysis, size of the pyramidal tract also becomes significant.

Also, CS morphology has for many years been suggested to be related to the specialized manual skills of primates. The giant Betz cells of M1 are likely to account for a significant proportion of corticomotoneuronal (CM) cells, and thus are likely to play a dominant role in skilled motor coordination. An important question though, is how closely CS soma size (as well as total number of CS neurons) is related to allometric scaling. When this issue was specifically examined, body weight accounts for over 30% of the variance in the number of CS neurons, and over 50% of the variance in their soma size (Nudo *et al.*, 1995). However, in primates, the number of CS neurons and their soma size deviate from the mammalian linear regression line. Primates have greater numbers and larger CS soma size than other mammals for their body weight. Strikingly, CS soma size and number of CS neurons track even more closely with neocortical surface area, which accounts for nearly 70% of the variance in number and over 80% of the variance in soma size in mammals. When this analysis is restricted to primates, neocortical surface area accounts for over 90% of the variance in CS soma size. (There was no significant difference between primates and nonprimates in the relationship between neocortical surface area and number of CS neurons.) Similar findings were found in a recent study of the allometric relationships of the size of Betz cells (Sherwood *et al.*, 2003). The authors suggests that Betz cells become larger in relation to body weight, brain weight, and encephalization quotient, but may not be related to digital dexterity, as others have suggested. Instead, the authors

propose that enlarged Betz cells may play a role in specialized locomotor behaviors in primates.

Before completely discounting the notion that CS soma size or Betz cell size is unrelated to specialized primate motor skills such as digital dexterity, one should consider that correlates of neocortical growth (brain weight, neocortical surface area, encephalization quotient) are not independent of the expansion and differentiation of motor and motor-related structures in primates. In fact, it is possible that morphologic alterations in motor structures (increased size of Betz cells and CS soma, differentiation of motor areas, increased intracortical circuitry, interconnected differentiated motor areas, increased corticofugal output, etc.) were major driving forces giving rise to a larger neocortex, at least in primates. Thus, number and size of CS neurons may still be important correlates of specialized motor skills such as digital dexterity, at least in the human lineage.

Mammals also differ widely in the pattern of CST terminations, and these differences appear to be related to digital dexterity. Heffner and Masterton (1975) showed that the mode of termination of CST fibers – both the extent of the fibers caudally in the cord and the ventral-most lamina in which they terminate – closely parallel the digital dexterity of a species. It is certainly reasonable to presume that animals with dexterous control of distal musculature might have direct cortical innervation of the (lateral) motoneurons that innervate this musculature. While this correlational study provides a rational hypothesis, the data were derived from older degeneration techniques that lack the sensitivity of more modern tract-tracing methods.

More recent neuroanatomical and electrophysiological data seem to corroborate this hypothesis. First, CST neurons that originate from a true motor cortex terminate in deeper laminae of the spinal cord (Ralston and Ralston, 1985). Most terminate in intermediate laminae in monkeys, somewhat more ventral to the termination of CS neurons originating in somatosensory cortex. However, a subset of CS neurons terminate in the ventral horn in the vicinity of motoneurons, and especially those motoneuron pools innervating muscles of the upper extremity. CS neurons originating in the primary motor cortex have the densest terminations to the deep spinal cord lamina where motoneurons innervating hand and finger muscles are located (Maier *et al.*, 2002).

There are also clear differences in the termination pattern of CS neurons among primate species. In some primates, a small subset of CS neurons terminates monosynaptically on motoneurons in the spinal cord. Such CM cells are present most notably in those primate species with the most highly developed digital skills. For example, squirrel monkeys accomplish grasping of objects with a prehensile, or power grip. A precision grip, in which the index finger is opposed to the thumb, is rarely if ever performed (Fragaszy, 1983). In this species, CM connections are relatively weak (Bortoff and Strick, 1993). Macaque monkeys display a precision grip and a number of other complex behaviors with the hand. This species possesses much more dense CM connections (Nakajima *et al.*, 2000). It has also been proposed that increased CM innervation is paralleled by decreased proprioceptive control of spinal cord motoneurons (Lemon and Griffiths, 2005).

Although rodents have surprisingly sophisticated motor control during grasping, and elaborate behavioral descriptions of complex movements of the digits have been reported (Walsh and Ebner, 1970; Iwaniuk and Whishaw, 2000), the termination of CS fibers differs from those in primates. CS neurons in rats tend to terminate in more dorsal laminae. Although there is a sparse termination in lamina IX, there is no evidence of monosynaptic connections between CS fibers and motoneurons (Yang and Lemon, 2003). Thus, control of the distal musculature may have evolved independently in various mammalian orders, and thus, neuroanatomical control mechanisms may differ among these species.

3.28.7 Summary and Conclusions

The evolutionary history of vertebrate motor systems is notable for its remarkable conservation of descending systems originating in upper levels of the neuraxis. All extant vertebrates apparently possess a similar subset of descending pathways that originated early in the evolution of the subphylum. Based on the available evidence from selected extant species, it would appear that the basic vertebrate plan was augmented by additional pathways, but antecedent pathways rarely became degenerate. The mammalian radiation was accompanied by the appearance of a new type of brain structure, a six-layered neocortex. In the earliest mammals, neocortex provided descending fibers to the medulla, and increasingly to the spinal cord. In multiple mammalian orders, a true motor cortex emerged, possibly through parallel or convergent evolution. Motor cortex became increasingly differentiated from the somatosensory cortex, and at least in primate species, terminated closer and closer to motoneuron pools, eventually providing fast, monosynaptic control of spinal cord motoneurons by the

cerebral cortex. At the same time, multiple motor areas became differentiated in the frontal cortex of primates, each with its own unique contribution to cortical motor control.

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3.29 The Evolution of the Basal Ganglia in Mammals and Other Vertebrates

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Glossary

agnathans Jawless fish, including the extant lamprey and hagfish.

amniotes Collective term for vertebrate groups whose members develop inside an amniotic sac, and which is a synonym for birds, reptiles, and mammals.

anamniotes Collective term for vertebrate groups whose members do not develop inside an amniotic sac, and which is a synonym for fish and amphibians.

basal ganglia A subcortical telencephalic region that in mammals includes two cell groups, the caudate and putamen (together referred to as the striatum), and a third cell group known as the globus pallidus.

caudate-putamen The input subdivision of the basal ganglia (together referred to as the striatum).

cerebral cortex The part of the mammalian pallium that is organized into six layers and mediates higher-order learning, perception, and motor control.

chondroicthyans Cartilaginous fish, such as skates, rays, sharks, and chimeras.

direct pathway The striatal output pathway to the internal pallidal segment and the substantia nigra, which facilitates desired movement of enkephalin. Opiate neuropeptide characteristically present in the GABAergic spiny striatal neurons of the so-called indirect striatal output neurons that project to the external pallidal segment.

globus pallidus The output subdivision of the basal ganglia.

indirect pathway The striatal output pathway to the external pallidal segment, which inhibits unwanted movement.

neostriatum Outdated synonym for the caudate-putamen that reflects the repudiated notion that the caudate-putamen appeared after the globus pallidus in evolution.

paleostriatum Outdated synonym for the globus pallidus that reflects the repudiated notion that the globus pallidus appeared before the caudate-putamen in evolution.

pallidum Short-hand term referring to the globus pallidus.

pallium Term referring to the part of the telencephalon (cerebrum) that lies above and/or around the basal ganglia.

sauropsids Collective term for birds and reptiles.

striatum Short-hand term for the input part of the basal ganglia, also known as the caudate-putamen in mammals.

subpallium Term referring to the part of the telencephalon that lies below the pallium.

substance P Tachykinin neuropeptide characteristically present in the GABAergic spiny striatal neurons of the so-called direct striatal output neurons that project to the internal pallidal segment and the substantia nigra.

substantia nigra Cell group in the midbrain tegmentum, in mammals consisting of two parts, a pars compacta rich in dopaminergic neurons and a pars reticulata rich in GABAergic neurons.

subthalamic nucleus Cell group in the lower thalamus of mammals that receives input from the external pallidal segment and projects to the internal pallidal segment

tetrapods Collective term referring to the limbed vertebrate groups, and it includes amphibians, reptiles, birds, and mammals.

3.29.1 Introduction

The basal ganglia is a subcortical telencephalic region that includes two cell groups, the caudate and putamen (together referred to as the striatum), and a third cell group known as the globus pallidus. Whereas additional cell groups such as the nucleus accumbens, the olfactory tubercle, and the ventral pallidum are sometimes considered to be part of the basal ganglia (Heimer *et al*., 1985, 1997; Reiner *et al*., 1998), for present purposes these will be considered the limbic or ventral basal ganglia and regarded as a separate entity from the basal ganglia. Understanding of the organization and function of the basal ganglia of mammals has increased tremendously in the past 100 years. When the term basal ganglia first became commonplace in the late 1800s, it loosely referred to various subcortical telencephalic cell groups (i.e., the basal nuclei) that included the amygdala and claustrum (and in some cases the thalamus), as well as what are now regarded as the basal ganglia (Parent, 1986). Without reliable methods for tracing brain connectivity, knowledge of the basal ganglia largely consisted of efforts to identify the same cell groups in diverse species, with some inferences made about a role in motor function from clinical findings (Vogt, 1911; Wilson, 1912). This early view suggested that the basal ganglia and cerebral cortex exerted separate control on motor function: the motor cortex via projections to brainstem and spinal cord via the pyramidal tract, and the basal ganglia via nonpyramidal circuits. Thus, motor control was thought to be effected by pyramidal and extrapyramidal motor systems.

With the advent of silver staining of degenerating fibers in the 1950s, it was soon recognized that the caudate and putamen receive extensive cortical input and have extensive projections to the globus pallidus and substantia nigra (Carman *et al*., 1963; Nauta and Mehler, 1966; Parent, 1986). The globus pallidus, in turn, was found to project to thalamic cell groups projecting to motor cortices (Nauta and Mehler, 1966). These findings indicated that the role of the basal ganglia in motor control involved cortical input and was mediated by a return projection to motor cortex. Neurochemical methods developed in the 1960s revealed a major nigral input to striatum that used dopamine (DA) as a neurotransmitter and led to the discovery that loss of this input was the basis of Parkinson's disease (Carlsson, 1959; Carlsson *et al*., 1962; Dahlstrom and Fuxe, 1964). Understanding of the cellular makeup of the basal ganglia, the neurotransmitters used by its neurons, and delineation of its circuitry at a cellular level accelerated greatly with the application of immunohistochemistry in the 1970s, and of *in situ* hybridization histochemistry in the 1980s, to the study of the basal ganglia (Parent, 1986; Reiner and Anderson, 1990; Gerfen, 1992; Graybiel, 1990). Finally, over the past 10 years, great advances have been made in identifying the genes controlling the regional identity and development of the striatum, globus pallidus, and cerebral cortex. These genes include *Dlx1* and *Dlx2*, which specify the striatum and pallidum, and *Nkx2.1*, which specifies the globus pallidus (Rubenstein *et al*., 1994; Smith-Fernandez *et al*., 1998; Puelles *et al*., 2000). Other genes control pallial identity, such as *Emx1*, *Emx2*, and *Tbr1*, and are expressed at high levels in developing cerebral cortex (Rubenstein *et al*., 1994; Smith-Fernandez *et al*., 1998; Puelles *et al*., 2000). Projection neurons of the cerebral cortex and other pallial telencephalic areas characteristically use glutamate as their neurotransmitter, whereas those of the subpallium (to which the basal ganglia belongs) characteristically are GABAergic (Swanson and Petrovich, 1998).

This accumulation of data, together with insights from physiology, pharmacology, and molecular biology, led to circuit level models of basal ganglia function that relate the major aspects of basal ganglia cytology and circuitry to normal and pathological basal ganglia function (Albin *et al*., 1989; Crossman, 1990; DeLong, 1990). In these models, the striato-pallidal output circuitry is recognized as being organized into two channels. One arises from substance P (SP)-containing GABAergic striatal neurons that project to the large GABAergic neurons of the internal segment of globus pallidus (GPi) that promote movement. The second output arises from enkephalinergic (ENK^+) GABAergic striatal neurons that project to the large GABAergic neurons of the external segment of globus pallidus (GPe) that inhibit unwanted movement (Figure 1). The GPe neurons have indirect outputs to GPi neurons via the subthalamic nucleus (STN) and for this reason the SP^+ striatal projection to the GPi is called the direct pathway and the ENK^+ striatal projection to GPi via the GPe–STN connection is called the indirect pathway. Identification of the cell types of the basal ganglia by their neurotransmitter

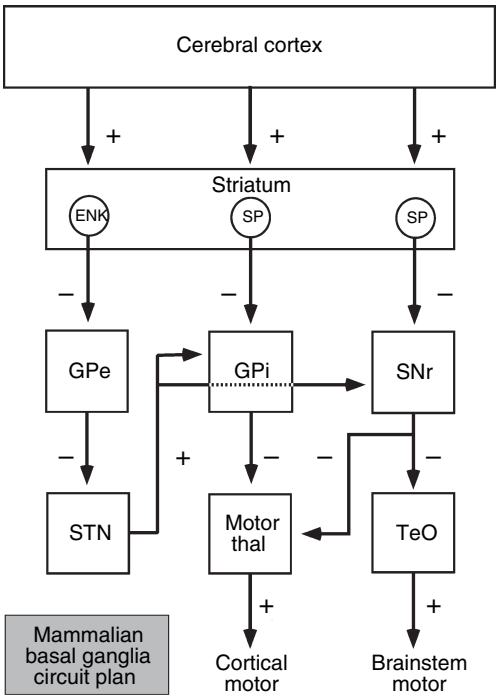

Figure 1 Circuit diagram illustrating the basic direct–indirect pathway organization of basal ganglia functional circuitry in mammals. The plus and minus signs indicate whether the specific projections of the basal ganglia circuitry are glutamatergic excitatory (+) or GABAergic inhibitory (–). ENK, enkephalinergic neurons; GPe, external globus pallidus segment; GPi, internal globus pallidus segment; SNr, substantia nigra pars reticulata; SP, substance P-containing neurons; STN, subthalamic nucleus; TeO, optic tectum; Motor thal, motor thalamus.

content and the genes controlling their identity led to the realization that the interneurons of both the striatum and the cerebral cortex, which tend to be GABAergic, migrate in from the Nkx2.1-expressing zone from which the globus pallidus also forms (Marin *et al*., 2000; Marin and Rubenstein, 2001). For striatum, these include three major neurochemically distinct types of interneurons: large cholinergic neurons, large GABAergic neurons co-containing parvalbumin (PARV), and small GABAergic neurons co-containing the neuropeptides somatostatin and neuropeptide Y and the nitric oxide (NO)-synthesizing enzyme NO synthetase (Kawaguchi *et al*., 1995).

Before the advent of modern neuroanatomical hodological methods, several investigators formulated a theory of basal ganglia and overall telencephalic evolution based strictly on the size, position, and cytological appearance of the major telencephalic regions (Edinger *et al*., 1903; Edinger, 1908; Ariëns-Kappers *et al*., 1936). This theory resulted in terminologies for both mammalian and nonmammalian telencephalons that have proven enduring, even in the face of their thorough repudiation by modern data. In this traditional terminology, the globus pallidus in mammals is alternatively referred to as the paleostriatum, whereas the caudate–putamen is called the neostriatum. These terms stem from the notion that the parts of the telencephalon had evolved in serial order during vertebrate evolution: the globus pallidus in jawed fish, the neostriatum in amphibians, and the primitive cerebral cortex in reptiles (Ariëns-Kappers *et al*., 1936). Mammals were thought to have elaborated cerebral cortex into neocortex, whereas birds were thought to have elaborated the basal ganglia by addition of a new territory known as the hyperstriatum. This view of telencephalic evolution has been refuted by modern neuroanatomical, molecular biological, and neurochemical studies, but vestiges of it have survived in the frequent use of the terms paleostriatum and neostriatum to refer to the globus pallidus and caudate–putamen in mammals and in the telencephalic terminology that was formerly used in birds (Reiner *et al*., 2004). Older views of telencephalic evolution also promoted the idea that a process of encephalization had occurred during vertebrate evolution, with more rostral structures taking over functions carried out by more caudal regions (Ariëns-Kappers *et al*., 1936; Herrick, 1948, 1956). In the case of telencephalic evolution, the cerebral cortex was thought to take over behaviors that had been carried out by the basal ganglia in a stereotyped fashion in the stem amniote common ancestors of mammals, birds, and reptiles, and still were carried out by the basal ganglia in modern birds and reptiles. The implication of this for mammals was the expectation that the cerebral cortex and basal ganglia size should be dissociated or inversely related.

The neuroanatomical tools that have been used to clarify the cellular neurochemistry and connectivity of the basal ganglia of mammals and determine the genetic control of regional identity in the developing mammalian telencephalon have been used to study telencephalic organization and development in members of other vertebrate groups as well (Parent, 1986; Reiner *et al*., 1998; Marin *et al*., 1998a, 1998b). These studies have dramatically revised our understanding of basal ganglia evolution and have profound implications for the traditional view of basal ganglia evolution that still is all too often promulgated in neuroanatomy textbooks. In the following paragraphs, I discuss evidence showing that both a striatum and a pallidum have been basal ganglia constituents since early in vertebrate evolution. I also examine data emphasizing the functional interrelatedness of cerebral cortex and basal ganglia, with both enlarging in parallel during brain expansion in the mammalian radiation.

3.29.2 Anamniote Basal Ganglia Evolution

3.29.2.1 Agnathans

There are two extant groups of jawless fish: hagfish and lamprey. Modern taxonomic studies suggest lamprey and hagfish to be only distantly related, with lamprey being a sister group of jawed vertebrates (Forey and Janvier, 1994). Telencephalic organization in these two agnathan groups reflects their taxonomic distance (see Evolution of the Deuterostome Central Nervous System: An Intercalation of Developmental Patterning Processes with Cellular Specification Processes). Neurochemical studies clearly identify a ventral telencephalic region rich in SP^+ perikarya in lamprey (Table 1; Figure 2; Nozaki and Gorbman, 1986; Auclair *et al*., 2004). This region receives a dopaminergic input from the midbrain and has a return projection to these dopaminergic neurons (Pierre *et al*., 1994; Pombal *et al*., 1997a). This region also expresses lamprey homologues of *Dlx1/2* (Murakami *et al*., 2001; Neidert *et al*., 2001) and contains some cholinergic interneurons (Pombal *et al*., 2001). For these reasons, this region appears to be homologous to the mammalian striatum. Although a globus pallidus in lamprey has not been demonstrated unequivocally (Nieuwenhuys and Nicholson, 1998; Murakami *et al*., 2001), SP^+ woolly fibers ventrolateral to the striatum in a field of GABAergic neurons within a region that has been called the ventral pallium delineate a field that may be pallidal (Pombal *et al*., 1997b). Alternatively, SP^+ woolly fibers ventromedial to the striatum define a field that may be pallidal (Figure 2; Nozaki and Gorbman, 1986). By contrast, SP and ENK immunolabeling fails to unequivocally identify a striatum or pallidum in hagfish (Wicht and Northcutt, 1994). Although lampreys clearly possess a striatum, the region is small and neuron sparse and the midbrain dopaminergic input is meager. Telencephalic inputs or descending projections of the lamprey striatum have not been investigated and not much is known of lamprey basal ganglia functional circuitry (Table 1).

3.29.2.2 Chondroicthyans

Cartilaginous fish possess simple tubular paired telencephalic hemispheres, as do lobe-finned fish and amphibians, and the ventrolateral sector of the telencephalon contains both a striatum and a globus pallidus, by neurochemical and hodological criteria (Table 1; Figure 2; Reiner and Carraway, 1985; Northcutt *et al*., 1988; Reiner *et al*., 1998). The striatal sector is located nearest the ventricle and is cell sparse, but contains SP^+ and ENK^+ neurons that give rise to projections to a cell plate lying external to the striatal field (Figure 2). This cell plate appears comparable to the globus pallidus, both because of this striatal input and because the neurons of the pallidal field contain the neurotensin-related hexapeptide LANT6 (Lys^8–Asn^9–neurotensin$^{8-13}$), which is present in mammalian pallidal neurons (Northcutt *et al*., 1988; Reiner and Carraway, 1985, 1987; Reiner, 1987a; Rodriguez-Moldes *et al*., 1993). In this pallidal field, the SP^+ and ENK^+ inputs overlap, indicating that GPi- and GPe-type neurons are intermingled. Moreover, the striatum receives a dopaminergic input from the midbrain and these dopaminergic neurons receive a return projection from SP^+ striatal neurons (Meredith and Smeets, 1987; Northcutt *et al*., 1988; Smeets and Reiner, 1994; Steusse *et al*., 1994). The pallium occupies the dorsolateral sector of the telencephalon in cartilaginous fish, its development is controlled by homologues of some of the same genes controlling pallial development in mammals (Derobert *et al*., 2002), and this region is larger and more complex in the more advanced cartilaginous fish (Northcutt, 1981a; Northcutt *et al*., 1988). Although not experimentally demonstrated, it seems likely that *Dlx* homologues control subpallium development in cartilaginous fish, given their expression in lamprey and bony fish subpallium (Murakami *et al*., 2001; Neidert *et al*., 2001; Stock *et al*., 1996) and their demonstrated existence in cartilaginous fish (Stock, 2005). Interneuron populations of the striatum in cartilaginous fish have not been extensively studied, but appear to be sparse at best (Reiner *et al*., 1998).

3.29.2.3 Osteicthyes – Ray-Finned versus Lobe-Finned Fish Divergence

Bony fish diverged into two very different groups early in their evolution (Nieuwenhuys, 1966; Northcutt, 1981a). One group, the lobe-finned fish, possesses the same paired tubular evaginated telencephalons as cartilaginous fish and includes the ancestors of amphibians. The other group, the ray-finned fish, evolved a very different telencephalic morphology, referred to as the everted telencephalon (see Evolution of the Nervous System in Fishes). In both groups, a basal ganglia has been demonstrated by modern hodological, neurochemical, and developmental criteria (Table 1; Figure 2; Reiner *et al*., 1998). For lobe-finned fish, the topography and cytology of the basal ganglia resemble those in cartilaginous fish (Reiner and Northcutt, 1987), whereas the peculiarities of the ray-finned fish telencephalon have rendered basal ganglia

Table 1 Summary of the major features of basal ganglia organization and whether those features are present in the major extant vertebrate groups possessing an evaginated telencephalon

Animal group	*Striatum*	*Pallidum*	*SP^+ striato-pallidal pathway*	*ENK^+ striato-pallidal pathway*	*SP^+ striato-nigral pathway*	*DA^+ nigro-striatal pathway*	*Glu^+ thalamo-striatal pathway*	*BG pretecto-tectal pathway*	*Glu^+ cortico-striatal pathway*	*GPe and GPi neuron location*	*GPe- STN-GPi pathway*	*GPi-thalamo-cortical pathway*
Lamprey	Present	Present	Present	Present	Present	Modest	Unknown	Unknown	Unlikely	Unknown	Unknown	Unlikely
Cartilaginous fish	Present	Present	Present	Present	Present	Modest	Unknown	Unknown	Unlikely	Intermixed	Unknown	Unlikely
Lobe-finned bony fish	Present	Present	Present	Present	Present	Modest	Unknown	Unknown	Unlikely	Intermixed	Unknown	Unlikely
Amphibians	Present	Present	Present	Present	Present	Modest	Present	Present	Negligible	Intermixed	Unknown	Absent
Reptiles	Present	Present	Present	Present	Present	Prominent	Present	Present	Present	Intermixed	Likely present	Likely present
Birds	Present	Present	Present	Present	Present	Prominent	Present	Present	Present	Intermixed	Present	Present
Mammals	Present	Present	Present	Present	Present	Prominent	Present	Indistinct	Present	Segregated	Present	Present

The table emphasizes that basal ganglia evolution was highly conservative among anamniotes, with the major changes occurring at the anamniote–amniote transition. A few additional changes have occurred in the evolutionary transition from stem amniotes to mammals. BG, basal ganglia; DA, dopaminergic; ENK^+, enkephalinergic; Glu^+, glutamatergic; GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; SP^+, substance P-containing; STN, subthalamic nucleus.

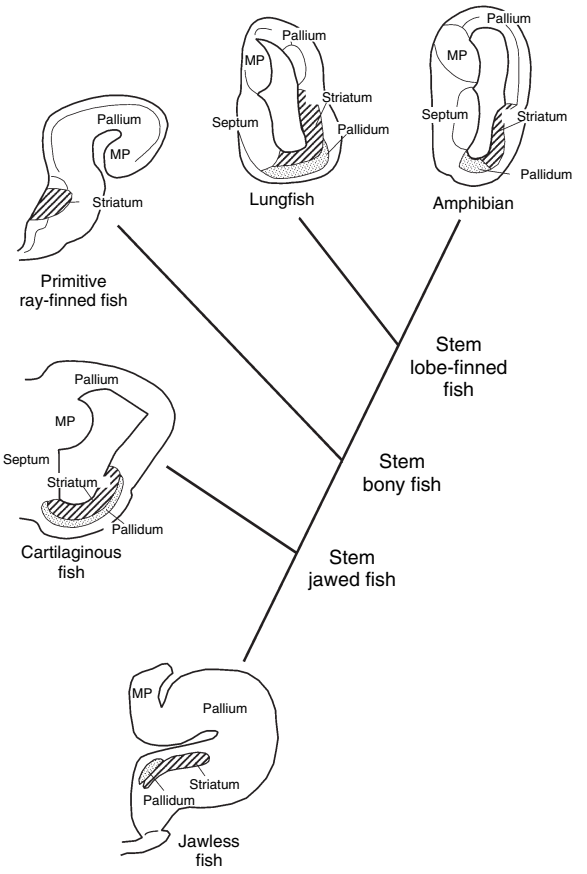

Figure 2 Schematics of frontal sections through the basal ganglia of the right telencephalic hemisphere in representative species from five anamniote groups: a lamprey (jawless fish), a shark (cartilaginous fish), a polypterid (ray-finned bony fish), a lungfish (lobe-finned bony fish), and a frog (amphibian), arranged according to their evolutionary divergences. The basal ganglia in all groups with an evaginated telencephalon (lamprey, shark, lungfish, and frog) consists of a striatum and a pallidum located in the basal telencephalon, beneath the pallial regions. Note that the pallium contains a medial (hippocampal) pallium (MP) in all amniote groups, with the medial pallium located laterally in ray-finned fish due to their telencephalic eversion. A striatum is evident in the ventral uneverted part of the telencephalon in ray-finned fish, but a pallidum is not well defined. Medial is to the left and dorsal to the top in all schematized sections.

topography and cytology less reminiscent of those in cartilaginous fish (Reiner and Northcutt, 1992). For example, in lungfish, the only lobe-finned fish whose basal ganglia has been studied (Reiner and Northcutt, 1987), the ventrolateral sector of the telencephalon contains both a striatum and a globus pallidus, by neurochemical and hodological criteria (Table 1). As in cartilaginous fish, the striatal sector contains SP^+ and ENK^+ neurons, but unlike in cartilaginous fish these are located in a cell-rich periventricular zone of neurons. The SP^+ and ENK^+ striatal neurons give rise to projections to a ventrocaudal neuronal cell group that appears comparable to globus pallidus (Figure 2), both because of this striatal input and because its neurons contain the neuropeptide LANT6. In this pallidal field, the SP^+ and ENK^+ inputs overlap, indicating that GPi- and GPe-type neurons are intermingled. Moreover, the striatum receives a dopaminergic input from the midbrain and these dopaminergic neurons receive a return projection from SP^+ striatal neurons (Reiner and Northcutt, 1987). The major populations of interneurons characterizing the striatum of amniotes may be scarce in lobe-finned fish basal ganglia (Reiner and Northcutt, 1987; Reiner *et al.*, 1998). The pallium occupies the dorsal sector of the telencephalon, but it is unknown whether it projects to the striatum.

In ray-finned fish, a striatum containing SP^+ and ENK^+ neurons (Reiner and Northcutt, 1992; Reiner *et al.*, 1998), likely to be GABAergic (Martinoli *et al.*, 1990; Medina *et al.*, 1994), having reciprocal connections with midbrain dopaminergic neurons (Reiner and Northcutt, 1992; Reiner *et al.*, 1998; Rink and Wullimann, 2001), and expressing a *Dlx1/2* homologue has been identified (Stock *et al.*, 1996; Wullimann and Mueller, 2004). Consistent with a dopaminergic input, the striatum in ray-finned fish is enriched in D1 and D2 dopamine receptors (Kapsimali *et al.*, 2000; Vacher *et al.*, 2003). A pallidal field may be present in the subpallium, since a ventral part of the subpallium expresses an *Nkx2.1* homologue (Wullimann and Mueller, 2004). The pallium in at least the more advanced ray-finned fish (i.e., teleosts with a large pallium) projects to the striatum and thalamic projections to the striatum appear to be present in all ray-finned fish (Northcutt, 1981b; Rink and Wullimann, 2004). The major populations of interneurons characterizing the striatum of amniotes are scarce in ray-finned fish basal ganglia (Reiner and Northcutt, 1992; Reiner *et al.*, 1998). The extent to which the divergent evolution of the ray-finned fish telencephalon has affected the circuitry or function of the basal ganglia is uncertain. Nonetheless, as in mammals, loss of DA^+ input to ray-finned fish striatum results in 'Parkinsonian' symptoms, i.e., slowed movements or bradykinesia (Pollard *et al.*, 1992). In any event, since ray-finned fish are not on the evolutionary line to mammals, further consideration of ray-finned fish basal ganglia anatomy is not entirely relevant here.

3.29.2.4 Amphibians

The telencephalon in amphibians is tubular in shape and its neurons largely occupy a periventricular position. This similarity to lobe-finned fish reflects the

evolutionary origin of amphibians from lobe-finned fish (Figure 3; see Evolution of the Amphibian Nervous System). The major features of basal ganglia organization demonstrated for lobe-finned fish and cartilaginous fish have also been demonstrated for amphibians (Table 1; Marin *et al*., 1998b; Reiner *et al*., 1998). Additionally, considerable hodological and developmental data are available for amphibians that definitively clarify many aspects of amphibian basal ganglia organization vis-à-vis that of amniotes (Table 1). For example, the ventrolateral sector of the telencephalon contains both a striatum and a globus pallidus, by neurochemical, hodological, and molecular developmental criteria. As in cartilaginous and lobe-finned fish, immunolabeling shows that the striatal sector contains SP^+ and ENK^+ neurons (Marin *et al*., 1997, 1998a, 1998b; Reiner *et al*., 1998), with the identity of the striatum further confirmed by the expression of glutamic acid decarboxylase (GAD) and an amphibian homologue of *Dlx1/2* (Papalopulu and Kintner, 1993; Bachy *et al*., 2002; Brox *et al*., 2003). Moreover, the SP^+ and ENK^+ striatal neurons give rise to projections to a ventrocaudal cell group that appears to be comparable to the globus pallidus (Figure 2), because of this striatal input, because it contains large GABAergic neurons, and because it expresses a homologue of *Nkx2.1* (Marin *et al*., 1998a, 1998b; Gonzalez *et al*., 2002; Brox *et al*., 2003). As is true in other anamniote groups, the SP^+ and ENK^+ inputs overlap in this pallidal field, indicating that GPi- and GPe-type neurons are intermingled. The amphibian striatum receives a dopaminergic input from the midbrain and these dopaminergic neurons receive a return projection from SP^+ striatal neurons (Table 1; Gonzalez and Smeets, 1994; Marin *et al*., 1998a, 1998b; Reiner *et al*., 1998). Both striatum and pallidum are, nonetheless, typically much more cell poor in amphibians, and the dopaminergic input more modest, than they are in amniotes. The types of interneurons characterizing the striatum of amniotes also seem to be scarce in amphibian basal ganglia (Reiner and Northcutt, 1992; Marin *et al*., 1997; Gonzalez *et al*., 2002; Reiner *et al*., 1998). The pallium occupies the dorsolateral sector of the telencephalon, but the major excitatory input to the striatum appears to arise from the thalamus rather than the pallium (Kicliter, 1979; Wilczynski and Northcutt, 1983; Marin *et al*., 1998b; Reiner *et al*., 1998). The basal ganglia in amphibians appears to have its major output to motor areas via a projection to the pretectum and a homologue of the substantia nigra pars reticulata, both of which affect head and eye movements by an input to tectal neurons with descending projections (Wilczynski and Northcutt, 1983; Marin *et al*., 1998b; Reiner *et al*., 1998). As in mammals, loss of dopaminergic input to amphibian striatum results in bradykinesia (Barbeau *et al*., 1986).

3.29.3 Amniote Basal Ganglia Evolution

3.29.3.1 Reptiles

Whereas the telencephalic hemispheres of reptiles also possess the same tubular evaginated structure as in amphibians during early development, the pallial and subpallial sectors of the telencephalon become much more cell rich than those in amphibians (Figures 3 and 4; Reiner *et al*., 1998). The

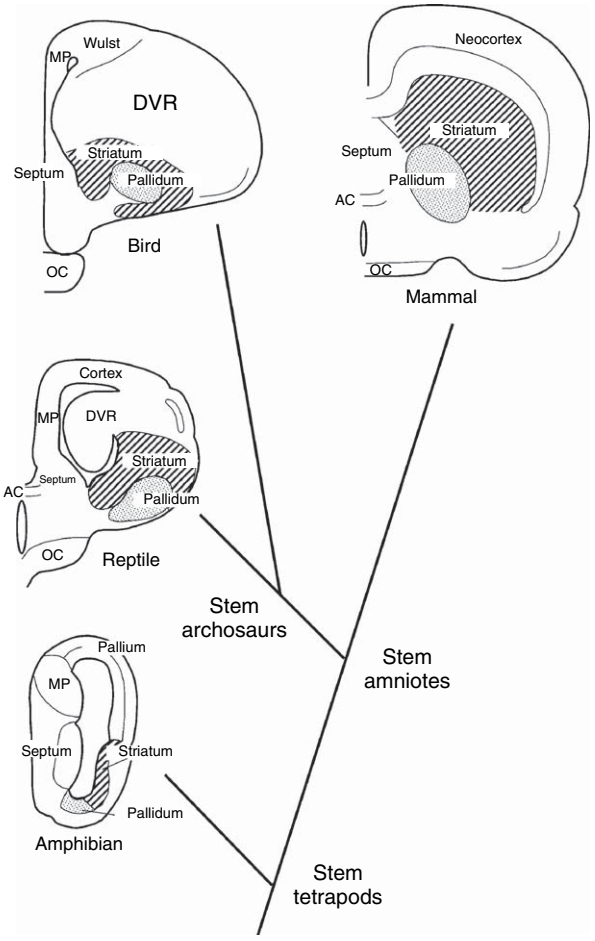

Figure 3 Schematics of frontal sections through the basal ganglia of the right telencephalic hemisphere in representative species from four tetrapod groups: amphibian (a frog), reptile (a turtle), bird (a pigeon), and mammal (a rat), arranged according to their evolutionary divergences. The basal ganglia in all four groups consists of a striatum and a pallidum located in the basal telencephalon, beneath the pallial regions. The pallidum, however, tends to be more laterally located in reptiles and birds than in amphibians and mammals. The phylogenetic distribution of pallidal laterality suggests that this trait arose in the reptilian lineage and was retained in birds. Medial is to the left and dorsal to the top in all schematized sections. AC, anterior commissure; MP, medial pallium; OC, optic chiasm.

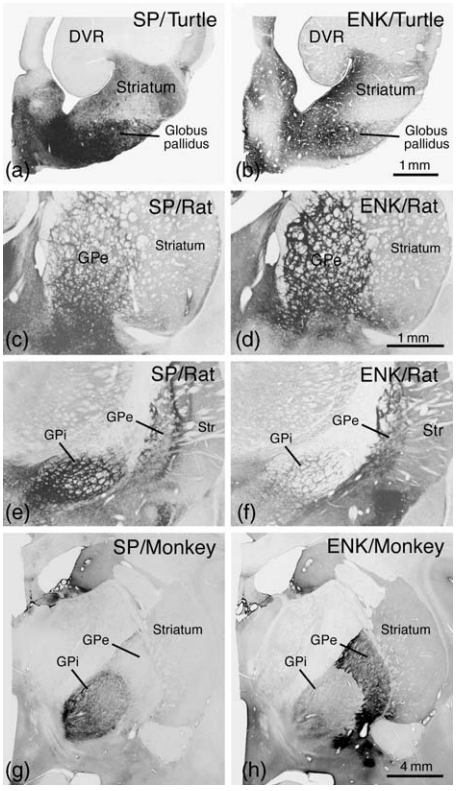

Figure 4 Images of frontal sections through the basal ganglia of one telencephalic hemisphere in a turtle (a), (b), rat (c–f), and rhesus monkey (g), (h). Sections were immunohistochemically stained for substance P (SP) (left-hand column) or enkephalin (ENK) (right-hand column). Note the intense SP^+ and ENK^+ immunoreactivity in the ventrolateral wall of the telencephalon that defines the region of the striatum and distinguishes it from the dorsal ventricular ridge (DVR) of the overlying pallium in the case of turtle. As in mammals, the striatum region is rich in SP^+ immunoreactivity due to the presence of numerous SP^+ and ENK^+ neurons and their processes. Note also that the globus pallidus in turtle is rich in both SP^+ and ENK^+ fibers, indicating that in turtles the ENK^+ fiber recipient GPe-type and SP^+ fiber recipient GPi-type pallidal neurons are intermingled. By contrast, in rats and monkeys GPe and GPi pallidal neurons are spatially segregated, more so in rat than in monkey. Medial is to the left and dorsal to the top in all images. GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; Str, striatum.

basal ganglia of reptiles reflects its inheritance from amphibians, but shows some differences that reflect the elaboration of the thalamus and pallium in reptiles (Table 1; see Evolution of the Nervous System in Reptiles). For example, as in amphibians, the ventrolateral sector of the reptile telencephalon contains both a striatum and a globus pallidus, by neurochemical, hodological, and developmental molecular criteria (Reiner *et al.*, 1998; Smith-Fernandez *et al.*, 1998). Also as in amphibians, immunolabeling shows that the striatal sector contains SP^+ and ENK^+ neurons (Reiner, 1987b; Russchen *et al.*, 1987; Reiner *et al.*, 1998), with the identity of the striatum further confirmed by the expression of GAD or GABA (Bennis *et al.*, 1991), and a homologue of *Dlx1/2* (Smith-Fernandez *et al.*, 1998). Moreover, the SP^+ and ENK^+ striatal neurons give rise to projections to a ventrocaudal cell group that is comparable to globus pallidus, because of this striatal input, because it contains large GABAergic neurons (Bennis *et al.*, 1991), and because its neurons contain LANT6 (Reiner and Carraway, 1987; Reiner *et al.*, 1998). The globus pallidus is, however, somewhat more laterally migrated than in amphibians (Figure 3). As is true in anamniotes, the SP^+ and ENK^+ inputs overlap in globus pallidus, indicating that GPi- and GPe-type neurons were intermingled in the stem amniotes (Figure 4). The reptile striatum receives a much more substantial dopaminergic input from the midbrain than in amphibians and these dopaminergic neurons in turn receive a substantial return projection from SP^+ striatal neurons (Smeets and Reiner, 1994; Reiner *et al.*, 1998). As in mammals, dopaminergic effects on striatum are mediated by D1 and D2 dopamine receptors, with DA agonists inducing hyperkinesia and DA antagonism yielding hypokinesia, indicating a similar role of the dopaminergic system in modulating striatal output as in mammals (Andersen *et al.*, 1975; Richfield *et al.*, 1987; Reiner *et al.*, 1998).

A distinguishing feature of the basal ganglia in reptiles is that it is much larger and more neuron rich than that in amphibians. Concomitant with the basal ganglia enlargement, the pallium in reptiles is also enlarged and is the source of a major excitatory input to the striatum, with the thalamus also providing excitatory input (Gonzalez *et al.*, 1990; Butler, 1994a, 1994b; Reiner *et al.*, 1998). Moreover, the glutamate receptors employed by specific types of striatal neurons to respond to this excitatory input in reptiles are very similar to those in mammals (Fowler *et al.*, 1999). These features of living reptiles indicate that a major telencephalic enlargement and elaboration of corticostriatal circuitry occurred by the evolutionary appearance of stem amniotes (Figure 5; Reiner, 2002). The three major types of striatal interneurons characteristic of the mammalian basal ganglia (cholinergic, $PARV^+$, and somatostatinergic) are also present in the striatum in living reptiles, although they are not as highly abundant as in mammals (Powers and Reiner, 1993; Reiner and Carraway, 1987; Reiner *et al.*, 1998). The basal ganglia in reptiles has its major output to motor areas via a projection to the pretectum and to the tegmentum (a substantia nigra pars reticulata (SNr) homologue), which affect head and eye movements

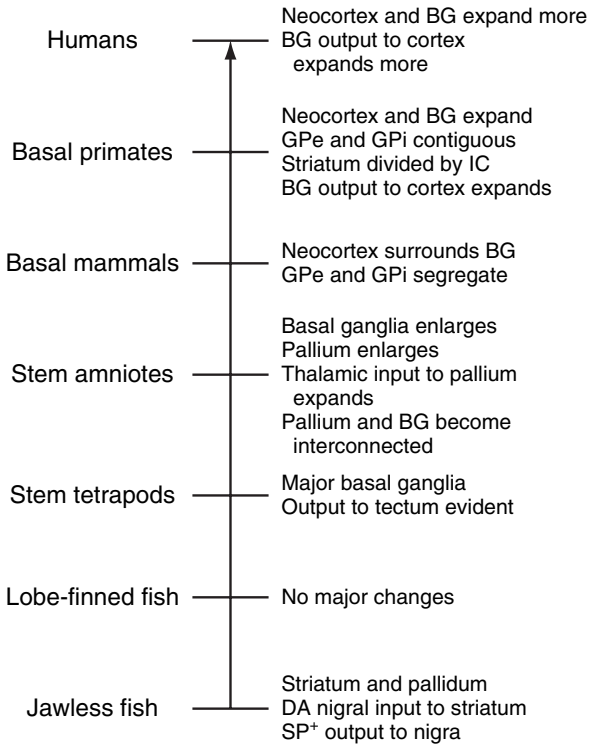

Figure 5 Time line indicating the points in the evolutionary line from jawless fish to humans at which major changes occurred in the basal ganglia.

by input to tectal neurons with descending projections to brainstem premotor cell groups (Reiner *et al*., 1980, 1998; Medina and Smeets, 1991).

3.29.3.2 Birds

Birds evolved from archosaurian reptiles, of which crocodilians are the only other living group (Chiappe, 1995). Unsurprisingly, therefore, the basal ganglia in birds highly resembles that in reptiles, with the main differences in the available data stemming from the overall telencephalic enlargement in birds and the deeper insights into basal ganglia anatomy and function stemming from the more extensively studied nature of birds (Table 1; Figure 4). For example, as in reptiles, the ventrolateral sector of the avian telencephalon contains both a striatum and a globus pallidus, as defined by neurochemical, hodological, and developmental molecular criteria (Reiner *et al*., 1998). Also as in reptiles, immunolabeling shows that the striatal sector contains SP^+ and ENK^+ neurons (Reiner *et al*., 1998), with the identity of the striatum further confirmed by the expression of GAD or GABA (Veenman and Reiner, 1994), and a homologue of *Dlx1/2* (Smith-Fernandez *et al*., 1998; Puelles *et al*., 2000). Moreover, the SP^+ and ENK^+ striatal neurons give rise to projections to a ventrocaudal cell group that is comparable to globus pallidus, because of this striatal input, because it contains large GABAergic neurons that also contain LANT6, and because its neurons express *Nkx2.1* (Karten and Dubbeldam, 1973; Reiner and Carraway, 1987; Veenman and Reiner, 1994; Reiner *et al*., 1998; Puelles *et al*., 2000). As in reptiles, this cell group is more laterally migrated than in lobe-finned fish, amphibians, or mammals, indicating that the pallidum was more medially located in stem amniotes and that the lateral migration of pallidal neurons evolved in the reptile–bird lineage (Figure 4). The three major types of striatal interneurons characteristic of the mammalian basal ganglia (cholinergic, $PARV^+$, and somatostatinergic) are present in striatum in living birds and as abundant as those in mammals (Medina and Reiner, 1994; Reiner and Carraway, 1987; Reiner *et al*., 1998). The reptilian striatum receives a dopaminergic input from the midbrain and these dopaminergic neurons receive a return projection from SP^+ striatal neurons (Reiner *et al*., 1994, 1998). As in reptiles and mammals, dopaminergic effects on striatum are mediated by D1 and D2 dopamine receptors, with DA agonists inducing hyperkinesia and DA antagonism yielding hypokinesia, indicating a similar role of the dopaminergic system in modulating striatal output as in mammals (Nistico *et al*., 1983; Richfield *et al*., 1987; Dietl and Palacios, 1988; Yanai *et al*., 1995; Reiner *et al*., 1998; Sun and Reiner, 2000; Reiner, 2002).

The pallium occupies the dorsolateral sector of the telencephalon, is much expanded in birds, and is the source of a massive excitatory input to the striatum (Veenman *et al*., 1995; Veenman and Reiner, 1996), with the thalamus also providing excitatory input (Wild, 1987; Reiner *et al*., 1998). The communication between pallium and striatum in birds appears to be mediated by the same two corticostriatal cell types as in mammals (Cowan and Wilson, 1994; Veenman *et al*., 1995; Reiner *et al*., 2001, 2003) and by the same cell type-specific glutamate receptors in striatum as in mammals (Reiner, 2002). The avian basal ganglia has its major output to motor areas via a projection to the pretectum and the tegmentum (an SNr homologue), which affect head and eye movements by input to tectal neurons with descending premotor and motor projections (Reiner *et al*., 1998). Additionally, birds possess a correspondent of the mammalian striato-pallido-thalamic circuit to motor cortex (Medina *et al*., 1997; Medina and Reiner, 2000), which suggests that one may be present in reptiles as well (see Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor

Cortices?). These various striatal output pathways to pretectum, tegmentum, and thalamus are all direct pathway type outputs that appear to facilitate behavior (Reiner *et al*., 1998). Birds have also been shown to possess a subthalamic nucleus and indirect pathway circuitry, as well (Jiao *et al*., 2000). The organization of the avian basal ganglia into direct and indirect striatal output circuits closely resembling those in mammals, and the presence of both SP^+ and ENK^+ striatal outputs in reptiles as well, suggests that the direct–indirect pathway plan of basal ganglia functional organization was already present in stem amniotes (Reiner, 2002).

3.29.3.3 Mammals

A few major changes in telencephalic morphology appear to have occurred in the evolution of the mammalian basal ganglia from the inferred stem amniote condition. First, from a simple, poorly laminated state, the pallium evolved into the multilayered neocortex that came to wrap around the basal ganglia (Karten, 1969; Puelles *et al*., 2000; Reiner, 2000, 2002). As a consequence, the basal ganglia occupies a more central position in the telencephalon than it does in nonmammals (Figures 2–4). The development of the basal ganglia versus the neocortex, however, reflects its ancestral basal position. The globus pallidus develops from an *Nkx2.1*- and *Dlx1/2*-expressing bulge (called the medial ganglionic eminence, or MGE) at the lower aspect of the lateral telencephalic wall and the striatum develops from *Dlx1/2*-expressing bulge that does not express *Nkx2.1* just above the MGE (called the lateral ganglionic eminence). By a process of extensive lateroventral migration from the pallium, where it meets the subpallium, the neocortex comes to surround the basal ganglia in mammals (Alvarez-Bolado and Swanson, 1996). Unlike in nonmammals, in which GPi and GPe neurons are intermingled, in mammals these pallidal populations occupy separate sectors, in either of two arrangements (Figure 5). In primates, the GPe and GPi are contiguous and distinguishable by their differential neurochemistry, namely, an enrichment in ENK^+ terminals from striatum in GPe and an enrichment in SP^+ terminals from striatum in GPi (Parent, 1986; Reiner *et al*., 1999; Hardman *et al*., 2002). All other mammal groups show a pallidal arrangement that must therefore be the primitive pattern for mammals. In this primitive pallidal pattern, the GPi and GPe are spatially separated, with the GPi enveloped by the internal capsule and seemingly dragged medially to a thalamic proximity. The gap between the two pallidal segments is so large that they have customarily been identified by different names than in primates: the globus pallidus instead of the GPe and the entopeduncular nucleus instead of the GPi. The globus pallidus is, however, clearly homologous to GPe and the entopeduncular nucleus to GPi. As a result, some neuroanatomical atlases for rodents embrace the primate names for the pallidal segments (Paxinos and Watson, 1998). Since it seems generally sound and parsimonious to call homologous structures by the same name (Reiner *et al*., 2004), this policy will be employed here as well.

The primate basal ganglia is also distinguished by being divided by the internal capsule into parts, called the caudate and putamen (Figure 4). Nonprimate mammals vary in the extent to which the striatum is divided by the internal capsule into a caudate and putamen, generally as a function of cortical development (Ariëns-Kappers *et al*., 1936; Parent, 1986). In many mammals with a lissencephalic cerebral cortex, such as some rodents, insectivores, bats, and monotremes, the caudate and putamen are not separated by the internal capsule and rather are pierced by myriad separate thalamocortical and corticothalamic fascicles that coalesce to form the internal capsule ventromedial to the striatum. In other mammalian groups, such as carnivores, ungulates, and some South American rodents, which independently evolved a gyrencephalic cortex, the striatum is divided by an internal capsule, but the point of division varies and differs from that in primates. This variation in internal capsule placement reinforces the notion that it, as well as cortex enlargement and convolution, evolved separately in different mammalian orders (Northcutt and Kaas, 1995). Since mammalian groups that are presumptively closer to the basal mammalian condition, such as monotremes and insectivores, do not show striatal division by the internal capsule, an undivided striatum is likely to be primitive for mammals.

There appear to be no noteworthy differences between mammals and sauropsids in the major types of neurons making up the striatum and pallidum (Table 1). In both amniote groups, the striatum consists of two main neurochemically distinct types of neurons, the SP^+ and the ENK^+ GABAergic neurons, and three types of interneurons, the cholinergic, the $PARV^+$, and the somatostatinergic (Graybiel, 1990; Gerfen, 1992; Reiner *et al*., 1998). In both groups, the projection neurons far outnumber the interneurons and pallidal neurons are large and GABAergic. Moreover, in mammals as in sauropsids, basal ganglia circuitry is organized into the direct–indirect pathway plan (Albin *et al*., 1989; DeLong, 1990; Gerfen, 1992; Reiner *et al*., 1998).

Massive glutamatergic inputs to striatum arise from cortex and thalamus, and a massive dopaminergic input to striatum arises from the substantia nigra pars compacta (Gerfen, 1992; Reiner *et al*., 1998), and the general role of the basal ganglia in motor learning and control seems similar in all amniotes. Mammals do differ from birds and reptiles in that they lack an obvious correspondent of the basal ganglia output to midbrain via pretectum (Table 1; Figure 5; Reiner *et al*., 1998), and their output to motor cortices via the thalamus seems instead more prominently developed, especially in primates (Albin *et al*., 1989; DeLong, 1990; Gerfen, 1992; Reiner *et al*., 1998). Additionally, mammalian striatum is compartmentalized into a network of interlaced zones called striosomes and a much larger sector in which the striosomes are embedded called the striatal matrix (Graybiel, 1990; Gerfen, 1992). These two striatal sectors, which differ in their connectivity with cortex and midbrain, consist of neuronal populations that are more uniformly interspersed in birds and reptiles (Reiner *et al*., 1998). Thus, striosomes are not evident in the striatum of birds or reptiles, but are in all mammals (Künzle, 2005).

Little is known about diversity in basal ganglia organization among mammals, largely because most hodological and neurochemical studies in mammals have focused on only a few groups: rats, mice, cats, and monkeys. Stephan (1979) compared the volume of the striatum in various insectivore, prosimian, and simian species. Although the raw data indicated that striatal volume as a percentage of the telencephalon decreased from approximately 8% to 3% from insectivores to humans, scaling according to body weight revealed an increase in striatal size (relative to body size) from insectivores to prosimians to simians. Along these lines, Stephan (1979) specifically noted that the human striatum would be 14 times larger than that of a basal insectivore of human size. The human cerebral cortex would be larger yet, approximately 30 times the size of that in a basal insectivore. Striatal enlargement, thus, in evolution from basal mammals through primates has contributed to overall telencephalic enlargement, but less so than has expansion and areal diversification of the neocortex (Stephan and Andy, 1969). Neocortical expansion and diversification, in particular, exceed striatal enlargement in the primate radiation from prosimians to simians. Another morphometric study using a slightly different approach also concluded that both neocortex and striatum had expanded progressively in the primate radiation, with the neocortical expansion outpacing the striatal expansion, especially in humans (Clark *et al*., 2001). These findings confirm, as suggested by the connectivity data, that cortex and striatum are functionally linked and that cortex does not take over the role of striatum, as had been presumed in early twentieth century ideas about telencephalic evolution (Ariëns-Kappers *et al*., 1936; Herrick, 1948, 1956).

Hardman *et al*. (2002) performed a quantitative morphological study on the size and neuronal abundance of several additional basal ganglia cell groups or targets in rats and several primate species. Immunolabeling for various cell type-specific markers was used to objectively define the extent of the different cell groups measured, which included the GPe, GPi, STN, substantia nigra pars compacta, and SNr. Corrected for overall brain size, the sizes of the GPe, GPi, and STN in relation to one another were relatively constant across the groups examined, with GPe being larger (and more neuron rich relative to brain neuronal abundance) than GPi and with GPi being larger than STN in each species. The substantia nigra was, however, relatively larger and more neuron rich (relative to brain neuronal abundance) in rodents than in the primates examined. This is likely to reflect a relatively greater importance of striato-SNr circuitry in motor control than striato-GPi circuitry in rat than in primate. Whether this is generally true of nonprimates is uncertain, but it suggests a possibly increased role of basal ganglia outflow to motor cortex in primates than nonprimates (Figure 5).

3.29.4 Mammalian Basal Ganglia Evolution – Outdated Concepts and Terminology

The preceding overview of basal ganglia evolution in vertebrates reveals that the striatum and pallidum are ancient structures, with both apparently being present in jawless fish ancestral to modern jawed vertebrates (Table 1; Figure 5). Thus, the notion that the pallidum (i.e., the so-called paleostriatum) evolved first and is older than the striatum (i.e., the so-called neostriatum) is incorrect. Because the terms paleostriatum and neostriatum reflect and perpetuate outdated ideas about basal ganglia evolution, we recommend their abandonment. Although the evidence was not reviewed here, the notion that the paleocortex (olfactory or pyriform cortex), archicortex (hippocampus), and neocortex evolved successively in evolution is also flawed, since paleocortex and archicortex seem to be equally ancient parts of the vertebrate telencephalon (Northcutt, 1981a; Northcutt and Kaas, 1995; Rodriguez *et al*., 2002). Because the term neocortex refers to a new, uniquely mammalian structure, this

term is, however, debatably suitable. Similarly, the notion that the basal ganglia are a part of the motor system separate from the descending cortical pyramidal tracts is belied by the extensive interconnections of the cortex and basal ganglia and by the output of the basal ganglia to motor cortices. Thus, the classification of the basal ganglia as part of a motor system called the extrapyramidal system is also suspect. The history of basal ganglia evolution seems to be characterized by an increase in neuron number as the telencephalon expanded during the anamniote–amniote transition, with the elaboration of prominent cortical glutamatergic inputs and midbrain dopaminergic inputs, and by an increased role for telencephalic circuitry in motor control all occurring in stem amniotes (Figure 5). In mammals, especially the primate lineage, this trend has been furthered. Nonetheless, the basic direct–indirect pathway circuit plan by which the basal ganglia regulates movement may have already been in place in early anamniotes.

Acknowledgments

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3.30 Evolution of the Cerebellum

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Glossary

allometry The approach to relate the size of (subdivisions of) the brain relative to the size of the body (body parts).

cerebellum The small brain. Posterior division of the brain. Connected to the brainstem through the cerebellar peduncles. The mammalian cerebellum is characterized by many transverse fissures of different depths. Vertebrate cerebella share the lattice character of the cerebellar cortex: its main elements, the Purkinje cells and the granule cells are oriented at right angles. Cerebellar-like structures, such as the dorsal cochlear nuclei, contain these elements in a less geometric structure.

clone The cells produced by one progenitor cell.

endocast The cast of the interior of a skull, which may reveal some aspects of the size and shape of the brain, formerly contained therein.

folia The smallest, leaf-like units of the cerebellar surface. A folium is oriented at right angles to the long axis of a folial chain and delimited by two transverse fissures.

folial chain Two deep longitudinal fissures subdivide the mammalian cerebellum in a median region, the vermis and two lateral hemispheres (Bolk, 1906). Transverse fissures of different depth subdivide both vermis and hemispheres in folia, lobules, and lobes. The cortex between adjacent folia within vermis and hemisphere, always is continuous. The term 'folial chain' accentuates this continuity.

folial pattern The combination of longitudinal and transverse fissures divides the cerebellar surface into its units. Variations in size and internal composition of these units, determine their positioning and, thus, the gross appearance of the cerebellum. The resulting pattern of these units is named after the smallest units, the folia.

function The function of the cerebellum can be studied at different levels. The algorithm performed by the cerebellar cortex is the most basic function, but is still unknown. Recorded activity or connections of certain parts of the cerebellum may indicate whether this unknown algorithm is used within the context of a certain functional system. Lesions or diseases affecting the

	cerebellum or its parts cause symptoms and signs that can be interpreted as a consequence of an alleged function of the cerebellum.
lineage	The origin of a particular cell type from a particular progenitor cell.
lineage restriction	The spatial restriction by temporary boundaries of groups of progenitor cells and their offspring.
lobe, lobule	Collections of folia delimited by deep, transverse fissures. The subdivision of the cerebellum in lobes, and of the folial chains in lobules is an arbitrary decision, depending on the importance given to particular transverse fissures of varying depth and their continuity in vermis and hemispheres.
module	Repeating neural unit with a specific structure, composition, and connections.
nomenclature	Set of terms used to indicate a coherent set of structures. Classical nomenclatures use resemblance of structures to everyday objects. Comparative anatomical nomenclatures use criteria derived from the variability and the development of a structure. Use of a particular nomenclature supposes knowledge of these criteria.
zonal pattern	Purkinje cells are distributed in multiple parallel zones that extend perpendicular to the transverse cerebellar fissures. Criteria to distinguish these zones are (1) the projection of the Purkinje cells of a particular zone to a particular cerebellar or vestibular target nucleus, (2) the innervation of each Purkinje cell zone by climbing fibers from a particular subdivision of the inferior olive, (3) the chemoarchitecture of the Purkinje cells of a particular zone. The disposition and extent of the longitudinal zones determine the zonal pattern. Zonal patterns defined by different criteria generally are congruent.

3.30.1 Introduction

This article aims to deal with the structure and function of the mammalian cerebellum from an evolutionary point of view. This approach can be only tentative since there are few clues from endocasts of fossil skulls; consequently, the fossil record can give only limited evidence on the evolution of soft tissues. In some vertebrate endocasts, for example, there is a possible cerebellar component that is encased within the petrous bone. In those living mammals in which it is present, this cerebellar subdivision is part of the dorsal paraflocculus, although it is sometimes mislabeled as flocculus in the literature. Since there is little data on the relative size of the entire cerebellum or its parts that can be derived from fossil evidence, and there are no guaranteed living primitive forms, we emphasize insights from comparative anatomy and development. As Bolk (1906) pointed out, there is a common plan to the structure of all mammalian cerebella. Moreover, the histology and the microcircuitry of the cerebellar cortex are preserved features among vertebrates. Comparative anatomy reveals the relative size of the cerebellum and its subdivisions and the variations upon the common plan among mammals, and hence may provide clues as to its evolution. Studies on the connectivity of the cerebellum give indications on the use of the stereotyped cerebellar cortical circuit in different functional systems, and may provide clues on adaptations in cerebellar structure during evolution. Cerebellar morphogenesis and its genetic control provide information on the crucial stages where mutations make these adaptations possible. The link between structure and function, so evident for many other parts of the central nervous system, unfortunately is lacking for the cerebellum. This aspect, the missing link in cerebellar evolution, is discussed in the final section of this article.

3.30.2 Gross Anatomy of the Mammalian Cerebellum

Figure 1 shows drawings of the human cerebellum. The cerebellum is made up of an extensive cerebellar cortex and its associated deep nuclei, which are located within the white matter. Only a small percentage of the cortex is visible on the surface, since most of the cortex is buried on the banks and in the depths of the fissures. The cortex is divided into a medial vermis and lateral hemispheres. Vermis is thus labeled because of its alleged resemblance to a worm. In the human brain, the vermis is overshadowed by the massive cerebellar hemispheres.

The cerebellum is also divisible into three lobes: anterior, posterior, and flocculonodular.

Figure 2 illustrates a midsagittal section through the cerebellum showing the cortex folded into lobes and lobules by deep fissures. Two of these fissures are of particular importance, because they segregate the three functionally important anterior–posterior divisions. The deepest of these is the primary fissure. The cerebellum in front of the primary fissure is the anterior lobe. Behind the primary fissure is the posterior lobe. The smaller posterolateral fissure separates the posterior lobe from the flocculonodular lobe.

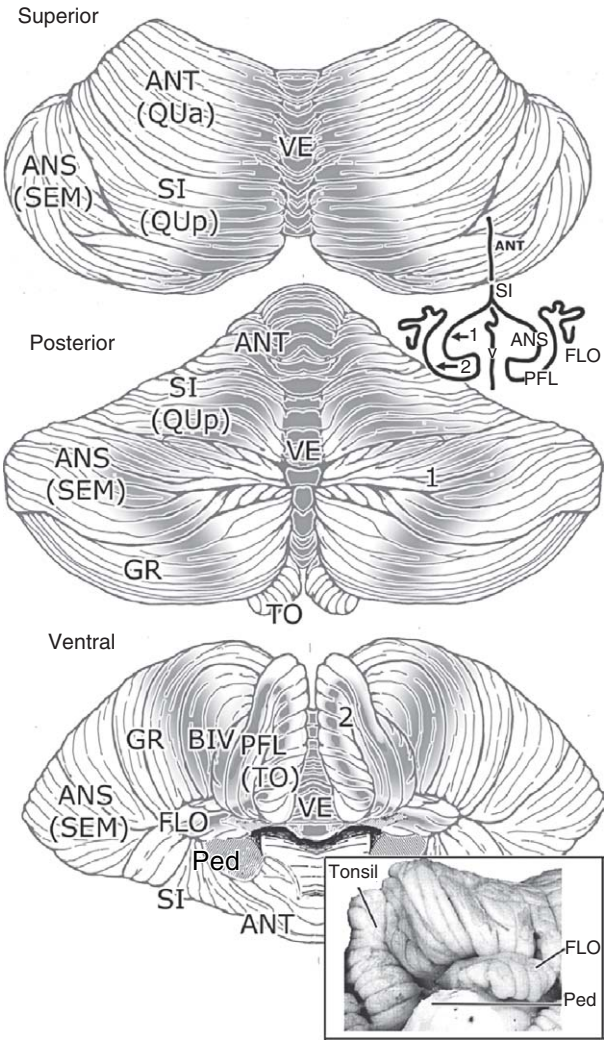

Figure 1 Three diagrams of the human cerebellum. Lobules are indicated with the nomenclature of Bolk (1906), classical names are given in parentheses. The grey band indicates the direction of the folial chains of vermis and hemispheres. The two loops in the folial chain of the hemisphere are indicated as 1 and 2. Insets show Bolk's (1906) wire diagram of the fundamental structure of the mammalian cerebellum and a photograph of the folial loop of the tonsilla. ANS, ansiform lobule; ANT, anterior lobe; BIV, biventral lobule; FLO, flocculus; GR, gracile lobule; Ped, cerebellar peduncles; PFL, paraflocculus; QUa, anterior quadrangular lobule; QUp, posterior quadrangular lobule; SEM, semilunar lobules; SI, lobulus simplex; TO, tonsilla; VE, vermis. Inset Reproduced from Rohen, J. W. and Yokochi, C. 1988. Human Anatomy. Photographic Atlas of Systematic and Regional Anatomy, 2nd edn. Schattauer. Voogd, J. 2003. The human cerebellum. *J. Chem. Neuroanat.* 26, 243–252.

Figure 3 shows a transverse section through the human cerebellum, demonstrating the deeply folded cerebellar cortex, with the cerebellar nuclei embedded within the white matter. The cortex is larger than the nuclei. The pattern of projection from the cortex to the nuclei is orderly. The most medial cortex projects to the middle (fastigial) nucleus and to the lateral vestibular nucleus. More laterally, the cortex projects to the interposed nuclei, called globose and emboliform in the human cerebellum. The cerebellar hemispheres project to the most lateral nucleus, the dentate nucleus. The functional units of the cerebellum are a series of long, parasagittal strips of cortex and their afferent and efferent connections. These will be discussed in detail in a later section.

Over several hundred years, anatomists have been identifying various finer subdivisions of the cerebellum, and there are many and varied systems of nomenclature for these subdivisions (Angevine *et al.*, 1961). Names were assigned long before there was any recognition of whether these subdivisions might be of functional importance. Thus, the most rostral region of the cerebellum was called lingula (originally linguetta) by Malacarne (1776) because it looked to him like a cat's tongue. Most of the traditional nomenclature is based on such superficial resemblance to a tonsil, a ball of wool, or

similarity to a particular geometric shape, such as a pyramid.

3.30.3 Comparative Anatomy of the Folial Pattern

The cell types and histological structure of the cerebellar cortex are similar among all vertebrates, and they are virtually identical among mammals and lower vertebrates. There is, however, great variability in the relative size and in the morphology of its lobes and lobules. The avian cerebellum, for instance, lacks a clear border between vermis and hemispheres. It consists of a series of simple folia, arranged like the pages of a book. The very small hemispheres are represented by the lateral, unfoliated cortex, and the auricle is the homologue of the mammalian flocculus.

Differences in shape and connections of the cerebellum among mammals are obvious (Voogd *et al.*, 1998). The comparative anatomy of the folial pattern of the mammalian cerebellum is based on gross inspection and dissection (Smith, 1903a, 1903b; Riley, 1928, 1929), and on its development (Kuithan, 1894; Stroud, 1898; Bradley, 1904; Bolk, 1906; Larsell, 1937, 1952, 1953, 1970). Larsell and Jansen (1972) combined both approaches. Bolk studied development in order to confirm his ideas on the basic folial pattern of the mammalian cerebellum. For Larsell, the basic pattern is revealed in its development. Inspection and dissection served to confirm this pattern in the adult.

Bolk (1906) emphasized the lack of a distinct border between vermis and hemispheres in the anterior lobe, and in the region immediately behind the primary fissure, known as lobulus simplex, which belongs to the posterior lobe. The transverse fissures in the anterior lobe and simplex run uninterruptedly over the entire width of the cerebellum. Caudal to the lobulus simplex, the cerebellum splits into the median folial chain of the vermis and the two folial chains of the hemispheres. By simple dissection, Bolk revealed the continuity of the folial chain in vermis and hemispheres in all of the 69 mammalian species that he studied (Figures 1, inset, and 4).

The vermis (lobule VII), immediately caudal to the lobulus simplex, is straight in many vertebrates, but it is bent in several carnivores, ungulates, and primates. The caudal lobules of the vermis (VIII, the pyramis; IX, the uvula; and X, the nodulus) show far less variation among species. The folial chain of the hemisphere forms two continuous loops, with a paramedian

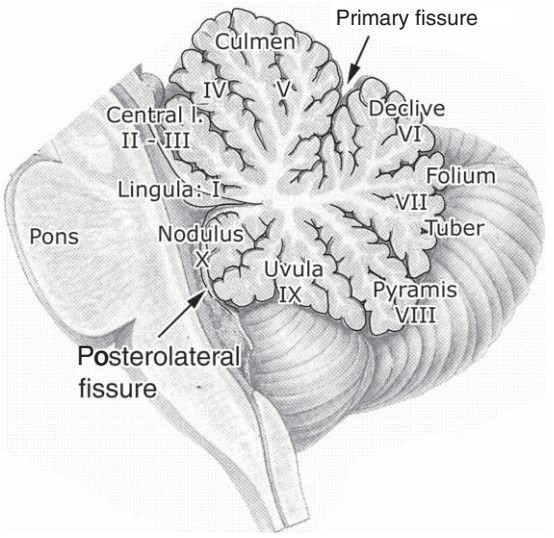

Figure 2 Sagittal section through the human brainstem and cerebellum. Modified from Nieuwenhuys, R., Voogd, J., and van Huijzen, chr. 1988. The Human Central Nervous System. Springer.

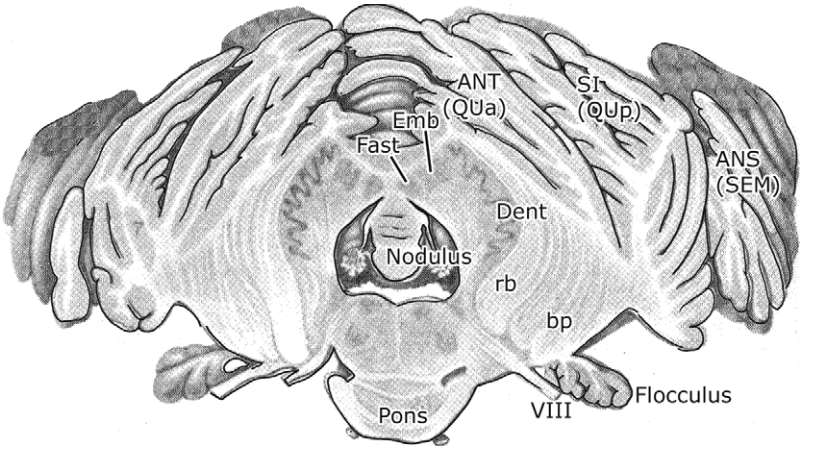

Figure 3 Transverse section through the human brainstem and cerebellum. ANS, ansiform lobule; ANT, anterior lobe; bp, brachium pontis; Dent, dentate nucleus; Emb, emboliform nucleus; Fast, fastigial nucleus; QUa, anterior quadrangular lobule; QUp, posterior quadrangular lobule; rb, restiform body; Sem, semilunar lobule; SI, lobulus simplex. Modified from Nieuwenhuys, R., Voogd, J., and van Huijzen, Chr. 1988. The Human Central Nervous System. Springer.

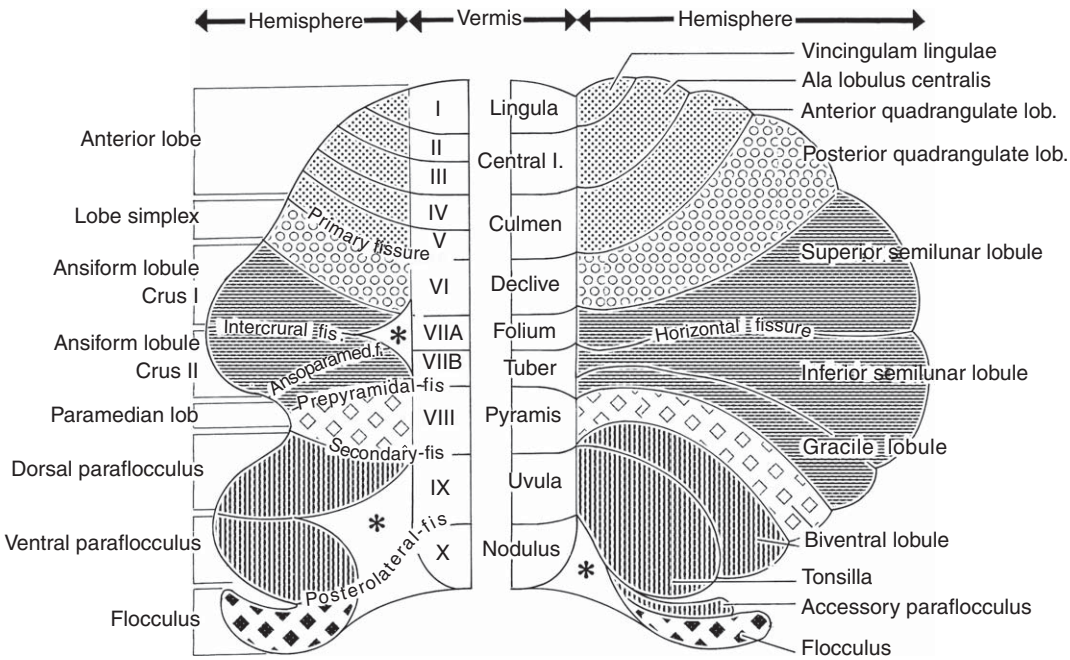

Figure 4 Diagram of the comparative anatomical nomenclature of the cerebellum (left panel) and of the classical nomenclature (right panel). The asterisks indicate superficial medullary areas not covered by cortex. From Voogd, J., Nieuwenhuys, R., van Dongen, P. A. M., ten Donkelaar, H. J. 1998. Mammals. In: The Central Nervous System of Vertebrates (*eds*. R. Nieuwenhuys, H. J. ten Donkelaar, and C. Nicholson), pp.1637–2097. Springer.

segment between the two loops. The rostral loop is known as the ansiform lobule, the caudal loop corresponds to the paraflocculus. The intermediate segment is the paramedian lobule. The caudal-most segment of the folial chain of the hemisphere is turned back upon the paraflocculus as the flocculus, Bolk's uncus terminalis. This basic mammalian folial pattern is present in all mammalian species (Figure 5). In Bolk's view, the anterior lobe and the lobulus simplex form a single growth center. Bolk considered the more caudal lobules of the folial chains of vermis and hemispheres to be mutually independent growth centers. Sultan and Braitenberg (1993) elaborated on Bolk's concept of the folial chains and illustrated this configuration in many mammalian species (Figure 6).

According to Larsell, the posterolateral and primary fissures are the earliest fissures to appear. Together with fissures that form later, they subdivide the cerebellum into 10 subdivisions, from the most rostral lobule I (lingula) to the most caudal lobule X (nodulus). Lobules I–V constitute the anterior lobe; lobules VI–IX are the vermian part of the posterior lobe; lobule X corresponds to the nodulus. Each of the vermian lobules is associated with a lobule in the hemisphere, indicated with the same Roman numeral, with the prefix H. Larsell emphasized the mediolateral continuity of the lobules of vermis and hemisphere. In his words, ". . . it is (also) clear in the adult and in the fetus that the lateral parts, namely lobulus ansiformis, paraflocculus and the lateral continuation of the pyramis are merely lateral extensions of the medial portion" (Larsell, 1937, p. 605).

Bolk attached more importance to the independence of the lobules of the folial chains of vermis and hemispheres. This independence was also emphasized in his studies on the development of the folial pattern of the human cerebellum (Bolk, 1906). He distinguished three rostrocaudal regions. In the rostral cerebellum, comprising the anterior lobe and the lobulus simplex, all of the fissures first appear in the midline and then grow out laterally. In an intermediate region, corresponding to lobule VII and rostral VIII (the pyramis) and the ansiform and paramedian lobules, the interlobular fissures arise medially, but the intralobular fissures arise independently in vermis and hemispheres. In the caudal region of the cerebellum, all of the fissures arise independently in vermis and hemispheres.

There are variations among mammals in the length and width of certain segments of the folial chains. The greatest amount of variability is seen in vermian lobule VII, in the ansiform lobule, and in the paraflocculus. Lobulus simplex, the vermian lobules VIII–X, the paramedian lobule, and the flocculus are less variable, although the width of the folia may vary. In some species, these variations

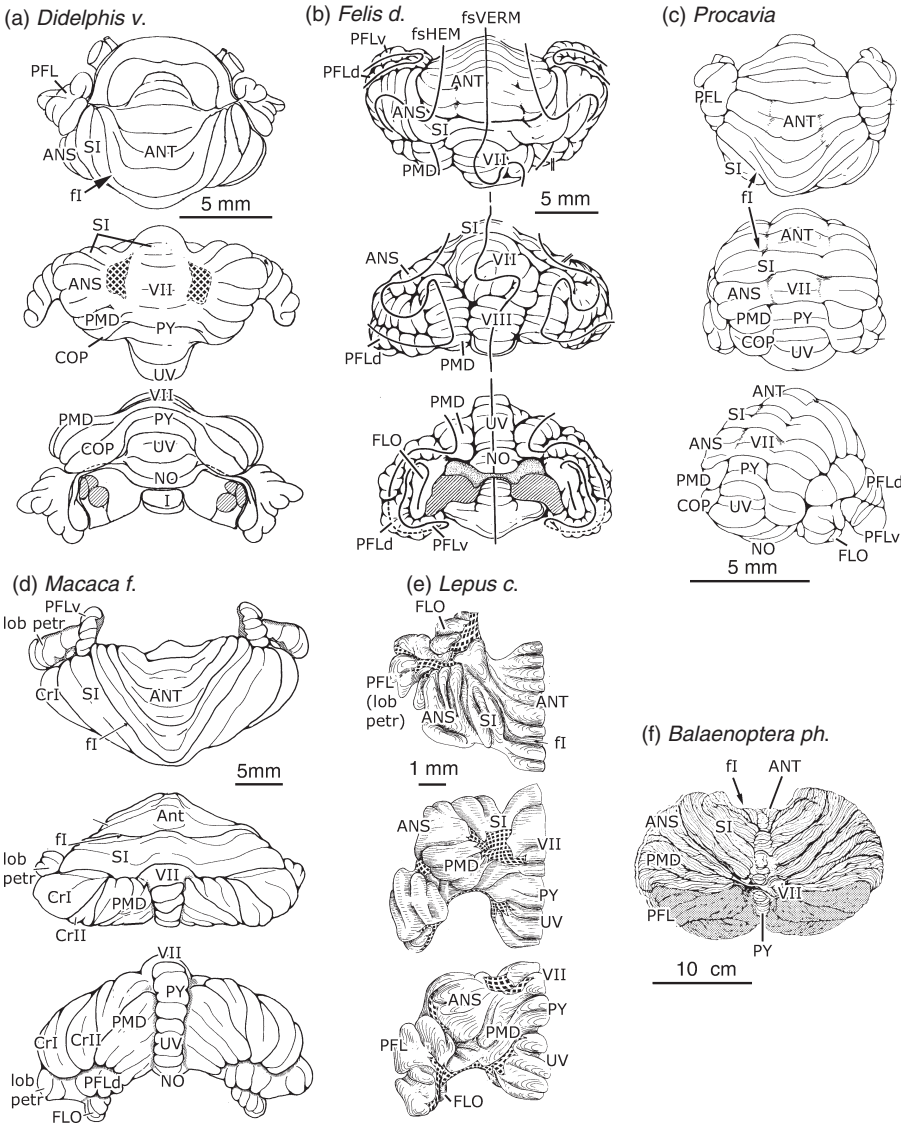

Figure 5 Anterior, dorsal, and caudal (ventral) views of different mammalian cerebella. In (b) the direction of the folial chains of vermis and hemispheres is indicated for the cerebellum of the cat. Medullary areas not covered by cortex are indicated by filled squares in (a) and (e). Note approximately equal width of the folial chains of vermis and hemispheres in opossum (a), cat (b), coney (c), and rabbit (e), and greater width of the hemiphere in monkey (d) and whale (f). A folial loop in the ansiform lobule is lacking in the coney (c). Size and shape of the visual vermis (lobule VII) differs in different mammals: it is large and convoluted in the cat (b), small and straight in the other species. Cetacea are characterized by the large, overall size of the cerebellar hemisphere, especially of the paraflocculus (shaded in f). ANS, ansiform lobule; ANT, anterior lobe; COP, copula pyramidis; CrI (II) crus I (II) of the ansiform lobule; fI, primary fissure; fsHEM, folial chain of the hemisphere; fsVerm, folial chain of the vermis; lob petr, petrosal lobule; NO, nodulus; PFL (dv), paraflocculus (dorsalis, ventralis); PMD, paramedian lobule; PY, pyramis; SI, lobulus simplex; UV, uvula; VII, lobule VII. a, Reproduced from Larsell, O. 1970. The Comparative Anatomy and Histology of the Cerebellum from Monotremes through Primates. University of Minnesota Press. b–d, Reproduced from Voogd, J., Nieuwenhuys, R., van Dongen, P. A. M., and ten Donkelaar, H. J. 1998. Mammals. In: The Central Nervous System of Vertebrates (*eds.* R. Nieuwenhuys, H. J. ten Donkelaar, and C. Nicholson), pp.1637–2097. Springer. e, Reproduced from Thunissen, I. 1990. Vestibulocerebellar and Vestibulo-Oculomotor Relations in the Rabbit. Thesis, University of Rotterdam. f, Reproduced from Jansen, J. and Brodal, A. 1954. Aspects of Cerebellar Anatomy. Grundt Tanum.

can be related to the zonal organization of the individual lobules. The details of zonal organization will be discussed in a later section.

Bolk's fundamental plan of the cerebellum was confirmed in Riley's (1928, 1929) studies of the gross anatomy of the mammalian cerebellum and can be recognized in the plots of the number and the width of the folia of several mammalian species of Sultan and Braitenberg (1993), illustrated in Figure 6.

Parallel fibers are the axons of granule cells. They extend within the molecular layer, where they

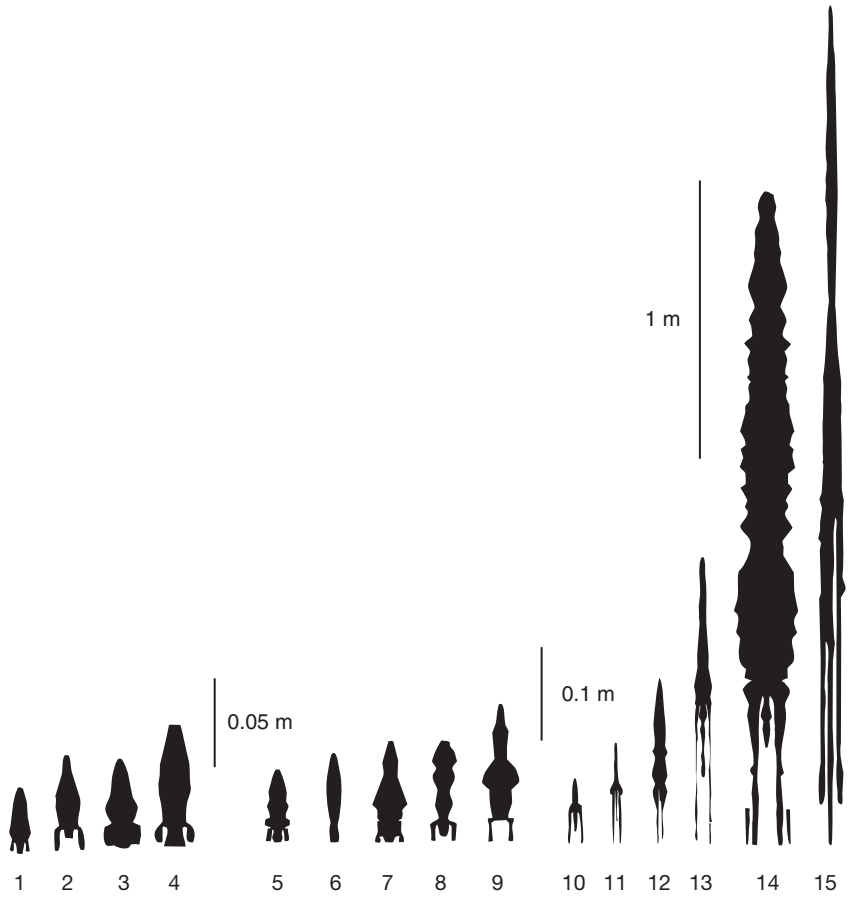

Figure 6 Outlines of the shapes of the cerebellar cortices, obtained by connecting the ends of the most prominent folia. The scale is the same in the laterolateral and anteroposterior direction. Note division of posterior cerebellum in the folial chains of vermis and hemispheres, and the relative width and length of these chains in different species. 1, Mouse; 2, bat; 3, flying fox; 4, guinea pig; 5, rabbit; 6, pigeon; 7, hare; 8, chinchilla; 9, squirrel; 10, dog; 11, cat; 12, macaque; 13, sheep; 14, human; 15, bovine. Magnifications differ for the diagrams 1–4, 5–9, and 10–15. Adapted from Sultan, F. and Braitenberg, V. 1993. Shapes and sizes of different mammalian cerebella. A study in quantitative comparative neuroanatomy *J. Hirnforsch.* 34, 79–92.

contact Purkinje cell dendrites. Mammals vary in the extent to which there is continuity of the parallel fibers between the vermis and hemispheres. This continuity is complete in the anterior lobe, the lobulus simplex, and between lobule VIII (the pyramis) and the paramedian lobule. The cortex is entirely or partially interrupted between lobule VII and the ansiform lobule, and between the caudal vermis (lobules IX, the uvula, and X, the nodulus) and the paraflocculus and the flocculus (Figures 5a and 5e) (Glickstein and Voogd, 1995).

3.30.4 Allometry and Cerebellar Size

Elephants and mice each have a four-chambered heart that works on the same general principles to pump blood around the body. The brain of an elephant, like its heart, is much larger than that of a mouse. To compare the weight of the brain or any body part between species, it is necessary to consider its relative, not its absolute size. Subdivisions of the brain will also vary with its total size. Allometry is an approach to dealing with such comparisons by plotting the size of each organ against total body size (see The Evolution of Human Brain and Body Growth Patterns). The same approach can be used to compare the relative size of one or another subdivision of the brain. The general form of the exponential equation that is used is typically in the form $\log Y = k \log X + \log b$, where X and Y are the two structures to be compared and k is the slope of a linear fit to the data. Thus, vast differences can be plotted in the same graph, and an exponential equation becomes linear. If brain weight is plotted against body weight in a log-log plot across a large number of mammalian species, there appears to be a satisfying linearity. But because a whale may weigh over 100 000 times more than a bat, important deviations from the linear fit may not be obvious. The same problem arises in studies in which the

volume of a brain subdivision is plotted against total brain weight (Finlay and Darlington, 1995).

Clark *et al.* (2001) compared the volume of brain subdivisions across several species of insectivores, tree shrews, and primates. They argue that although the telencephalon, and especially the cerebral cortex is relatively large in primates, the cerebellum remains a constant fraction of brain volume. On the basis of this analysis, they grouped mammalian species into several subtypes that they called cerebrotypes. Clark *et al.*'s conclusion about mammalian cerebrotypes was criticized on several fronts. De Winter and Oxnard (2001) used the same data set in a principal component analysis. Their results suggested that a grouping of species by locomotor types is more appropriate than the cerebrotypes postulated by Clark *et al.* Moreover, as Barton (2002) pointed out, Clark *et al.*'s data does, in fact, demonstrate an increase in the relative volume of the cerebellum among the species studied, but the increase proceeds at a slower rate than that of the cerebral cortex. Sultan (2002) has questioned the very basis of Clark *et al.*'s analysis. He argues convincingly that relative volume or weight is not an appropriate measure for the functional importance of a given brain subdivision. A crude volume estimate of subdivisions is inappropriate, since his own work clearly demonstrates that valid comparisons should be based not on volume but on surface extent of the cerebellum.

3.30.5 Cell Types and Cerebellar Circuitry

3.30.5.1 Histology of the Cerebellar Cortex

The histological structure of the cerebellar cortex was described and summarized by Ramon y Cajal (1911). Cajal paid attention to the histology of the cortex in lower vertebrates, but his 1911 description is mainly based on the situation in mammals. The following account also describes the cell types and the circuitry of the mammalian cerebellar cortex.

The cerebellum is made up of two fundamental subdivisions: a broad sheet of cells, the cerebellar cortex, and a group of deep cerebellar nuclei that are buried within the white matter (see The Evolution of the Cerebellum in Anthropoid Primates). The cerebellar cortex of all vertebrates shares several fundamental features (Nieuwenhuys *et al.*, 1998). Purkinje cells in all species constitute the main output element. The Purkinje cells receive two types of excitatory afferents: the climbing fibers, originating from the contralateral inferior olive, and the parallel fibers, which are the axons of granule cells. Granule cells receive their input from many sources outside of the cerebellum, all of which terminate as mossy fibers. The axons of Purkinje cells terminate in the cerebellar and vestibular nuclei.

Figures 7a and 7b are sketches showing the structure of the cerebellar cortex. Purkinje cell bodies form a single layer throughout the entire extent of the cortex. They have large flask-shaped cell bodies, and their axons constitute the only output from the cortex to the cerebellar nuclei. Purkinje cells are GABAergic and inhibitory. Their dendrites branch extensively in a plane perpendicular to the cerebellar folium. Their axon projects to the nuclei, giving off axonal collaterals within the cortex. By far the most numerous cells in the cerebellum, indeed in the entire mammalian brain, are granule cells. These are small cells, measuring about 7 μm in diameter, packed densely in the granular layer of the cortex. The axon of granule cells ascends to the outer molecular layer of the cortex, where it branches in a characteristic T fashion, and extends as a parallel fiber within the molecular layer. Parallel fibers are labeled as such because they are oriented parallel to the course of the cerebellar folia to which they project. The ascending axon of the granule cell and its parallel fiber branches contact Purkinje cell dendrites and dendrites of interneurons. In addition to Purkinje and granule cells, there are three other types of neurons, all three of which are inhibitory. Golgi cell bodies reside in the granular layer. Their dendrites extend into the molecular layer, where they are contacted by parallel fibers. Their axons ramify within the granular layer, where they terminate on dendrites of granule cells. Golgi cells use glycine as their inhibitory neurotransmitter. Basket and stellate cell bodies are in the molecular layer. Their dendrites and axons extend in a plane perpendicular to the long axis of the folia. Basket and stellate cells use GABA as their neurotransmitter. Basket cell axons give off several branches, each of which surrounds Purkinje cell bodies like a wicker basket. They have powerful inhibitory connections that are concentrated at the axon hillock of the Purkinje cell. Stellate cells inhibit the Purkinje cell dendrites.

Purkinje cells are activated by way of two totally independent systems of afferent fibers. Mossy fibers originate from the spinal cord, multiple centers in the lower brainstem, and the pontine nuclei. Mossy fibers branch extensively and terminate on the dendrites of the granule cells, which in turn connect to the Purkinje cells by way of parallel fibers. Some mossy fibers provide collaterals to the cerebellar nuclei. Each Purkinje cell also receives a completely different type of input, a single climbing fiber,

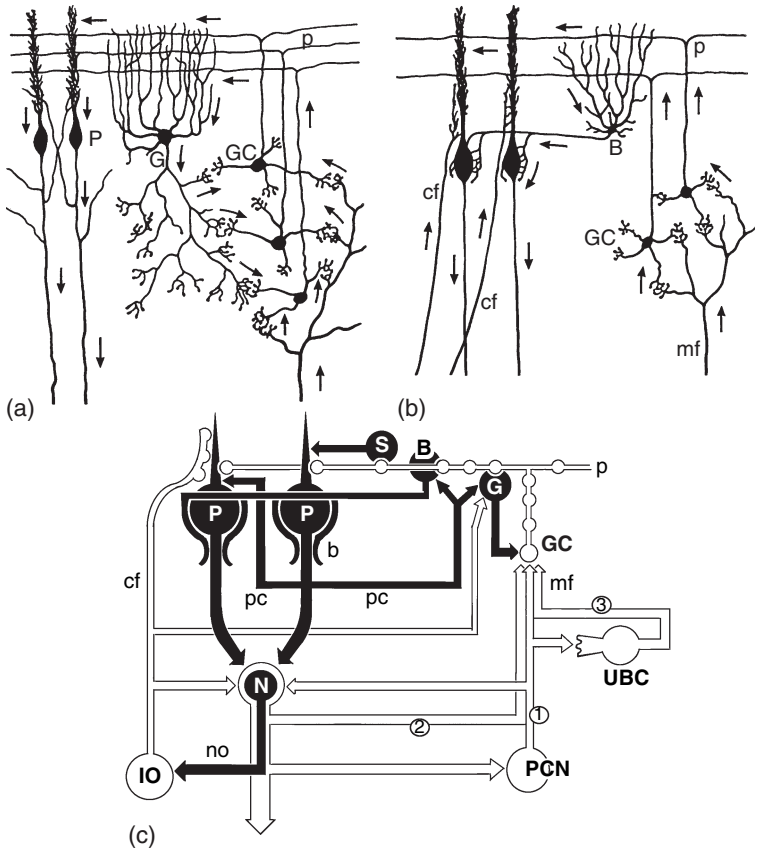

Figure 7 a, Diagram showing the main mossy fiber-granule-cell–Purkinje-cell circuit and the innervation of the granule cells by the axonal plexus of the Golgi cell. b, Diagram of the climbing fiber innervation of the Purkinje cells, and the basket cells with their axonal baskets surrounding the Purkinje cell perikarya. c, Diagram of the cerebellar circuitry. Inhibitory neurons are indicated in black. B, basket cell; b, pinceau of basket all axons; cf, climbing fiber; G, Golgi cell; GC, granule cell; IO, inferior olive; mf, mossy fiber; no, nucleo-olivary axons; pc, recurrent Purkinje cell axon collaterals; P, Purkinje cell; p, parallel fibers; PCN, precerebellar nuclei; S, stellate cell; UBC, unipolar brush cell; 1, extracerebellar mossy fiber; 2, nucleo-cortical mossy fiber; 3, mossy fiber collateral of unipolar brush cell. a and b, Redrawn from Ramon y Cajal, S. 1911. Histologie du système nerveux de l'homme et des vertebrés. Maloine.

because their terminations ascend along the dendrites of the Purkinje cells, making multiple and direct contacts. Climbing fibers all arise solely from the inferior olivary nucleus on the opposite side of the cerebellum, giving off collateral fibers to the cerebellar nuclei as they ascend to the cortex.

Both inputs to the Purkinje cells, the mossy fiber–parallel fiber system and the climbing fibers, are excitatory, but they terminate on different segments of the Purkinje cell dendritic tree. The climbing fiber terminates on short, stubby spines on the proximal, smooth portion of the dendrites. Parallel fibers contact long-necked spines of the distal spiny branchlets of the Purkinje cell dendritic tree.

The histology of the cerebellar cortex is very similar in mammals and in lower vertebrates. Purkinje cells, granule cells, and Golgi cells have been identified in all vertebrate genera. The main differences concern the lamination of the cortex, the spatial segregation of Purkinje cells and granule cells in certain forms, and the shape of the Purkinje cell dendritic tree. In fish, the proximal smooth dendrites with their climbing fiber afferents are located within the Purkinje cell layer and the spiny branchlets ascend into the molecular layer. In mammals, smooth and spiny branches are found throughout the molecular layer. In birds there is an intermediate arrangement in which the smooth branches are restricted to the lower half of the molecular layer (Nieuwenhuys *et al.*, 1998).

Cerebellar nuclei are not present in all lower vertebrates. In fish, the connections of the cerebellum with other parts of the central nervous system take their origin from cells that are located within the Purkinje cell layer (eurodendritic cells). Unlike the cerebellar nuclei, they are not buried in the white matter, but their axons, like those from nuclear cells in mammals, project to targets outside of the cerebellum. A similar type of cortical neuron with long, extracerebellar connections has been described only

in a fourth cerebellar layer of Pinnipedia (Ogawa, 1934).

The cerebellar nuclei are arranged from medial to lateral, with their major input from the overlying cerebellar cortex. The most medial cortex of the cerebellar vermis projects to the medial nucleus of the cerebellum, also called the fastigial nucleus (Figure 8). The lateral vestibular nucleus, which is in fact a fourth deep nucleus, receives its input from the lateral vermis. The most lateral fibers of the cerebellar hemisphere project to the lateral cerebellar nucleus, also called the dentate nucleus. Between the fastigial and dentate nucleus are the anterior and posterior interposed nuclei, also called globose and emboliform in the human cerebellum. The interposed nuclei receive Purkinje cell axons from the intermediate zone of the cerebellar cortex. Size and structure of the cerebellar nuclei are directly related to the size and the configuration of their Purkinje cell input and to the weight and the construction of the functional motor, sensory, and cognitive systems which serve as their targets.

3.30.5.2 Purkinje Cell Zones. Morphology, Connections, and Chemical Identity

The output of the cerebellum is organized as a series of independent modules. Each module consists of one or more longitudinal zones of Purkinje cells, oriented perpendicular to the transverse fissures, its cerebellar or vestibular target nucleus, and a climbing fiber system, innervating both the Purkinje cells

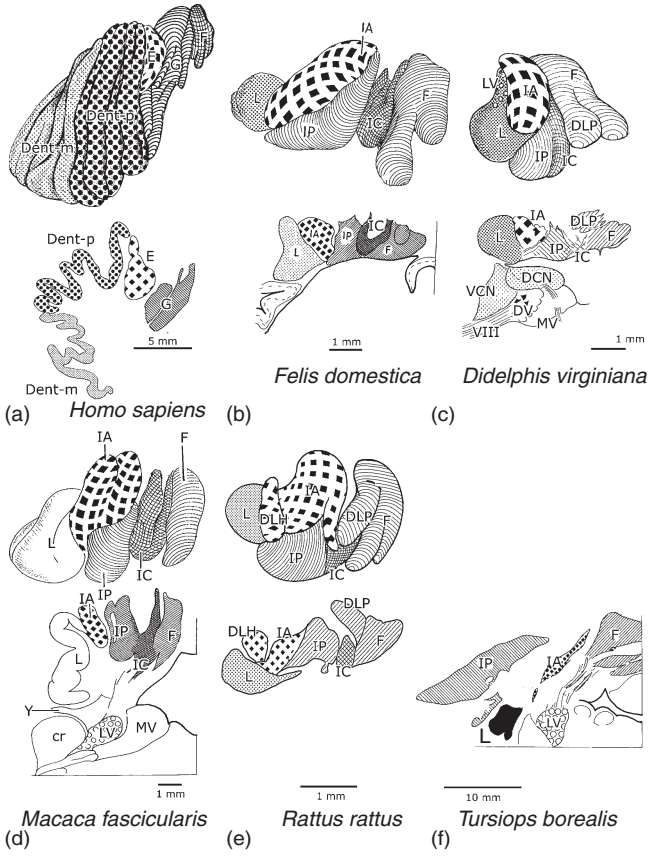

Figure 8 Diagrams of graphical reconstructions of the cerebellar nuclei (upper panels) and of a transverse section through the nuclei (lower panels) in different mammals. The interstitial cell groups (IC) are a nucleus located between the fastigial and interposed nuclei, which serves as the target nucleus of the X and CX zones. The dorsolateral hump (DLH) and the dorsolateral protuberance of the fastigial nucleus (DLP) have only been described in rodents and/or marsupials (c, e). Group Y is particularly well developed in primates (d). It serves as one of the target nuclei of the flocculus. For the dolphin (f; *Tursiops truncatus*), no graphical reconstruction was available. cr, restiform body; DCN, dorsal cochlear nucleus; Dent-m, macrogyric portion of human dentate nucleus; Dent-p, microgyric portion of human dentate nucleus; DLH, dorsolateral hump; DLP, dorsolateral protuberance of fastigial nucleus; DV, spinal vestibular nucleus; E, emboliform nucleus; F, fastigial nucleus; G, globose nucleus; IA, anterior interposed nucleus; IC, interstitial cell groups; IP, posterior interposed nucleus; L, lateral cerebellar nucleus; LV, lateral vestibular nucleus; MV, medial vestibular nucleus; VCN, ventral cochlear nucleus; Y, group Y. a, Reproduced from Voogd, J. 2004a. Cerebellum and precerebellar nuclei. In: The Human Nervous System (*eds*. G. Paxinos and J. K. Mai), pp. 321–392. Elsevier. b–f, Reproduced from Voogd, J., Nieuwenhuys, R., van Dongen, P. A. M., and ten Donkelaar, H. J. 1998. Mammals. In: The Central Nervous System of Vertebrates (*eds*. R. Nieuwenhuys, H. J. ten Donkelaar, and C. Nicholson), pp.1637–2097. Springer.

and the target nucleus of the module. Among all mammals studied, there is a similar pattern of longitudinal Purkinje cell zones in the mammalian cerebellum (Voogd, 1967; Buisseret-Delmas and Angaut, 1993; Voogd *et al.*, 1996, 2003; Voogd and Glickstein, 1998; Sugihara and Shinoda, 2004; Voogd and Ruigrok, 2004). Three of these zones occupy the vermis (Figure 9). The medial A zone is present along its entire length and projects to the medial (fastigial) nucleus and restricted portions of the vestibular nuclei. Its climbing fibers originate from the caudal medial accessory olive (MAO). The X zone is the next lateral zone. It projects to the junction of the fastigial and the posterior interposed nucleus (interstitial cell groups) and receives climbing fibers from an intermediate region of the MAO. The most lateral zone of the vermis is the B zone. It is only present in restricted anterior and posterior portions of the cerebellum, projects to the lateral vestibular nucleus of Deiters, and receives a climbing fiber projection from the caudal dorsal accessory olive (DAO). The cerebellar hemispheres are made up of seven or eight zones. The most medial of these, the A2 zone (not illustrated in Figure 9), projects to a dorsolateral protuberance of the fastigial nucleus (Figure 8) and is innervated by climbing fibers originating from the medial subnucleus C of the caudal MAO. Three successively more lateral zones, C1, C3, and Y, all project to the anterior interposed nucleus and all receive their climbing fiber afferents from the rostral DAO. The CX zone occupies a strip, immediately medial to C1. It shares its connections with the X zone. Like the B zone, the X, CX, C1, C3, and Y zones are only present in restricted anterior and posterior segments of the hemisphere (i.e., in the anterior lobe and the lobulus simplex and in the caudal ansiform lobule and the paramedian lobule, see below). The C2 zone, which is located between C1 and C3, is connected with the posterior interposed nucleus and receives climbing fibers from the rostral MAO. Two zones in the lateral hemisphere, D1 and D2, project to rostromedial and caudolateral portions of the lateral cerebellar (dentate) nucleus and are innervated by climbing fibers from the ventral and dorsal lamina of the principal olive, respectively. C2 and the D zones are present over the entire length of the cerebellar hemispheres.

This fundamental pattern, with very little variation, is present in all of the species studied. The main differences concern the relative width and the length of the zones in certain regions of the vermis and hemisphere. There are variations among mammals in the length of the A zone, which is located in the middle regions of the vermis, particularly in lobule

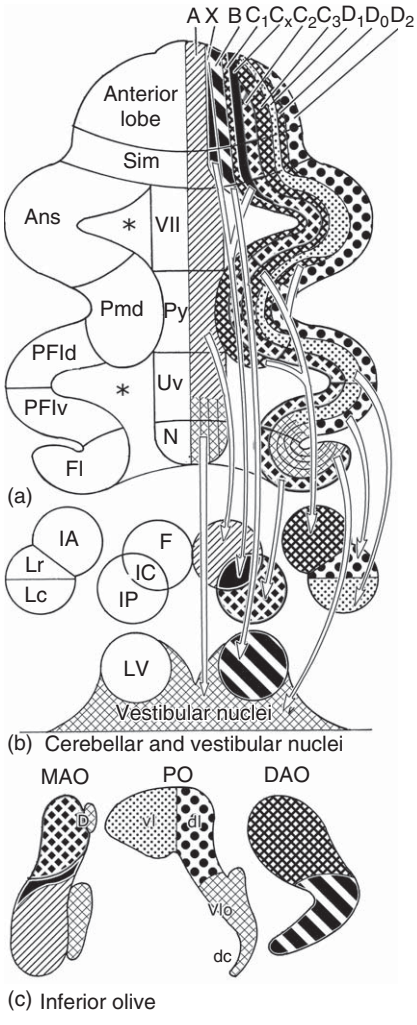

Figure 9 Diagram of the zonal organization in the corticonuclear and olivocerebellar projections. a, Diagram of the flattened cerebellar cortex. b, Diagram of the cerebellar and vestibular nuclei. c, Diagram of a projection of the monkey inferior olive in the horizontal plane. The longitudinal corticonuclear and olivocerebellar projection zones are indicated with capitals (A, X, B, C_X, $C_{1–3}$, Y, $D_{1,2}$). The zones, their target nuclei, and the subnuclei of the inferior olive that project to these zones are indicated with the same shadings. The diagram applies equally to the cerebella of rat, cat, rabbit, and monkey, with the exception of the floccular zones, the most medial one of which is lacking in the monkey. The A_2 zone, only present in the rat, is not indicated. Asterisks, areas without cortex. A, A zone; Ans, ansiform lobule; B, B zone; $C_{1–3–X}$, $C_{1–3–X}$ zones; $D_{1,2}$, $D_{1,2}$ zones; D, dorsomedial cell column; DAO, dorsal accessory olive; dc, dorsal cap; dl, dorsal lamina of the principal olive; F, fastigial nucleus; Fl, flocculus; IA, anterior interposed nucleus; IC, interstitial cell groups; IP, Posterior interposed nucleus; Lc, caudal lateral cerebellar (dentate) nucleus; Lr, rostral lateral cerebellar (dentate) nucleus; LV, lateral vestibular nucleus; MAO, medial accessory olive; N, nodulus; PFlv, ventral paraflocculus; PFld, dorsal paraflocculus; PMD, paramedian lobule; PO, principal nucleus of the inferior olive; PY, pyramis; Sim, lobulus simplex; UV, uvula; vl, ventral leaf of principal olive; vlo, ventrolateral outgrowth; X, X zone. Reproduced from Voogd, J., Nieuwenhuys, R., van Dongen, P. A. M., and ten Donkelaar, H. J. 1998. Mammals. In: The Central Nervous System of Vertebrates (*eds.* R. Nieuwenhuys, H. J. ten Donkelaar, and C. Nicholson), pp.1637–2097. Springer.

VII. This lobule is associated with the control of eye movements. In many carnivore, ungulate, and primate species, this increase in rostrocaudal length leads to the formation of an S-shaped curve in this portion of the vermis (Figure 5b; Voogd and Barmack, 2005). The B, C1, C2, and Y zones are represented only in the rather conserved anterior (anterior lobe and lobulus simplex) and posterior (pyramis, lobule VIII, and paramedian lobule) regions of the cerebellum (Voogd, 2003). They share similar corticonuclear and olivocerebellar connections, which are relatively constant.

Most variations in length and width concern the C2 and the D zones. The C2 zone and its connections with the posterior interposed nucleus and the rostral MAO and the associated brainstem circuitry are hypertrophied in cetaceans, where this zone is associated with the large size of the paraflocculus (Figures 5f, 8, 10, and 11) (Korneliussen, 1967, 1968a, 1968b). The C2 zone in whales was indicated by Korneliussen as the lateral intermediate zone (Figure 10). The D1 and D2 zones, along with the associated regions of the dentate nucleus and the principal olive are greatly enlarged in primates. They are responsible for the great size of the ansiform lobule in nonhuman primates, but also for the width of the folia of the anterior lobe, the lobulus simplex and the paramedian lobule in these animals (Figures 5d and 10). In the human cerebellum, this increase in width of the D zones also affects the homologue of the paraflocculus, the medial belly of the biventral lobule, and the tonsilla (Figure 1). The D1 zone, generally, is the more narrow of the two D zones (Voogd, 2003, 2004a, 2004b). Similarly, in the elephant, it is the dorsal lamina of the principal olive with its projection to the D2 zone that is specifically enlarged (Figure 11) (Verhaart, 1962). There is only one example of a zone, the A2 zone, that exists in some, but not other mammalian species. Judging from the presence of its target nucleus, the dorsolateral protuberance of the fastigial nucleus (Goodman *et al.*, 1993), the A2 zone is present in rodents, lagomorphs, and marsupials, but absent in carnivores and primates (Figures 8c and 8e) (Buisseret-Delmas, 1988a, 1988b).

Purkinje cells are not a homogeneous population; they differ in their biochemical properties. Two populations of Purkinje cells were distinguished by Hawkes and Leclerc (1987) on the basis of their immunoreactivity with an antibody against the zebrin I epitope. Zebrin-positive and Zebrin-negative Purkinje cells are distributed in alternating longitudinal zones (Figure 12). The zebrin pattern is correlated with the distribution of many different substances in Purkinje cells, such as enzymes (5′-nucleotidase and aldolase-C, or zebrin II), certain

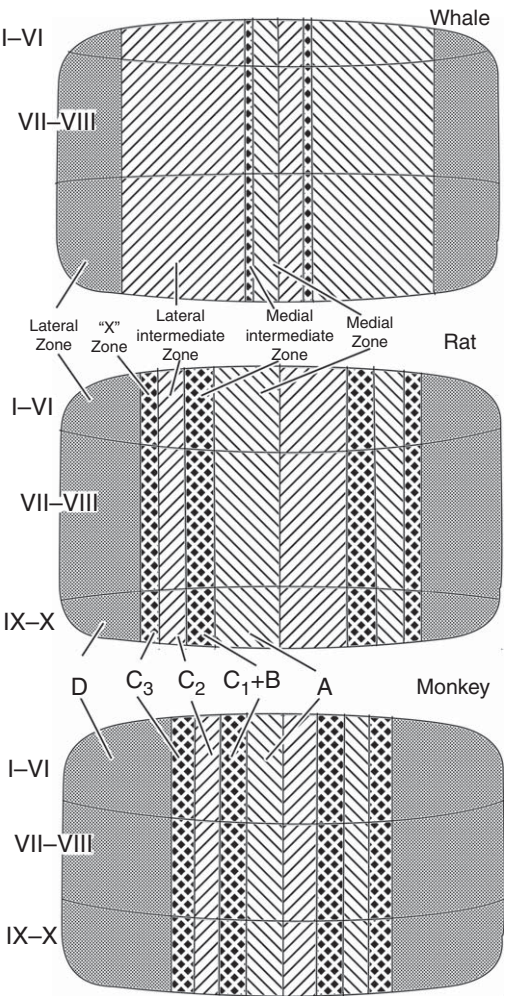

Figure 10 Diagram of the relative width of corticogenetic Purkinje cell zones in different mammals. During early development, the future Purkinje cell zones are present as Purkinje cell clusters at the still unfolded surface of the cerebellar anlage (see also Figure 16). The diagrams depict the relative width of these clusters. Corresponding zones are indicated with the same symbols. The nomenclature of Korneliussen for rat and whale differs from the traditional nomenclature, employed by Kappel. The diagrams of the whale and the rat are based on data from Korneliussen, H. K. 1967. Cerebellar corticogenesis in Cetaca, with special reference to regional variations. *J. Hirnforsch.* 9, 151–185. The data from the monkey are based on Kappel, R. 1981. The Development of the Cerebellum in *Macaca mulatta*. A Study of Regional Differentiation during Corticogenesis. PhD dissertation, University of Leiden.

glutamate transporters, growth factor receptors, etc. There are vast differences in expression of the zebrin II marker across the vertebrate subphylum. For example, basal vertebrates such as sharks and rays reveal uniform expression of the marker. In contrast, all mammalian species show a similar number of zebrin II zones, although the specific patterns are subtly different (Figure 13; Sillitoe *et al.*, 2003, 2005). Recent studies comparing the

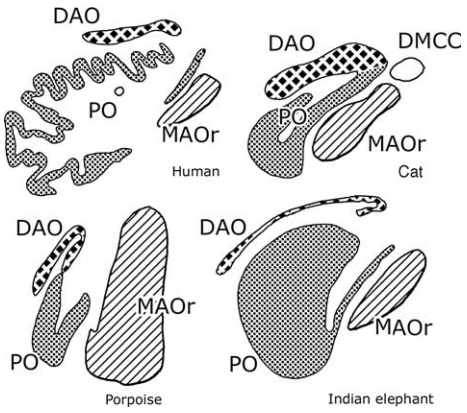

Figure 11 Transverse sections through rostral levels of the inferior olive in different mammalian species. DAO, dorsal accessory olive; DMCC, dorsomedial cell column; MAOr, rostral medial accessory olive; PO, principal olive. The human olive and the olive of the elephant were redrawn from Kooy (1917) . The other diagrams are reproduced from Voogd (2004b; a) and Voogd *et al.* (1998; b–f)

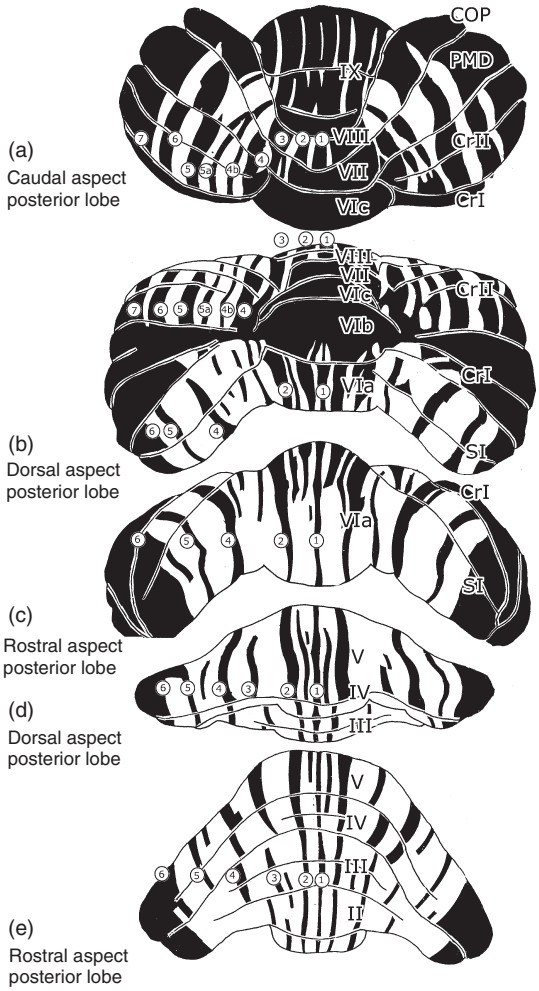

Figure 12 Zebrin-positive and zebrin-negative zones in the cerebellum of the rat. Caudal, dorsal, and rostral aspects of the posterior lobe (a–c) and dorsal and rostral aspects of the interior lobe (d, e) are illustrated. Numbers indicate zebrin-positive Purkinje cell zones P1–P7 of Hawkes and Leclerc (1987). COP, copula pyramidis; CrI, CrII, crus 1 and II of the ansiform lobule; PMD, paramedian lobule; S1, simple lobule; I–X, lobules I–X.

connections and the zebrin-identity of Purkinje cells in the rat showed a close correspondence between the two patterns. Purkinje cells of the B, X, CX , C1, C3, and Y zones are zebrin-negative. Purkinje cells of the C2, D1, and D2 zones are zebrin-positive. The A zone is a composite of zebrin-positive and zebrin-negative areas. Crus I of the ansiform lobule and the paraflocculus and the flocculus, where only the C2 and the D zones are represented, are entirely zebrin-positive (Voogd *et al.*, 2003; Voogd and Ruigrok, 2004; Sugihara and Shinoda, 2004).

Purkinje cell zones are connected by way of the cerebellar and vestibular nuclei with the centers in the brainstem, the thalamus, and the spinal cord (Figure 14; see Voogd, 2003, 2004a, 2004b for reviews). The C1, C3, and Y zones project through the anterior interposed nucleus to the contralateral magnocellular red nucleus and, via the ventrolateral nucleus of the thalamus, to the primary motor cortex. They monitor activity in the rubrospinal and corticospinal tracts. The A, B, X, and CX zones maintain strong connections with the spinal cord, through the cerebellospinal, reticulospinal, and vestibulospinal tracts. Note that these Purkinje cell zones share somatotopically organized somatosensory climbing fiber projections. The connectivity of the C2 and D zones is different. Their target nuclei, the posterior interposed and dentate nuclei, project to centers at the junction of the mesencephalon and diencephalon which, in turn, give rise to strong descending systems to the inferior olive. For the dentate nucleus, the system relays in the parvocellular red nucleus, with the central tegmental tract as its descending system terminating in the principal olive; for the posterior interposed nucleus, the mesodiencephalic nucleus is the nucleus of Darkschewitsch, with the medial tegmental tract, terminating in the rostral MAO, as its descending system. In addition, the posterior interposed and dentate nuclei project to ventral thalamic nuclei with connections to motor, premotor, and prefrontal areas, including the frontal eye fields, and more limited projections to the parietal lobe. The closed cerebello-mesodiencephalic-olivary loops are under strong cortical influence of these same cortical areas (Voogd, 2003, 2004a).

Relative size and connectivity of the Purkinje cell zones and their target nuclei are indicative of adaptations of the cerebellum to changes in the organization of motor, sensory, and cognitive systems of the brain

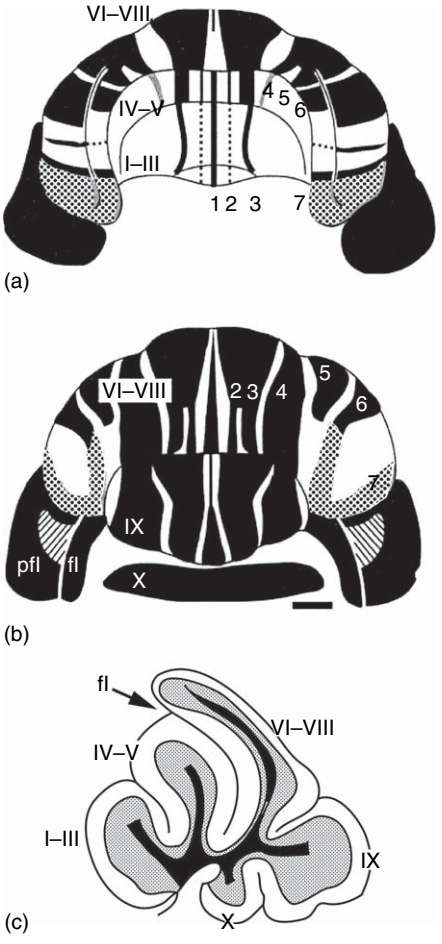

Figure 13 Reconstruction of the location of zebrin II immunoreactive Purkinje cell zones in anterior (a) and posterior (b) views of the cerebellum of the tenrec (*Echinops telfari*). The same zebrin-positive bands 1–7 can be recognized as in the rat (Figure 12). The cerebellum of this basal insectivore can be subdivided into the fused lobules I–II and IV–V of the anterior lobe, the combined lobules VI–VIII with the ansiform and paramedian lobules, the uvula (IX), the nodulus (X), the paraflocculus (pfl) and the flocculus (fl). a and b, Reproduced with permission from Sillitoe, R. V., Künzle, H., and Hawkes, R. 2003. Zebrin II compartmentation of the cerebellum in a basal insectivore, the Madagascar hedgehog, tenrec, *Echinops telfari*. *J. Anat.* 203, 283–296.

in different species. The prominence of the Purkinje cell zones of the vermis and the C1 and C3 zones in lower mammals, and the large hemispheres and the increase in length and width of the D1 and D2 zones in primates, parallels the shift from a local spinal and brainstem regulation of movement to a situation where movement is largely dependent on the cerebral cortex. Prominent vermal and C zones in lower mammals are associated with prominence of their target nuclei, such as Deiters' lateral vestibular nucleus and the magnocellular red nucleus and their spinal tracts. Prominent hemispheres, extensive D zones, and a large, convoluted and subdivided dentate nucleus are found in primates. Targets of the primate dentate nucleus include extensive and differentiated motor, premotor, and frontal association areas and the large parvocellular red nucleus, which links these cortical areas with the inferior olive and the cerebellum. It has been suggested that the functions of the primate dentate nucleus principally involve cognitive and emotional aspects of behavior (Schmahmann, 1997). However, the great development of the cerebellar hemisphere and the dentate nucleus can also be considered as an adaptation to visual and visuomotor exigencies in primates. Much remains unknown. The functional importance of the C2 zone, which overwhelms the cetacean cerebellum and the presence of additional Purkinje cell zones in rodents remain unexplained.

The caudal portions of the vermis and the hemisphere, i.e., the nodulus and the flocculus, are known as the vestibulocerebellum. The zonal organization of the nodulus and the flocculus represents a modification of the A and D zones, respectively (Figure 9). The afferent and efferent connections of the nodulus are mainly with the vestibular nuclei; the flocculus receives olivocerebellar systems mediating optokinetic information, and projects to vestibulo-ocular and vestibulospinal neurons mediating the optokinetic and the labyrinthine neck reflexes (Voogd and Barmack, 2005).

Both in the flocculus and the nodulus, the Purkinje cells are arranged in a complicated pattern of multiple longitudinal zones. For the flocculus, this is a highly conserved feature. It is present in the flocculus of mammals and birds (Voogd and Wylie, 2004). However, one distinguishing feature is present in the primate flocculus. The folial rosette, which links the flocculus with the paraflocculus (known as the ventral paraflocculus) has increased in size, while retaining its original floccular zonal pattern. This feature has been related to the development of foveal vision and smooth pursuit in primates. The flocculus still subserves the calibration of the vestibulo-ocular reflex, as it does in lower mammals; the ventral paraflocculus mediates smooth pursuit (Voogd *et al.*, 1987; Nagao, 1992; Rambold *et al.*, 2002). It exemplifies how a preserved, anatomical configuration can be used for another purpose.

There are limited data on variations in the zonal pattern between species, and our knowledge on the connectivity of sets of Purkinje cell zones and their target nuclei suggests that the combined A, B, C1, C3, and Y zones show little variation among species. The main differences concern the flocculus and the C2, D1, and D2 zones and their afferent and efferent connections. The functions of these zones and the kind of adaptations provided by their variations in width and length, however, remain largely unknown.

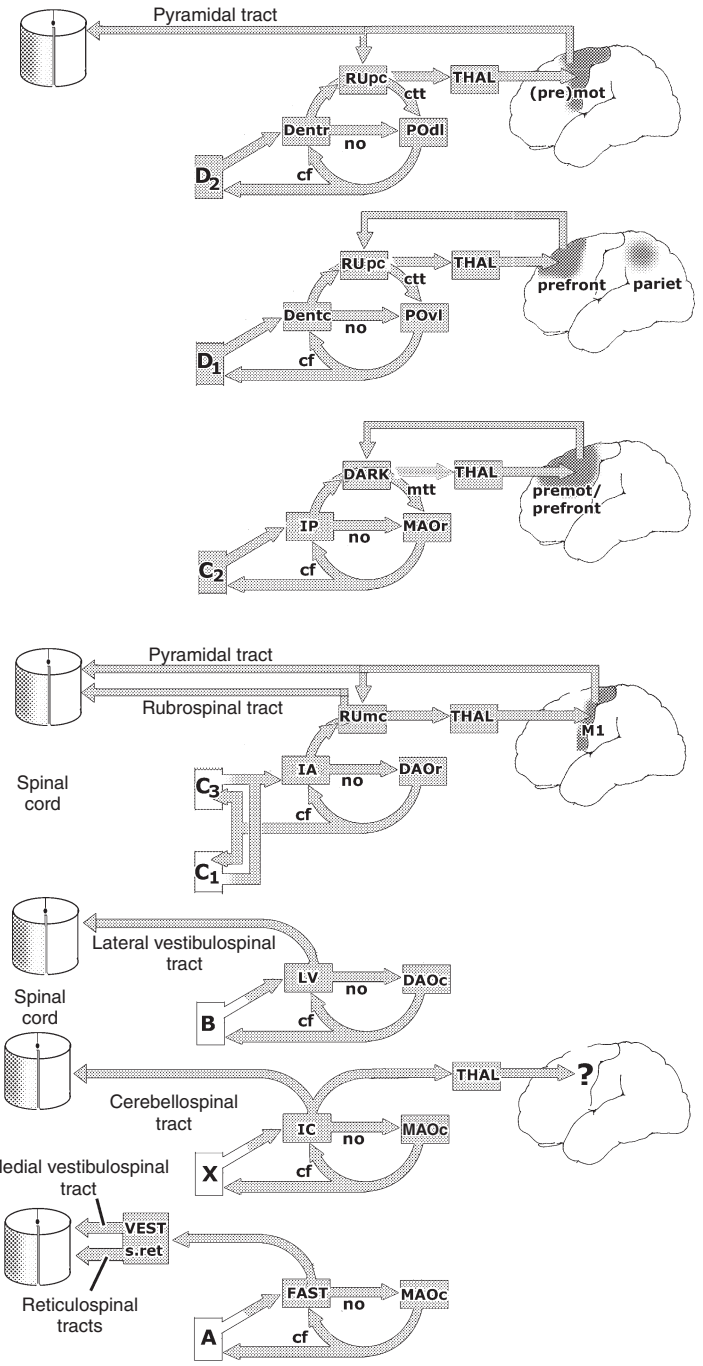

Figure 14 Diagrams of brainstem, thalamic, and cortical connections of the cerebellar Purkinje cell zones. The CX, A2, and Y zones are not included. All zones and their cerebellar and vestibular target nuclei are reciprocally connected with the inferior olive through a GABAergic nucleo-olivary pathway (no) and the climbing fiber projections with their collateral projections to the nuclei (cf). One set of zones (A, X, B, C1, C3, and D2) is connected with the spinal cord, through brainstem-spinal and/or corticospinal pathways. The D1, D2, and C2 zones and their target nuclei give rise to mesencephalo-olivary reciprocal circuits. These circuits are topically organized and include a relay in the parvocellular red nucleus (RUpc) or Darkschewitsch (DARK) nucleus at the mesodiencephalic junction. Similar reciprocal circuits do not exist for the other zones and their target nuclei. The D1, D2, and C2 zones, in addition, project to the cerebral cortex through the thalamus. For the D2 zone, these projections include the motor and premotor cortex; for the D1 zone they include the frontal eye field and prefrontal and parietal areas (see Voogd, 2004a, 2004b). (pre)mot, (pre)motor area; CA–X, zones A–X; cf, climbing fibers; ctt, central tegmental tract; DAOc/r, caudal/rostral part of the dorsal accessory olive; DARK, Darkschewitsch nucleus; Dentc, caudal dentate nucleus; Dentr, rostral dentate nucleus; FAST, fastigial nucleus; IA, anterior interposed nucleus; IP, posterior interposed nucleus; LV, lateral vestibular nuclues; M1, primary motor cortex; MAOc/r, caudal/rostral part of the medial accessory olive; mtt, medial tegmental tract; no, nucleo-olivary pathway; POdl, dorsal lamina of the principal olive; POvl, ventral lamina of the principal olive; prefront, prefrontal cortex; RUpc, parvocellular red nucleus; RUmc, magnocellular red nucleus; s.ret, reticular formation, THAL, thalamus; VEST, vestibular nuclei.

3.30.5.3 Mossy Fiber Afferents to the Cerebellar Cortex

The main afferent system of the cerebellum are the mossy fibers. The distribution of the mossy fibers differs from that of the climbing fibers. Mossy fibers generally distribute bilaterally, the mossy parent fibers collateralize into multiple longitudinal aggregates, and mossy fiber systems generally distribute to certain cerebellar lobules only (Wu *et al.*, 1999). Some mossy fiber systems contribute collaterals to the cerebellar nuclei on both sides. The multiple longitudinal aggregates of mossy fiber terminals are topographically related to the longitudinal pattern of Purkinje cell zones and their climbing fiber afferents, but our knowledge on this subject is still far from complete (Voogd, 2004a, 2004b).

Mossy fibers originate both from intrinsic and extrinsic sources. Collaterals of the axons of the cells of the cerebellar nuclei and the axons of a recently discovered cell type of the granular layer, the unipolar brush cell (Dino *et al.*, 2000), terminate as mossy fibers.

By far the largest single extrinsic source of mossy fiber afferents for humans, primates, and many other species is the pontine nuclei. Mossy fibers also originate from sensory relay nuclei, and as collateral systems from interneuronal pools of the spinal cord and the brainstem (the spino- and trigeminocerebellar tracts), certain reticular nuclei (the lateral, paramedian, and reticular tegmental nuclei), the vestibular nuclei, and the adjacent perihypoglossal nucleus (with its subsidiaries). Spino-, vestibulo-, and reticulocerebellar connections are constant among most mammalian species, although variations related to the prevalence of trigeminal over spinal connections in marsupials and rodents, as compared to carnivores, ungulates, and especially primates may also be expressed in the cerebellum (Voogd *et al.*, 1998). These mossy fiber systems terminate preferentially in anterior and posterior regions of the cerebellum, in relation to the A, B, C1, C3, and the Y zones, which, as pointed out above, constitute the stable backbone of the mammalian cerebellum.

On a higher resolution, a fine-grained microzonal topography is present in both the climbing and mossy fiber systems projecting to these zones, which subserve the local interaction of both afferent systems, believed to be at the core of cerebellar functioning (Ekerot and Larson, 1973 Garwicz *et al.*, 1998; Brown and Bower, 2001; Serapide *et al.*, 2001; Voogd *et al.*, 2003).

The pontocerebellar system is the final link in the main cerebrocerebellar pathway, although from a comparative anatomical point of view, it may have arisen as a tectocerebellar connection. In birds, two small medial and lateral pontine nuclei receive their afferents from the tectum and project to middle lobules (corresponding to lobule VII) and the caudal cerebellum (lobule IX, the uvula), regions that also receive an input from the pons in mammals (Freedman *et al.*, 1975). The tectopontine projection is preserved in mammals, but, in addition, the mammalian pontine nuclei now receive profuse projections from the cerebral cortex (Figure 15) (Münzer and Wiener, 1902; Mower *et al.*, 1979; Hartmann-von

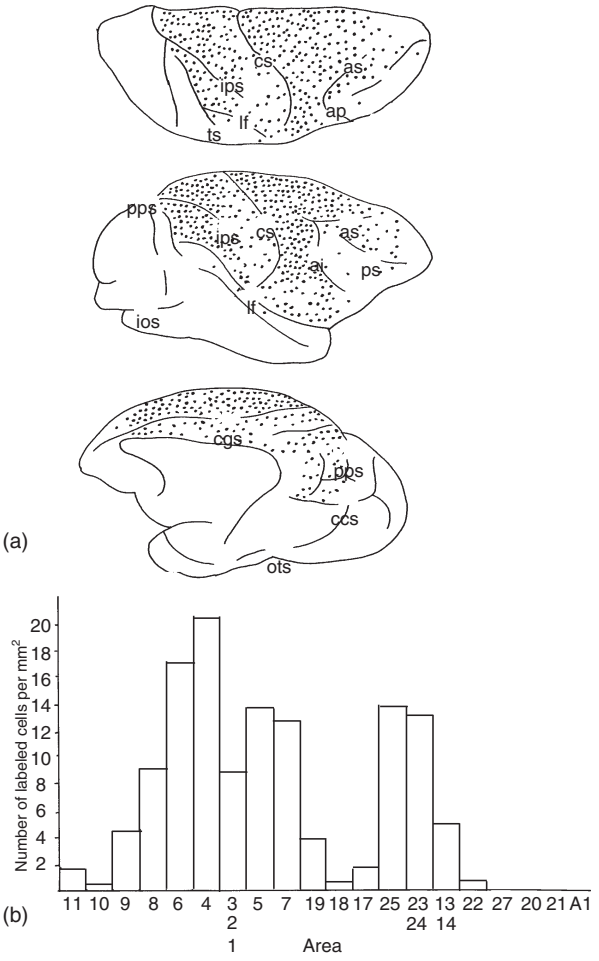

Figure 15 Distribution of corticopontine neurons in macaque monkeys. a, Distribution of retrogradely labeled neurons in the left cerebral hemisphere from a complete wheat germ agglutinin-coupled horseradish peroxidase filling of the pontine nuclei. b, Bar graphs illustrating the numbers of retrogradely labeled neurons in different cortical areas. ai, arcuate sulcus, inferior branch; as, arcuate sulcus, superior limb; ap, arcuate sulcus, inferior limb; ccs, calcarine fissure; cgs, cingulate sulcus cs, central sulcus; ios, inferior occipital sulcus; ips, intraparietal sulcus; lf, lateral (Sylvian) fissure; ots, occipitotemporal sulcus; pps, postparietal sulcus; ps, principal sulcus; ts, superior temporal sulcus. From Glickstein, M., May, J., and Mercier, B. 1985. Corticopontine projection in the macaque: The distribution of labelled cortical cells after large infection of horseradish peroxidase in the pontine nuclei. *J. Comp. Neurol.* 349, 51–72.

Monakow *et al.*, 1981; Glickstein *et al.*, 1980, 1985). These projections fall into two groups. One group, originating from the primary motor, the premotor, and the primary sensory cortices, contains collateral projection from the pyramidal tract (Ugolini and Kuypers, 1986). The second, originating from prestriate, posterior parietal, and prefrontal areas, may be considered in part as a collateral projection from the corticotectal system (Baker *et al.*, 1983; Keizer *et al.*, 1987). The largest evolutionary changes in mossy fiber connections occur in the second corticopontine cerebellar system. The pyramidal collateral system may subserve the coordination of skilled movements. The corticotectal collateral system probably is involved in the execution of sensory guided movements. The prominence of visual association and visuomotor areas of the cerebral cortex in the primate corticopontine projection indicates the importance of vision in the evolution of the primate cerebellum.

Pontocerebellar mossy fibers probably distribute to all cerebellar lobules, with the exception of the nodulus and the flocculus (Voogd, 1967; Kawamura and Hashikawa, 1981; Gerrits and Voogd, 1982, 1986; Glickstein *et al.*, 1994). A longitudinal zonal pattern in their termination was recently described by Serapide *et al.* (2001). In the anterior lobe and the lobulus simplex, they project to the apical and lateral portions of the folia, covering the spino-, reticulo-, and vestibulocerebellar mossy fibers, which terminate in more basal regions of the granular layer. A similar pattern may be present in the pyramis (lobule VIII) and the adjacent paramedian lobule. The apical coverage of pontocerebellar mossy fibers is much thicker in primates, where spino- and reticulocerebellar mossy fibers may never reach the cerebellar surface. Pontocerebellar mossy fibers terminate in lobule VII of the vermis and the uvula (lobule IX), in the ansiform lobule, and densely in the paraflocculus, the regions that display most variations in their folial pattern.

3.30.6 Embryological Origin of the Cerebellum

3.30.6.1 Determination and Origin of the Cerebellar Primordium

The past decade has seen rapid growth in our understanding of the molecular and cellular mechanisms underlying the embryological origins of the cerebellum. This has been fueled mainly by a great increase in knowledge on the mechanisms of action of key developmental control genes that sculpt the body plan in a large spectrum of animals ranging from insects to mammals.

In spite of the enormous species diversity with respect to size, foliation, and mediolateral patterning of the cerebellum, the following basic embryological scheme likely applies to all vertebrates. The cerebellum is derived from an embryonic territory at the junction of the developing midbrain and hindbrain, the so-called mid–hindbrain boundary, or MHB. Chick/quail transplantation experiments have revealed an organizing influence of a band of cells at the MHB called the isthmus (Marin and Puelles, 1994; Crossley *et al.*, 1996; Hidalgo-Sanchez *et al.*, 1999; reviewed in Joyner *et al.*, 2000). This region directs the formation of the midbrain, cerebellum, and anterior hindbrain and can induce midbrain and cerebellum tissue when placed ectopically. Genetic analysis in mice has shown that the position of the isthmic organizer is fixed at the boundary between the domains of expression of two homeodomain transcription factors, Otx2 and Gbx2 (Millet *et al.*, 1999; Broccoli *et al.*, 1999; reviewed in Joyner *et al.*, 2000). Otx2 is typically expressed throughout the embryonic forebrain and midbrain with a caudal border at the MHB; Gbx2 is expressed in the anterior hindbrain with a rostral limit at the MHB (Figure 16). Each of these factors mutually excludes the other from its territory. Using transgenic methods to artificially expand either expression territory results in malpositioning of the MHB and a variety of effects on development of the midbrain and cerebellum.

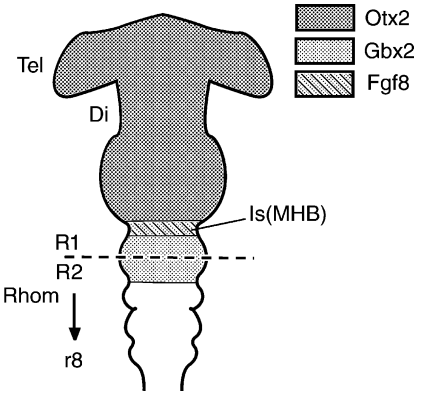

Figure 16 Position of the mid–hindbrain boundary (MHB) is genetically determined. Mutually exclusive domains of expression of Otx2 and Gbx2 define the position of the MHB and isthmus region of the developing vertebrate embryo. The organizer activity of the isthmus leads to the specification of cell fates in the midbrain, cerebellum, and rostral hindbrain. The major brain vesicles are indicated. Tel, telencephalon; Di, diencephalon; Mes, mesencephalon; Rhom, rhombencephalon; Is, isthmus. Shadings correspond to expression domains of developmental control genes. Redrawn and adapted from Joyner, A. L., Liu, A., and Millet, S. 2000. Otx2, Gbx2, and Fgf8 interact to position and maintain a mid–hindbrain organizer. *Curr. Opin. Cell Biol.* 12, 736–741.

All vertebrates, from fish to birds to mammals, have a similar embryonic pattern of brain vesicles that include the mesencephalon (midbrain) and rhombencephalon, the latter of which is made up of two secondary brain vesicles classically called metencephalon (cerebellum) and myelencephalon (hindbrain). The embryonic hindbrain is subdivided into eight transient swellings called rhombomeres, labeled R1 (the most rostral; really equivalent to the metencephalon) through R8 (Keynes and Lumsden, 1990; Lumsden and Krumlauf, 1996) (or R0–R7 in zebra fish; see Moens and Prince, 2002) (Figure 16). The cerebellum develops from the rostral rhombic lip, which corresponds with the part of the rhombic lip in R1. The rhombic lip is the dorsal rim of the alar plate, which gives attachment to the thin roof plate of the fourth ventricle. The border between the rostral and caudal rhombic lip is located at the greatest width of the fourth ventricle, between R1 and R2 (Figure 17). While expression boundaries of Hox and other control genes coincide with transient morphological constrictions between rhombomeres in many vertebrates, and genetic analysis indicates an intrinsic program controlling the segmental appearance of the hindbrain, there is no evidence of rhombomeric boundaries in the mature hindbrain. Thus, the major brain vesicles, the isthmic organizer, and rhombomeres are transient lineage restriction compartments for the generation of cellular diversity required for mature brain function (Fraser *et al.*, 1990; Keynes and Lumsden, 1990; Zervas *et al.*, 2004).

Several studies have examined the embryological origins of the cerebellum and its major afferent motor nuclei. For example, the inferior olive is generated from R8 and rostral spinal cord (Cambronero and Puelles, 2000), and the pontine nuclei are generated from R2–R8 (Marin and Puelles, 1994, 1995; Rodriguez and Dymecki, 2000). Although several studies using chick–quail chimera analysis indicate a contribution to the cerebellum from both the mesencephalon and metencephalon (or R1) (Martinez and Alvarado-Mallart, 1989; Hallonet and LeDouarin, 1993), this may be dependent upon the precise definition of the mes-/metencephalic boundaries. For example, another study in chick, using the marker Otx2 to define the caudal limit of the mesencephalon, indicated that the cerebellum is derived wholly from the metencephalon (or R1) (Millet *et al.*, 1996). A more recent study using an inducible fate-mapping technique in mice supports this view (Zervas *et al.*, 2004). This study suggests that lineage restriction boundaries restrict the intermingling of mes and met progenitor cells, and that the isthmus region (organizer), separating the mes and met, acts to further restrict any mixing of cells in these two neuromeres, in addition to influencing their distinct fates. Whether the cerebellum is derived wholly from R1 or not, the patterns of expression of key developmental control genes (En, Wnt, Otx, Gbx, Pax, Fgf8, etc.), which determine the positions of the isthmus and subsequent development of the midbrain and cerebellum, are highly conserved from fish to mammals. Thus, this highly conserved developmental program, activated in all vertebrates, guarantees the formation of the same basic brain substructures but allows for the expansion of features within these substructures required for species diversification.

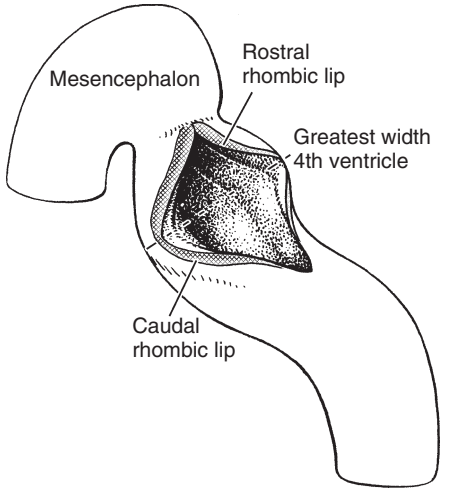

Figure 17 Diagram illustrating the position of the rostral and caudal rhombic lip.

Many of the genes that control specification of the cerebellar primordium are vertebrate orthologues of genes that control formation of the fruit fly body plan, such as Engrailed (En1, En2), Wingless (Wnt-1), Orthodenticle (Otx1, Otx2), etc. While no brain structure exists in the fruit fly that might remotely be considered a cerebellum, it is possible to trace the evolutionary origins of cerebellar development based on expression of these same genes in primitive chordates. In Amphioxus and Ascidians, for example, there is a single Otx gene expressed in the head region with a sharp posterior boundary of expression reminiscent of that in vertebrates (Williams and Holland, 1998). It is possible that the Otx territory in these primitive chordates is functionally homologous to the forebrain and midbrain in vertebrates. As in vertebrates, the rostral-most boundary of Hox gene expression in these species is posterior to the caudal boundary of Otx expression (Figure 18). While not yet identified in Amphioxus, Pax gene

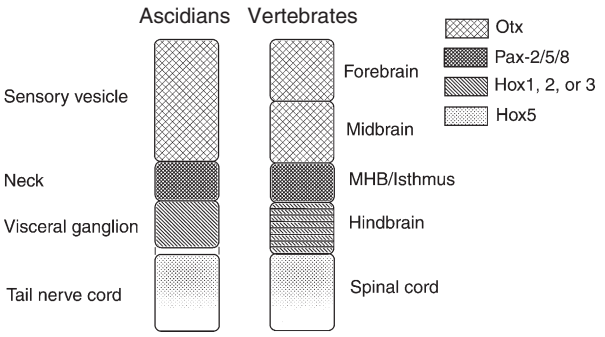

Figure 18 Comparison of the ancestral chordate neural tube structure to that of vertebrates. Homologous functional domains in the neural tubes of ascidians and vertebrates are hypothesized based on the observed expression domains of orthologous developmental control genes. The MHB of ascidians does not give rise to a cerebellum, and therefore new functions are acquired by this domain in vertebrates. Similarly, modification of these functions and the acquisition of new ones are likely to explain evolutionary changes within the vertebrate cerebellum. The subdivision of the rostral neural tube into forebrain and midbrain is hypothesized to be a novelty of vertebrates due to novel rostral expression domains of developmental control genes such as Dmbx 1 that emerge in the vertebrates (not shown). Di, diencephalon; Is, isthmus; Mes, mesencephalon; MHB, midbrain–hindbrain boundary; r1/8, rhombomere 1/8; Rhom, rhombencephalon; Tel, telencephalon. Redrawn and excerpted from Takahashi and Holland (2004).

expression in Ascidian embryos is wedged in between the Otx and Hox domains. Therefore, it is possible, but by no means proven, that this region could be homologous to the MHB of vertebrates, but without the capacity to generate a cerebellum.

This is analogous to the observation that these primitive chordates, which do not have a neural crest, nonetheless show expression of crest regulatory genes in the lateral plate (e.g., Snail; Langeland *et al.*, 1998; Erives *et al.*, 1998; for review, Shimeld and Holland, 2000). Thus, the Pax-positive MHB cells and the Snail-positive lateral plate cells of primitive chordates may be the evolutionary progenitors of cerebellum and neural crest, respectively.

Primitive basal vertebrates such as lampreys have a neural crest and a primordial cerebellum with primitive Purkinje cells (Larsell, 1967). So far, in the limited number of cases where it has been examined, expression of known Purkinje cell markers such as Zebrin II cannot be detected in these primitive cells (Lannoo and Hawkes, 1997). The failure to detect these cell markers is most likely due to species divergence of the specific biochemistry of Purkinje cells or of marker protein structure, rendering them undetected by antisera generated to vertebrate proteins. Nevertheless, all other vertebrate species that have been examined, from fish to humans, express most of the classic Purkinje cell markers, many of which deal with Ca^{2+} metabolism (calbindin, parvalbumin, IP3 receptor type 1, etc.).

3.30.6.2 The Genetics of Cerebellar Morphogenesis and Zone Formation

As described above, there is a great deal of evolutionary conservation of the cerebellar zonal pattern among vertebrate species. Studies from multiple avenues have converged to suggest that the division of the cerebellum into zones occurs by mechanisms that are intrinsic to that tissue and genetically encoded. If this is the case, then it is likely that functional differences among the species could be added by slight modifications of the genetic program underlying this pattern, resulting in expansions or contractions of zonal dimensions and/or cell numbers as the need arises. So far, however, the precise mechanisms defining zonal boundaries within the cerebellum remain elusive. Nevertheless, based on what is known, development of zones in the cerebellum can be viewed as a special case of the general process whereby developmental fields are parcelled into progressively narrower functional domains.

3.30.6.3 Fate Mapping and Clonal Analysis

Early in its development, the rostral rhombic lip changes in position from a mainly rostrocaudal orientation to a mediolateral one (Hochstetter, 1929). This change in position may be caused by mechanical factors and is related to the development of the pontine flexure and/or differential growth within the cerebellar primordium. More recently Mathis *et al.* (1997) and Sgaier *et al.* (2005) drew attention to this change in position in their interpretations of their fate maps of the cerebellar anlage and suggested that rostral rhombic lip ultimately corresponds to the medial cerebellum and posterior rhombic lip to the lateral cerebellum (Figure 19).

Fate maps of the matrix of the rhombic lip have a spatial and a temporal aspect. Studies of the development of the cerebellum have shown that its neurons are derived from the ventricular matrix of the rhombic lip in a definite sequence (Altmann and Bayer, 1978, 1985). The earliest are the nuclear cells, followed by the Purkinje cells and the Golgi cells. Interneurons of the molecular layer are generated by cells derived from the ventricular matrix, which divide in the white matter during late stages of cerebellar development (Zhang and Goldman, 1996). The cells of the external granular layer (EGL), the secondary matrix that produces the granule cells, are derived from the dorsal margin of the rhombic lip (from the URL, or upper rhombic lip or

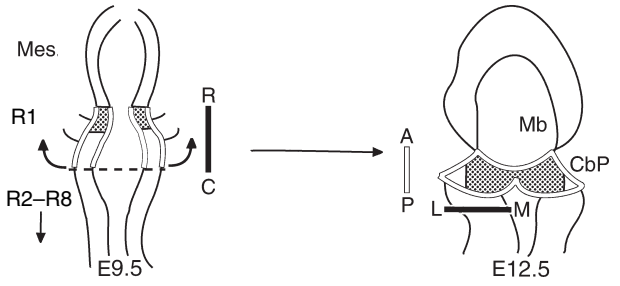

Figure 19 Orthogonal rotation of the A–P axis of R1 gives rise to the mediolateral orientation of cerebellar zones. Growth of the brain and cerebellar primordium in mouse between E9.5 and E12.5 results in a 90° rotation of the longitudinal axis of the rhombic lip. The darkly shaded region indicates a zone of cerebellar cell clones marked at E7.5 using an En2-promoter-based genetic fate mapping strategy. The open contour line indicates the rhombic lip and the cerebellar primordium. Note that the boundaries of the marked region change from a primarily anterior–posterior (AP) orientation to a mediolateral (ML) one. Mes, mesencephalon; Mb, midbrain; CbP, cerebellar primordium; R and C are rostral and caudal, respectively; A and P refer to anteroposterior axis; M and L refer to mediolateral axis. Adapted from Sgaier, S. K., Millet, S., Villanueva, M. P., Berenshteyn, F., Song, C., and Joyner, A. L. 2005. Morphogenetic and cellular movements that shape the mouse cerebellum; insights from genetic fate mapping. *Neuron* 45, 27–40, with minor modifications.

the germinal trigone) and subsequently migrate over the outer surface of the cerebellum.

Clonal analysis of cerebellar cells using a variety of techniques reveals a number of interesting relationships between cerebellar progenitors and both the determination of specific cerebellar cell types as well as their spatial organization. All of these studies have concluded that granule cells constitute a distinct lineage from all other cerebellar cell types, which is consistent with their initial origin from a spatially separate germinative neuroepithelium. In fact, mutation of the gene *Math1*, a molecular determinant of this unique lineage, results in a complete loss of this germinal region and thus granule cells, with no effect on the specification of Purkinje cells and deep nuclear neurons (Ben-Arie *et al.*, 1997). In contrast, in a study using replication defective retroviruses in chick, it was found that Purkinje cells and Bergman glia frequently occupied the same clone (Lin and Cepko, 1999). This study could find no strong evidence for a clonal relationship among the ventricular matrix-derived neurons. In another study designed only to detect relationships between neurons, however, Purkinje cells, deep nuclear neurons, Golgi neurons, and molecular layer interneurons in mice were often found to occupy the same clones (Mathis *et al.*, 1997). Thus, a picture emerges in which a common self-renewing progenitor in the ventricular matrix gives rise to most cerebellar cells except granule cells, and it does so asymmetrically and sequentially according to the known birth dates of these neurons. However, this view is now in need of some updating due to recent transgenic fate mapping studies. It is currently thought that large glutamatergic deep nuclear neurons, in addition to granule cells, are generated from the Math1-positive upper rhombic lip. Purkinje cells, inhibitory interneurons, and small inhibitory deep nuclear neurons are generated from a Ptf1a-positive ventricular zone immediately subjacent to the Math1 domain (Hoshino *et al.*, 2005; Machold and Fishell, 2005; Wang *et al.*, 2005).

One other feature of these clonal analyses is that clones were typically found to spread nearly the entire rostralcaudal dimension of the cerebellum, but were restricted in their spread along the mediolateral axis. No clones in any study were ever found to cross the midline, even in cases where clones spread rostrally into the midbrain or caudally into the hindbrain. In these cases, such large clones never occupied an entire hemicerebellum. Rather, these early labeled clones completely filled either a median territory (extending from the midline to roughly the edge of the vermis) or a lateral territory, but never both. Both territories extended the full rostralcaudal dimension of the cerebellum (Mathis *et al.*, 1997). The size of the median and lateral territories occupied by an intermediate-size clone varied with the actual clone size, and no fixed boundaries were observed that were respected by all or even several clones, except for the midline. Smaller clones cover less total space, but are similar in that they are oriented like zones, with distinct mediolateral boundaries but fanned out rostrally and caudally. When examined, no obvious relationship has been found to exist between these mediolateral clonal boundaries and boundaries defined by zonal markers, such as Engrailed, Eph's, and Gli, leading to the conclusion that individual cerebellar zones are not lineage restriction compartments (Lin and Cepko, 1999). Nevertheless, clones are always very limited in their spread along the mediolateral direction.

The finding of two precursor pools, a median one and a lateral one in each hemicerebellum, and the relative restriction of clone expansion in the mediolateral direction, may be consistent with what was observed using an inducible fate mapping technique in mouse (Sgaier *et al.*, 2005). This technique made use of the endogenous Engrailed promoters to inducibly and permanently label distinct territories of cerebellar precursors starting from embryonic day 7.5(E7.5) or later. Rather broad territories could be labeled (with lacZ) by this method, extending from the midline to variable lateral positions that depended upon the time of induction and the

promoter used. When the fate of cells that were labeled as early as E9.5 was determined by examination of lacZ expression in mature cerebellum, the mediolateral positions of cells at the time of their labeling were found to be unchanged in the adult. This is consistent with other studies that have observed gene expression in cerebellar cell clusters at E15 that are remarkably akin to the zonal pattern of expression of the same gene in adults (Oberdick *et al.*, 1993; Ozol *et al.*, 1999). From these studies, it seems likely that the rhombic lip is primarily divided by broad patterns of developmental gene expression rather than by lineage restriction, and the overlapping patterns of many such genes may result in embryonic zones with distinct cell fates whose positions are roughly maintained until adulthood. Time of birth of the cells may also play a role in the development of zonal patterns in the distribution of cortical neurons (see below).

It is formally possible that a transient lineage restriction boundary exists between the two clone pools within the rhombic lip, generating a medial and a lateral set of precursor cells, and that zone refinement occurs subsequent to this by overlapping patterns of expression of developmental control genes. At any rate, the rotation of a mostly rostrocaudally oriented pattern in the rhombic lip to one oriented mediolaterally during the period from E9.5 to E11.5 in mouse is consistent with many studies, as indicated above.

3.30.6.4 Late Embryonic Patterning and Cerebellar Morphogenesis

When Purkinje cells migrate toward the surface of the cerebellar anlage, they collect in a number of mediolaterally arranged clusters (Figures 10 and 20) (Korneliussen, 1967, 1968a, 1986b; Kappel, 1981; Feirabend *et al.*, 1985; Feirabend, 1990). Similar observations on the clustering of the Purkinje cells were made in studies using Purkinje cell-specific markers (Wassef and Sotelo, 1984; Smeyne *et al.*, 1991). Cell strands and/or fiber streams associate Purkinje cells of different clusters with different cerebellar nuclei. These connections of Purkinje cells are obvious even at these early stages, before synaptic connections have developed. The proliferation of enormous numbers of granule cells by the EGL leads to a great increase in the external surface area of the cerebellum and its folding into lobules and folia, most prominently in the rostrocaudal direction. This increase in the surface area causes the spreading out of the Purkinje cells of the clusters into a monolayer. The Purkinje cell clusters have been considered as the primordia of the adult Purkinje cell zones, and this is supported by studying their temporal morphogenesis through the use of zonal markers, as described above (Oberdick *et al.*, 1993; Ozol *et al.*, 1999).

Timing of Purkinje cell production by the ventricular matrix can be further subdivided into several successive stages, each of which gives rise to distinct clusters of Purkinje cells. Both in birds (Feirabend *et al.*, 1985; Feirabend, 1990; Karam *et al.*, 2000) and mice (Hashimoto and Mikoshiba, 2004), Purkinje cell clusters that are born at different dates typically form interdigitating patterns. In mice, early- and late-born clusters are located both in the vermis and in the hemisphere (Figure 21).

Purkinje cell clusters differ in their expression of different polarity genes such as En-2 (Millen *et al.*, 1995; Lin and Cepko, 1998). In the avian cerebellum, moreover, Purkinje cell clusters that are born

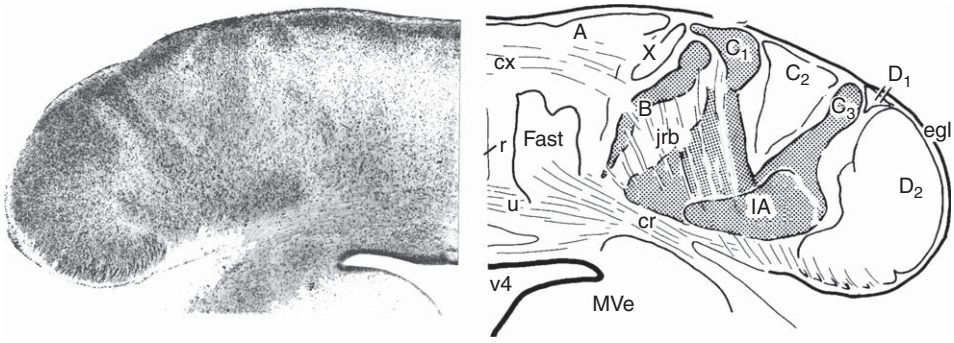

Figure 20 Coronal section through the cerebellum of a 55-day rhesus monkey fetus. Purkinje cell clusters are located at the surface of the cerebellum or are still located in a subcortical position. The cluster that will give rise to the future A zone is related to the fastigial nucleus. Cell strands connect the clusters C_1 and C_2 with their future target nucleus, the anterior interposed nucleus. The posterior interposed and dentate nuclei, the respective target nuclei of the C2 and the D zones are located at a different level. A–D, Purkinje cell clusters A–D; cr, restiform body; cx, cerebellar commissure; egl, external granular layer; Fast, fastigial nucleus; IA, anterior interposed nucleus; jrb, juxtarestiform body; r, midline recess; u, decussation of the uncinate tract; v4, fourth ventricle. From Kappel, R. 1981. The Development of the Cerebellum in *Macaca mulatta*. A Study of Regional Differentiation during Corticogenesis. PhD dissertation University of Leiden.

on different dates express different cell-adhesion molecules, such as the cadherins (Arndt *et al.*, 1998) and BEN (Chédotal, 1996, 1997), and repulsion molecules such as the ephrins and their receptors (Figure 22) (Karam *et al.*, 2000). The cadherins have been held responsible for the clustering of the Purkinje cells and the setting up of their corticonuclear projections (Arndt *et al.*, 1998; Luo *et al.*, 2004). The ephrins and BEN have been shown to be involved in the patterning of the olivocerebellar projection (Chédotal, 1996, 1997). The Engrailed gene products have been shown to be regulators of ephrin expression in other systems (Logan *et al.*, 1996). A sequence of events seems to emerge that determines the mediolateral zonal patterns in the distribution of the Purkinje cells and their connections. The fate maps of the rhombic lip, discussed in the previous section, and the temporal sequence in the production of the Purkinje cells of the different clusters form key features in their development. Further studies are needed to reconcile the spatial and temporal determinants of these zonal patterns.

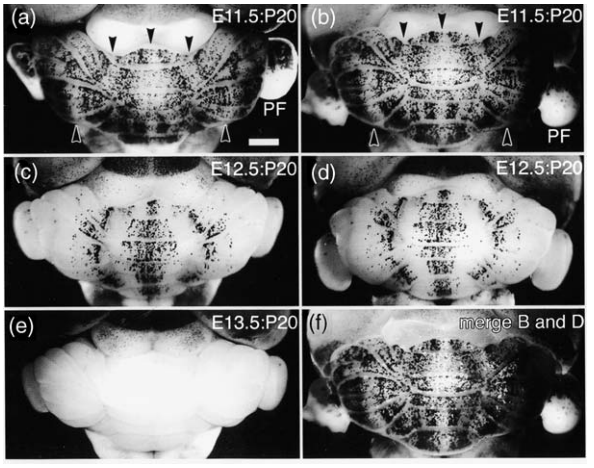

Figure 21 Comparison of the zonal localization of Purkinje cells with different birth dates. Purkinje cells are labeled with birth date-specific gene transfer of the adenoviral vector AdexCAG-Nl-lacZ injected into the midbrain ventricles of embryos at E11.5 (a, b), E12.5 (c, d), and E13.5 (e). At P20, each manipulated brain was stained by whole-mount for beta-gal. Purkinje cells born at E11.5 and at 12.5 are distributed in complementary zonal patterns. In (f) panels b and d are superimposed. The arrowheads in a and b indicate beta-gal-negative clusters. PF, paraflocculus. Scale bar: 1 mm. Reproduced with permission from Hashimoto, M. and Mikoshiba, K. 2004. Mediolateral compartmentalization of the cerebellum is determined on the 'birth date' of Purkinje cells. *J. Neurosci.* 23, 11342–11351.

It seems likely that some of the genes that control the very formation of the cerebellar primordium also play a role in the establishment and late embryonic refinement of cerebellar zones. En1, En2, Wnt1, Pax5, etc., are all known to be expressed in a zonal pattern during late embryogenesis of the mouse cerebellum (Millen *et al.*, 1995). That En and Wg (the fly orthologue of vertebrate Wnt genes) participate in establishing boundaries between fruit fly body segments and En/Wnt signaling is conserved in mice (Danielsen and McMahon, 1996) add fuel to the notion that these genes play a role in zone formation. So far, however, proof of a direct role has not been forthcoming. Null mutation of most of these genes results in deletion of the entire cerebellar primordium due to their very early action (McMahon and Bradley, 1990; Wurst *et al.*, 1994), and therefore effects on zones cannot be studied. However, ectopic overexpression of En-2 in mouse cerebellum starting from E15 has a mixed effect. It has no effect on the adult pattern of zones revealed by L7BG3 expression, a lacZ reporter gene driven by a truncated version of the Pcp-2(L7) promoter, but

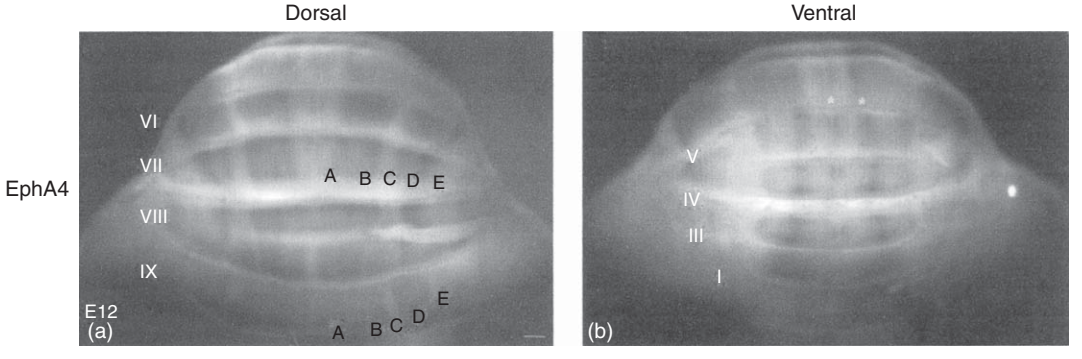

Figure 22 Dorsal and ventral views of EphA4 receptor localization (a, b) in whole-mount immunostaining on chick cerebella taken between stages 36 (E1O) and 38 (E12). Roman numerals refer to cerebellar lobules. EphA4-positive Purkinje cells in bands B and D are born early, EphA4-negative Purkinje cells in bands C and F are born late. The localization of Cadherin 6B (Arndt *et al.*, 1998) corresponds with the localization of EphA4. Reproduced with permission from Karam, S. D., Burrows, R. C., Logan, C., Koblar, S., Pasquale, E. B., and Bothwell, M. 2000. Eph receptors and ephrins in the developing chick cerebellum: Relationship to sagittal patterning and granule cell migration. *J. Neurosci.* 17, 6488–6500.

it has a severe effect on the pattern of stripes revealed by Zebrin II expression (Baader *et al.*, 1999). In addition, it results in an effect on the zonal organization of mossy fiber afferents, which could be explained by disruption of the expression of guidance molecules such as ephrin.

The development of the folial pattern of the cerebellum is closely related to the proliferation and migration of the granule cells in the EGL. An important aspect of transversely migrating EGL cells to the foliation of the cerebellum of the mouse was noticed by Sgaier *et al.* (2005). As previously revealed in chick, the transverse migration is mostly lateral to medial (Ryder and Cepko, 1994). However, one significant difference seems to be that in mouse there is a much greater flow of EGL cells from lateral regions in the posterior vermis (Sgaier *et al.*, 2005). This novel migratory trajectory was hypothesized to correlate with the expansion of the hemispheres in mammals.

The development and the patterning of the afferent climbing and mossy fiber connections were recently reviewed by Sotelo (2004). The inferior olive, the single source of the climbing fibers, and the lateral reticular, the external cuneate, the pontine tegmental reticular, and the pontine nuclei, all of which give rise to mossy fibers, are derived from the region of the caudal rhombic lip (R2–R8, as described above). Temporal and spatial sequences exist in their development from this region (Altman and Bayer, 1987a, 1987b, 1987c, 1987d). Neurons of the inferior olive and certain mossy fiber systems become specified as future mossy and climbing fibers at their birth. The molecular cues for pathfinding and translocation of the cell bodies to their definite positions have been extensively studied. Both mossy and climbing fibers enter the cerebellar anlage very early. Their transverse and longitudinal patterning occurs early, before synaptic connections are established, and, for the olivocerebellar climbing fiber projection at least, is related to the patterning of the Purkinje cells.

3.30.6.5 Conclusions on the Genetic Control of Cerebellar Development

1. Mutations in genes determining the production and differentiation of cells in the rostral and caudal rhombic lip, which are responsible for setting up the temporal and spatial gradients in the production of different cell types, expressing specific recognition and repulsion molecules, which would subserve the future patterning in their connections, are a possible substrate for natural selection.
2. The variations in the morphology of the mammalian cerebellum are mainly related to variations in length and the width of the C2, D1, and D3 Purkinje cell zones and their afferent climbing and efferent cerebellar nuclear connections in certain lobules of the cerebellum. Such variations are not observed in the zonal composition of the vestibulocerebellum, and the system of the A, C1, C3, and Y zones, which subserves the classical motor functions of the cerebellum. However, there are indications that these zones and their target nuclei can be integrated in newly developed systems, i.e., the development of the ventral paraflocculus in species with foveal vision. Generally, Purkinje cell zonation of the mammalian cerebellum is a highly conserved feature.
3. Mossy fiber systems, namely the corticopontocerebellar system, are subject to great variations among mammals. When the cerebellum is used for new or extended functions, adaptations in mossy fiber afferent systems may occur, which use the conserved, modular output system of the cortex for a new purpose.

3.30.7 The Functions of the Cerebellum

3.30.7.1 Historical Aspects

The earliest clues about functions of the cerebellum came from animal experiments. Rolando (1804) made lesions in the cerebellum of mammals and birds and found that the lesions impaired the animals' ability to move. Flourens (1824) agreed, but unlike damage to the spinal cord, the lesions do not abolish movement. Flourens agreed that the cerebellum is involved in the control of movement, but he argued that rather it is the 'coordination' of movement that is lost. Over the course of the nineteenth century, surgical technique improved, allowing more precise control of lesions and longer postoperative survival times. Luciani (1891) studied the long-term effects of cerebellar lesions in mammals. He believed that the observed lack of coordination caused by the lesion is best interpreted on the basis of more elemental deficits in muscle control. Luciani identified these deficits as asthenia, or muscular weakness, atonia, or loss of muscle tone, and astasia, the inability to fuse successive contractions, leading to a characteristic tremor.

Animal experiments strongly influenced the interpretation of the effects of cerebellar lesions in humans. Holmes (1917, 1939) studied the effects of cerebellar lesions on soldiers wounded in the First World War. Citing Luciani, Holmes interpreted most of the deficits in his patients as due to loss of the elementary functions of muscle tone, muscle strength, and loss of the continuity of movements. Babinski (1902) cited the experiments of

Flourens as the basis for his interpretations of the deficits caused by cerebellar lesions in humans. In addition to loss of coordination, Babinski added a characteristic symptom of cerebellar disease: the inability to execute rapid alternating movements, which he labeled adiadochokinesis.

All experimenters and clinicians agree that lesions of the cerebellum cause an impairment of movement. There is, however, no agreement on the underlying cause. Rodolfo Llinas and his colleagues (Llinas and Welsh, 1993; Welsh and Llinas, 1997) argue for a critical role for the inferior olivary nucleus and its efferent climbing fibers in the initiation and timing of voluntary movement. Bower (2002) and his colleagues (Parsons *et al.*, 1997) believe that the cerebellum is entirely a 'sensory' structure. According to this interpretation, the cerebellum receives sensory information that is used to predict the sensory consequences of a movement. Paulin (1993), who shares this view of the sensory role of the cerebellum, pointed out that the motor effects of lesions would be analogous to the effect on an automobile if its windshield were to be shattered. The car seems to perform poorly. Even though its motor, drive shaft, and wheels are intact, it is hard to steer. Thom Thach and his colleagues (Thach *et al.*, 1992) take a middle ground, arguing that the cerebellum serves as a sensory to motor coordinator. The parallel fibers are seen as allowing the coordination of movements among disparate body parts.

3.30.7.2 The Cerebellum and Plasticity

Humans and other mammals are capable of exquisitely precise control of movement. The nature of this control can best be studied in quantitative detail in eye movements. Saccades are rapid shifts of gaze that are characteristic of foveate animals. It has been estimated that humans execute as many saccades in a lifetime as they do heartbeats. In experiments with humans (McLaughlin, 1967) and monkeys (Straube *et al.*, 1997), it is clear that the saccades are highly accurate in finding a target. How is this accuracy maintained over a lifetime? If a human or monkey looks at a central fixation target and then makes a saccade to a target 15 degrees to the right or left, the saccade is typically made with great precision. If, when the eyes begin to move, the target is displaced by five degrees, the saccade is first made to the original position, and then a catch-up saccade brings the eyes to the new target position. Within a single session, the eyes now make a successively larger saccade. Saccadic adaptation requires the cerebellum (Barash *et al.*, 1999). After lesions restricted to lobule VII and caudal VI of the vermis, monkeys are completely unable to adapt to the altered target position. On average, saccades are made with some reduced accuracy to the presented target, but adaptation to the displaced target is no longer possible.

The failure of saccade adaptation reflects an important underlying function of the cerebellum. Each time a saccade is made, a measure of its accuracy is fed to lobule VII. Small errors due to perturbations such as fatigue are compensated and accuracy is restored.

Similar results show that the cerebellum is involved in other forms of motor calibration. The vestibulo-ocular reflex (VOR) is a mechanism whereby the stability of gaze can be maintained in the presence of head movements. As Melville-Jones and his colleagues have shown (Gonshor and Melvill-Jones, 1973; Melvill-Jones and Davies, 1976), as have Miles and his colleagues (Miles *et al.* 1980; Miles and Lisberger, 1981), the reflex can be modified by changing the direction or size of the image on the retina. The flocculus is an essential link in the long-term adaptation of the VOR. Paired Purkinje cell zones are able to adapt eye movements in the plane of the horizontal or the anterior semicircular canals, via the oculomotor neurons in the superior and medial vestibular nuclei (van der Steen *et al.*, 1994). The floccular zones also are a highly conserved system, present in mammals and birds alike (Voogd and Wylie, 2004). The circuit for smooth pursuit in monkeys includes the primary visual cortex, the middle temporal visual area (MT), and the frontal pursuit area in the arcuate cortex and converges upon the flocculus/ventral paraflocculus. Area MT extracts information about direction and speed of the target. The frontal pursuit area is concerned with the modulation of the visuomotor transmission for pursuit, but is dependent on feedback of the eye velocity command from the cerebellum or the brainstem for this task (Rambold *et al.*, 2002; Tanaka and Lisberger, 2002a, 2002b; Priebe *et al.*, 2003; Osborne *et al.*, 2004). The connections of the frontal pursuit area with the flocculus/ventral paraflocculus are not known, but may use the pontine nuclei (Leichnetz *et al.*, 1984; Glickstein *et al.*, 1985; Fries, 1990). The primate corticopontine system differs from mammals with nonfoveate vision in the presence of strong projections from parastriate and parietal areas belonging to the dorsal visual stream, including area MT. These visual corticopontine projections involve the rostral and lateral pontine nuclei, which project to the ventral and the adjacent dorsal paraflocculus. The primate ventral paraflocculus is an extension of the flocculus, using the same, conserved, zonally organized output system

(Voogd *et al.*, 1987). In lower mammals, it is represented by a single lobule (Gerrits and Voogd, 1982; Voogd and Barmack, 2005). The Purkinje cell zonation of the dorsal paraflocculus is quite different, and consists of the C2, D1, and D2 zones with an output through the posterior interposed and dentate nuclei. Visual corticopontine projections to the dorsal paraflocculus are already present in nonfoveate mammals (Burne *et al.*, 1981), and the output system of this lobule through the C2, D1, and D2 zones is the same in foveate and nonfoveate species. However, both the input and the output of the dorsal paraflocculus have differentiated and now include extensive areas of the parietotemporal and frontal association cortex, both as a source for the corticopontine mossy fiber input and as a target for the visual portions of the posterior interposed and dentate nuclei and, 'inter alia', as the origin of the corticomesodiencephalic principal olive climbing fiber paths to the C2, D1, and D2 zones (Voogd, 2003, 2004a). This differentiation in primates may provide the reciprocal pathways connecting the frontal pursuit area with the effective output through the flocculus/ventral paraflocculus.

3.30.7.3 Theories of Cerebellar Function

Some authors interpret the role of the cerebellum in the control of movement as being analogous to a problem of industrial control. Miall *et al.* (1993) propose that the cerebellum acts as a Smith predictor, taking their example from the field of industrial chemistry. In a typical arrangement, such as a petrochemical plant, there is a flow of material into a processor, which then acts on that material to produce an output. The output of the plant is monitored, but since online adjustments are too slow to correct for errors, the Smith predictor serves to compensate for the inherent delays. Rather than directly affecting the processor, there is a computer-based model of the processing system which controls production. The output of the plant is continuously monitored. Errors are fed into the model and corrections made. According to this view, the cerebellum is thought to contain an internal model of the motor system. Any deviations from an intended movement are fed into the cerebellum, which continuously compensates for errors in movement.

In addition to its obvious role in the control of movement, some have argued that the cerebellum, particularly the cerebellar hemispheres, plays a critical role in more complex functions, such as cognition, language, and emotion. Peter Strick and his colleagues (Dum and Strick, 2002; Strick, 2003) find the cerebellar hemispheres are preferentially connected by way of the lateral nuclei to the prefrontal cortex. This connection is interpreted as a closed loop; with the cerebral cortex accessing the cerebellar hemispheres and the cerebellar hemispheres projecting back to the same region of cerebral cortex by way of the lateral nuclei. They suggest that this loop plays a critical role in cognition and planning.

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3.31 Do All Mammals Have a Prefrontal Cortex?

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Glossary

class-common behavior	Behaviors and behavioral capacities demonstrable in all mammals.
species-typical behavior	Behaviors and behavioral capacities that are specific to one or a number of related species.

3.31.1 What is the Prefrontal Cortex?

One of the most obvious characteristics of the mammalian cortex is the regional variation in cellular organization (cytoarchitecture). Beginning in the latter part of the nineteenth century, anatomists used the cytoarchitectonic differences to subdivide the cortex into a mosaic of regions that were presumed to be functionally different (see review by Kemper and Galaburda, 1984). Brodmann, and later many others, attempted to use naming systems that could be used across different species but such comparisons are not easy and are open to many criticisms (e.g., Lashley and Clark, 1946). A principal problem is that mammalian species differ profoundly in the total amount of cortex, in the relative differentiation of cortex, and in the fine details of cytoarchitectonics. For example, a region such as the visual cortex (Brodmann's area 17 or V1) can be presumed to have similar functions across species because it receives its input from the eyes via the lateral geniculate nucleus of the thalamus, yet the species differences in cytoarchitecture are so great that on cytoarchitectonic grounds it is not obvious that the regions are the same (Kaas, 1987). Nonetheless, it is clear that the cortex is divided into distinct anatomically defined areas and that the borders between these areas are relatively sharp. The difficulty is in determining which areas are equivalent in different species. There are clear differences in the size and number of areas across species and it seems likely that most cortical regions subserve more than one function.

The problem of identifying regions of the cortex that have sensory or motor functions is relatively easy insofar as they may receive unimodal input from sensory receptor systems (such as eye, ear, and tongue) or send connections to effector organs (such as muscles). But what about regions that have multiple sensory inputs and are not directly connected to effector organs? This is the problem we encounter in identifying the prefrontal cortex of mammals. What defines this region reliably across species? Although the term prefrontal suggests the tissue at the very front of the hemisphere, this is not a definition that is based either on anatomy of the tissue or on function. The answer to this question must include a consideration of multiple criteria that encompass not only structural comparisons but also functional ones.

The term prefrontal has an uncertain origin dating back to at least the early 1900s and over time has meant different things to different people. Today, however, prefrontal is generally regarded to be synonymous with orbitofrontal as used by Rose and Woolsey (1948). These authors first proposed that the cortical projection of the mediodorsal nucleus of the thalamus (MD) would define the orbitofrontal cortex of mammals. These authors saw their definition as a way of solving the problem that the frontal region of primates beyond the motor and premotor cortex has a prominent layer IV, and thus was called frontal granular cortex, whereas in nonprimates layer IV is indistinct or absent (see The Evolution of Motor Cortex and Motor Systems). Because the MD projection was constant across the mammals that they examined and the granular cortex was not, they believed that their definition of orbitofrontal cortex solved the apparent conflict. Although Rose and Woolsey's definition is useful and unambiguous, it has been criticized (see review by Reep, 1984). The major problem is that the MD is not a homogenous structure and shows large differences in organization and connectivity across mammalian species. The differences in connectivity are not just in the afferents to

the MD but also in the efferents from different regions of the MD. Just because the MD-projection cortex is present across mammalian phylogeny does not mean that the cortex has the same function. We thus must use other criteria in parallel with the Rose and Woolsey definition.

Reep (1984) proposed a different solution to the definitional problem. He proposed that the connections with the amygdala and certain brainstem regions, such as the dopaminergic input from the ventral tegmentum, as well as cytoarchitecture be used to make cross-species comparisons. Using these criteria, he identified five different subregions (see Figure 1) present in monkeys, cats, and rats including the following: (1) a medial infralimbic region receiving input from the amygdala but not the MD; (2) a medial prelimbic region (Brodmann's areas 25 and 32) receiving input from the MD, amygdala, and dopaminergic brainstem; (3) a region of agranular cortex overlying the claustrum receiving input from the MD, amygdala, and dopaminergic brainstem; (4) the anterior cingulate region receiving input from the MD and the anterior thalamus; and (5) a region of cortex lying between the prelimbic and insular areas that receives input from the MD but not the amygdala. He noted that other regions, such as area 9 of the monkey, do not appear to follow any obvious patterns across species and thus may be unique to one or a few orders of mammals. Although Reep preferred to avoid defining the term prefrontal by this set of connections to the subregions, it is an obvious conclusion that could be reached.

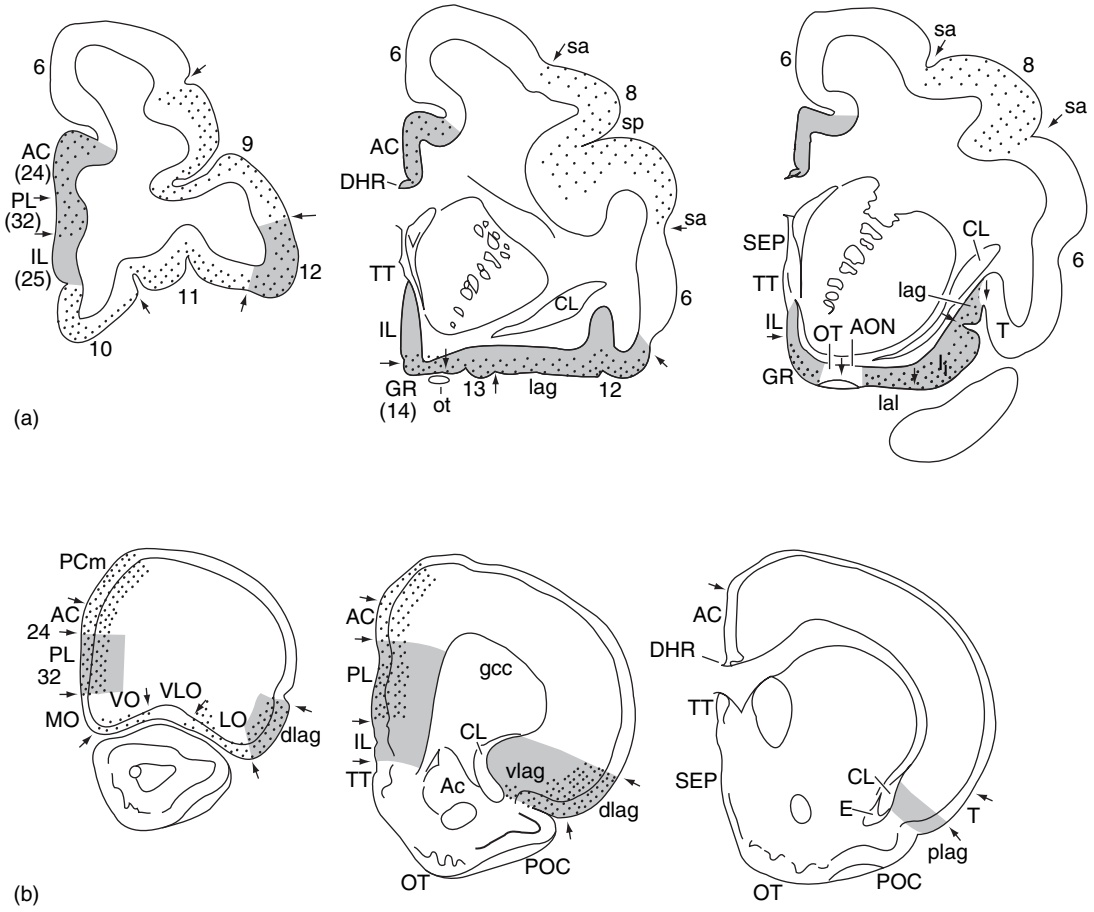

Figure 1 The prefrontal cortex. a, Serial sections through a rhesus monkey brain showing different cytoarchitectonic regions (Brodmann numbers are shown in parentheses). Dotted areas receive projections from MD; gray areas receive projections from the amygdala: b, Serial sections through a rat brain showing architectonic areas and MD and amygdala projection areas as in the monkey. Both species have similar regions of overlap in MD and amygdala projections as well as areas of no overlap. AC, anterior cingulate; Ac, anterior commissure; AON, accessory olfactory nucleus; CL, claustrum; DHR, dorsal hippocampal rudiment; dlag, dorsal agranular insular cortex; E, endo pyriform nucleus; gcc, genu of the corpus callosum; GR, gyrus rectus; lal, allocortical insular cortex; IL, infralimbic; lag, lateral agranular; LO, lateral orbital cortex; MO, medial orbital; OT, olfactory tubercle; PL, prelimbic; plag, posterior lateral agranular; POC, pyriform cortex; sa, arcuate sulcus; SEP, septum; sp, principal sulcus; T, taste cortical area; TT, tania tecta; vlag, ventral lateral agranular; VLO, ventral lateral orbital; VO, ventral orbital. Adapted from Reep, R. 1984. Relationship between prefrontal and limbic cortex: A comparative anatomical review. *Brain Behav. Evol.* 25, 1–80.

The Reep elaboration of the Rose and Woolsey definition of prefrontal is still problematic for two reasons. First, it does not include connections to other forebrain areas, especially the basal ganglia and sensory cortical areas, that are especially extensive with the MD- and amygdala-projection cortex. Unfortunately, these patterns of connection are well described only in primates and rodents but they are nonetheless instructive (for a review, see Uylings *et al*., 2003).

The frontal cortex as a whole has a special relationship with the basal ganglia because, although virtually all of the cerebral cortex projects to the basal ganglia, the frontal cortex receives most of the return projections via the thalamus (Middleton and Strick, 2000; see The Evolution of the Dorsal Thalamus in Mammals). These projections are present in species as diverse as monkeys and rats and, in addition, in both species there are a number of parallel, apparently functionally segregated circuits (Alexander *et al*., 1990; Middleton and Strick, 2000). For example, there are corticobasal ganglia circuits to the motor cortex, occulomotor cortex, anterior cingulate/medial orbitofrontal region, lateral orbitofrontal region, and dorsolateral region in both species.

In addition to the frontal–basal ganglia connections, in both primates and rats the prefrontal cortex is extensively connected with all cortical sensory regions (auditory, gustatory, olfactory, somatosensory, and visual), motor, and limbic cortical areas (e.g., Pandya and Yeterian, 1990; van Eden *et al*., 1992). Thus, in both species the prefrontal cortex receives multimodal corticocortical connections that make the prefrontal region appear to be a nodal station in several parallel networks (van Eden *et al*., 1992; Uylings *et al*., 2003).

In summary, the definition of prefrontal cortex remains inconclusive as no single criterion will suffice. The best definition is that the prefrontal cortex is the region in the cerebral cortex that includes the tissue that has projections from the MD and/or amygdala and that has extensive projections to the basal ganglia and the rest of the cerebral cortex. Given the extensive period of modification of cerebral regions in mammalian evolution, we should not be surprised that there is considerable variation in the details of prefrontal organization and function.

3.31.2 Unity and Diversity in the Prefrontal Cortex

Kaas (1987) proposed that a few basic areas of cortex are present in all mammals. These include primary and secondary visual and somatosensory areas (i.e., V1, V2, S1, and S2), at least one auditory and one taste area, a motor area, a transitional strip of cortex that relates the amygdala and hippocampus to other cortical areas, and a prefrontal cortex related to the mediodorsal nucleus of the thalamus. The assumption is that the common ancestor of modern mammals likely had all these areas and that they are maintained in extant species. Of course, evolution has had millions of years to modify these regions and as brains grew larger, especially in carnivores and primates, many more regions formed, probably by subdividing the prototypical regions. The prefrontal cortex expanded greatly in primate evolution and now has numerous subregions. Pandya and Yeterian (1985) proposed that the expansion of prefrontal subareas is related directly to the increase in sensory areas and the increase in posterior parietal cortex. The implication from this conclusion is that the prefrontal cortex must have some function in the integration of sensory information from different modalities and as more information is processed, the prefrontal cortex must enlarge.

Although the study of mammals in general suggests that all have a region that receives projections from the MD, that all have patterns of connections that have a general similarity, and that the prefrontal region expands as more sensory areas are added, studies of the brains of cetaceans (dolphins, whales, and porpoises) suggest otherwise (see Cetacean Brain Evolution). Cetaceans diverged from the line leading to primates approximately 90 Mya. Like the primate brain, the cetacean brain became large and highly encephalized over the course of evolution and when corrected for body size, many cetacean brains appear to be larger than the brains of chimpanzees or gorillas (e.g., Glezer *et al*., 1988; Marino, 2004). Marino and her colleagues have argued that a fundamental difference in brain development in cetaceans and primates is that, although there is a dramatic increase in the occipital, temporal, and parietal sensory regions, there is little elaboration of frontal lobe structures in cetaceans. Curiously, this lack of frontal lobe elaboration is correlated with a dramatic shrinkage of the olfactory system and in many cetacean species the olfactory structures are completely missing. In addition, limbic structures including the hippocampus and mammillary bodies are unusually small in the cetacean brain. What is not known is whether there is an MD-like structure in the cetacean brain and, if so, where it projects. Although there are studies on thalamic organization in cetaceans, they are all related to the sensory thalamus (e.g., Revishchin

and Garey, 1990) and there do not appear to be any studies of anterior thalamic nuclei.

The possible absence of, or at least the presence of, unusually small, prefrontal cortex and olfactory structures in cetaceans has obvious implications not only for cerebral evolution in general but for the question of why the prefrontal cortex evolved at all.

3.31.3 Why is there a Prefrontal Cortex?

A key concept in answering this question is to remember that evolution acts on the success of the organism, and a major component of the organism's fitness is related to its behavior. Although the brain produces behavior, the evolutionary pressure on brain evolution is indirect through selection of behavior. To understand why there is a prefrontal cortex, we must therefore ask what behaviors have been selected. Warren and Kolb (1978) proposed that although the details of behavior may differ somewhat, mammals share many similar behavioral traits and capacities that have a similar function. For example, all mammals detect and interpret sensory stimuli, relate this information to past experience, and act appropriately. Similarly, all mammals appear to be capable of learning complex tasks under various schedules of reinforcement (Warren, 1977) and all mammals are mobile and have developed mechanisms for navigating in space. Warren and Kolb (1978) proposed that behaviors and behavioral capacities demonstrable in all mammals could be designated as class-common behaviors. In contrast, behaviors that are unique to a species and that have likely been selected to promote survival in a particular niche are designated as species-typical behaviors.

The concept of class-common behaviors is useful in asking why there is a prefrontal cortex. There must be some set of environmental demands that are common across mammals and have encouraged the development of prefrontal cortex. At the same time, there must be differences in the details of prefrontal organization that are related to the differences in species-typical behaviors. For example, the prefrontal cortex has a role in social behavior (a class-common behavior) but mammals vary considerably the complexity of their social interactions (species-typical behavior). It seems likely that highly social mammals such as dogs will have different prefrontal organization than less social mammals such as domestic cats and, indeed, the volume of prefrontal cortex is considerably larger in dogs than in cats.

But what is the class-common function(s) of the prefrontal cortex? This is a difficult question but a review of theories of frontal lobe function have a common theme that the prefrontal cortex is involved in the temporal organization of behavior (e.g., Kolb, 1984; Goldman-Rakic, 1987; Fuster, 1989). The general idea is that the prefrontal cortex supports cognitive functions that are necessary to organize behavior in time. The temporal control of behavior requires at least six components (Kolb, 1990a). First, there must be an ongoing record of sensory experience that has occurred. This allows behavior to be disconnected from ongoing sensory stimulation and related to other stimulation that may have occurred previously. Second, there must be an ongoing record of what movements are being produced by the brain at any given moment, a record known as reafference. Reafference allows the prefrontal cortex to modify ongoing behavior in response to changes in the environment. Third, there must be selection of incoming experiential information, a process often referred to as attention. The importance of particular sensory information obviously varies depending on the environmental demands at any given moment. Fourth, there must be inhibition of some motor impulses and excitation of others to produce appropriate behavioral sequences. Fifth, behavior must be flexible with respect to both internal and external environments. It is one of the characteristics of mammals that we are able to adapt behavioral patterns to changing contexts. The importance of context-dependent behavior is clear in the complex social interactions of mammals. The makeup of the social group at any given time dictates the behavior of each animal. Species as diverse as apes and horses, for example, have a clear pecking order but the details of this social order vary with the precise grouping. Given the presence and position of certain animals, a given ape (or horse) may be bold and relaxed, whereas the presence of a single additional ape may lead to the same ape being quiet and nervous. An error in evaluating context can have grievous consequences. It is likely no accident that the prefrontal cortex has grown so large in primates, which are so highly social. Sixth, there must be an ongoing monitoring of the consequences of behavior. If reward is associated with a particular set of behaviors, but not with another, then the association between movements and consequences needs to be made.

In summary, whereas the principle of temporal organization of behavior provides a basis for the unity of prefrontal function, the necessary components for such a function provide the basis for diversity in the details of prefrontal function across mammals. The apparently unusually small (or absent) prefrontal region of cetaceans may provide

some insight into the class-common function of the prefrontal cortex although it is not immediately obvious what this might be. Another possibility is that the cetacean brain has evolved some other mechanism of integrating behavior over time that uses a fundamentally different set of cerebral processes, much as birds must be doing (see Divac and Mogensen, 1985).

3.31.4 How did the Prefrontal Cortex Arise in Evolution?

The existence of several discrete areas in the prefrontal cortex leads us to wonder whether there is some organizational principle that accounts for the different areas. Pandya and Yeterian (1990) have built on earlier ideas (e.g., Dart, 1934; Sanides, 1972) and conclude that in the mammalian brain the cerebral cortex has had a dual origin. They begin with the proposition that the cortex has evolved from two moieties – the hippocampus (archicortex) and the olfactory system (paleocortex). Each moiety is proposed to be tied to one of two basic functional issues common to all mammals, namely, the questions of 'what' and 'where'. During cortical evolution, the archicortex and paleocortex both give rise to tissue that Pandya and Yeterian describe as periallocortex (Figure 2). The next step is toward cortex that is nearly six-layered (proisocortex), which in turn leads to the most developed, six-layered isocortex. As these two parallel moieties develop, the olfactory-originated cortex develops the ventral and lateral prefrontal areas, whereas the hippocampus-originated cortex develops the anterior cingulate, ventral medial, and dorsolateral cortex. The two regions join on the dorsal surface in what are Brodmann's areas 8, 10, and 46.

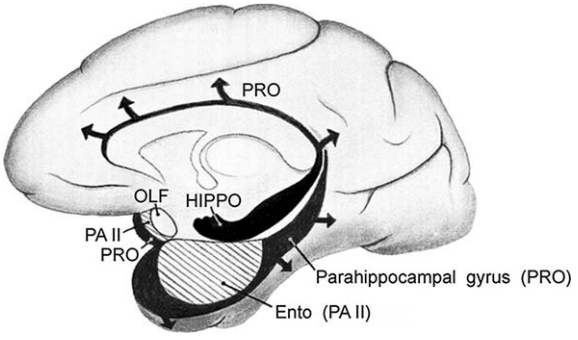

Figure 2 Schematic of a medial view of a rhesus monkey brain showing the hypothetical origin of the cortex from the olfactory system (OLF) and the hippocampal system (HIPPO). Adapted from Cerebral Cortex, vol. 4, 1985, pp. 3–61, 'Architecture and connections of cortical association areas.' Pandya D. N. and Yeterian E. H., with kind permission of Springer Science and Business Media.

In parallel to the prefrontal development is the development of the posterior sensory cortices. Although still poorly understood, the general idea is that the two frontal regions grow in conjunction with sensory regions that are related to the what and where questions. Thus, the paleocortical regions related to 'what' could be expected to develop gustatory, tactile, auditory, and visual connections, whereas the archicortical regions related to 'where' would be unlikely to develop connections with the olfactory and gustatory systems as their information would provide less reliable spatial information. This appears to be the case.

There are important implications of the Pandya and Yeterian ideas. First, the idea that there is an evolutionary development of dual cortical systems indicates that all mammals will have a functionally divided prefrontal cortex. Certainly all mammals that have been studied in detail do seem to have such a distinction (e.g., Kolb, 1984), although the relative size of the two subfields varies considerably (e.g., Benjamin and Golden, 1985). Second, there will be species differences in cortical (including prefrontal) organization and expansion depending on the relative importance of different sensory inputs to the species-typical 'what' questions. Obviously animals living in visually deprived conditions (such as underground) will have less prefrontal–visual interaction and likely more prefrontal–olfactory or tactile interaction. Third, animals that travel extensively in space or make discrete movements to points in space will likely have a more extensive archicortical system. The differences in species-typical behavioral selection of one or the other prefrontal subfields will likely have important implications for understanding much of the unity and diversity in prefrontal functioning. Finally, the fact that the cetacean brain has a marked reduction in both the olfactory system and the hippocampus is intriguing when seen in the light of the dual moieties and the apparently very small frontal lobe. If we assume that early mammals began with the two moieties that expanded into the modern terrestrial mammalian brain, then we can speculate that the two moieties may have regressed as the cetacean brain evolved. The puzzling thing is why did this occur? One intriguing aspect is that the two regions of the mammalian brain that continue to produce neurons in adulthood are the olfactory bulb and the hippocampus. How might these neurons be related to prefrontal functioning, if at all, and what are the implications for regression of these zones in cetaceans?

Jerison (1991) has proposed a complementary model of cortical evolution. His basic thesis is that the major change in mammalian brain evolution is the increase in the number of separate representations (maps) of the sensory world. Whereas relatively primitive mammalian brains (such as that of the hedgehog) may have only a couple of visual maps, the large brains of primates have 10 or more maps. The problem with developing so many maps is in making a single sensory–perceptual representation of the sensory world, a problem often called the binding problem. Jerison proposes that one solution is to bind events together in place and time so that events that are coincident in place and time, but represented in different places in the brain, are experienced as a single event. Recall that we noted earlier that an important class-common function of the prefrontal cortex is the temporal organization of behavior. We can speculate that the prefrontal cortex plays a role in binding sensory information from different sensory modalities together in time. Thus, as the sensory areas expanded to make more maps, the prefrontal cortex expanded to mediate the binding of the maps together. The Pandya and Yeterian model emphasizes the separate evolution of what and where systems in the prefrontal cortex and these represent a type of cognitive map of sensory information. They too must be bound together and the merging of the archicortical- and paleocortical-derived regions in the dorsolateral frontal regions (areas 9, 10, and 46) may contribute to the merging of these maps.

A final consideration in the question of whether the initial mammalian brain had a prefrontal cortex. The Pandya and Yeterian proposal would suggest that it does. Monotremes (egg-laying mammals) might be presumed to be most similar to the initial mammalian brain. Curiously, when Divac and his colleagues examined one monotreme, the Australia spiny anteater (echidna), they found not only that the echidna had an MD-projection cortex but that it had nonthalamic connections that are similar to those of the placental mammals, including Old World monkeys. In contrast, the other monotreme species, the platypus, has a small MD-projection cortex (Bohringer and Row, 1977). This may reflect the relatively smaller size of the MD in the platypus and the lesser importance of olfaction in the largely aquatic platypus (Rowe, 1990), a conclusion that would be concordant with the small frontal area in cetaceans. Studies of other relatively primitive mammals such as marsupials and insectivores have identified an MD-projection cortex (e.g., Broomhead, 1974; Joschko and Sanderson, 1987; Dinopoulos, 1994), suggesting that the initial mammal likely had a prefrontal-like cortex (but see Preuss, 1995; Krubitzer, 1998).

3.31.5 Brain Size and the Scaling of the Prefrontal Cortex

The relationship between brain size, cortical surface area, and behavior has fascinated comparative neuroanatomists for over 100 years. As a rule of thumb, as the brain becomes larger, the number of cortical subregions increases. Jerison (1991) has argued that the increase in cortical surface area is related to more cortical sensory subregions, each of which provides a different map of the sensory world. Pandya and Yeterian (1990) see the increase in sensory maps as being directly related to the increase in prefrontal regions. Kaas and Preuss (2003) propose that as brain size increases, there is a serious problem with connectivity as it is known that connections take up more space than cell bodies themselves (e.g., Klyachko and Stevens, 2003). Because most cortical connections are with functionally similar (and adjacent) regions, one solution to the volume of connections problem is to make connections as short as possible. It follows that if subregions of cortical areas are formed, then the number of connections can be reduced. Thus, as the brain increases in size, we can see for several reasons that there will be more subregions and that this will affect the organization of the prefrontal cortex. We can expect that mammals with large brains will have more prefrontal regions and that there may be species-typical differences in the functional organization of the prefrontal cortex.

But is there a linear relationship between the increase in brain size and the increase in the area of prefrontal cortex? Bush and Allman (2004) addressed this question by comparing the brains of 43 primate and 15 carnivore species. Their analysis shows that in primates the frontal cortex increases in size relative to the rest of the brain, whereas in carnivores it does not. There are no data for other mammalian orders but the Bush and Allman results indicate that not all mammalian orders have an equivalent relative volume of frontal cortex. Indeed, we have seen earlier that cetaceans have a very small frontal lobe given their brain size and the monotreme echidna has a surprisingly large area of MD-projection cortex. The difference in relative volume of prefrontal cortex is almost certainly related to function and the challenge for the future will be to determine whether there are significant functional differences in frontal functioning across

species. As noted above, it is likely that there is a unity in function related to class-common functions of the prefrontal cortex and the differences are related to species-typical specializations in prefrontal function. Identifying these functional differences has not proven easy and is a major challenge in behavioral neuroscience (e.g., Kolb, 1990a; 1990b; Uylings *et al*., 2003).

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3.32 The Evolution of Neural Systems for Sleep and Dreaming

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Glossary

amniotes Vertebrates in which the developing embryo is protected from desiccation by a series of membranes, as an adaptation for terrestrial life. The group comprises reptiles, birds, and mammals.

arc Activity-regulated cytoskeletal-associated protein, a calcium-dependent immediate-early gene directly involved in synaptic remodeling and memory consolidation. Also known as Arg 3.1.

insight Sudden realization of the solution of a problem, achieved by way of a novel strategy not previously envisaged by conscious thought.

memory consolidation Maturation process by which recent memories are strengthened, amplified, propagated, and restructured so as to become long-lasting traces.

neuronal reverberation Poststimulus recurrence of spatiotemporal patterns of neuronal activity related to the stimulus. The phenomenon can be observed in multiple forebrain regions, such as the cerebral cortex, hippocampus, putamen, and thalamus.

plasticity-related genes Set of genes directly or indirectly involved in synaptic remodeling, and required for memory formation. Includes arc and zif268.

proteasomes Complex cellular structures that catabolize proteins. Proteasomes play an essential role in the regulation of the cell cycle, signal transduction, and gene expression.

wish fulfillment According to Sigmund Freud, dreams represent an unconscious attempt to resolve conflicts by way of the realization of repressed wishes.

zif268 Transcription factor required for memory consolidation, encoded by a calcium-dependent immediate-early gene. Homologues include egr1, Krox-24, NGFI-A, and zenk.

3.32.1 Introduction

> Macrobius and Artemidorus [sustained that] dreams were divided in two classes. The first was believed to be influenced only by the present (or the past), and was unimportant in respect to the future; it included the [...] insomnia, which directly reproduces a given idea or its opposite (e.g., hunger or its satiation), and the phantasmata, which elaborates the given idea fantastically (as e.g. the nightmare) [...]. The second class of dreams, on the other hand, was determinative of the future. To this belonged direct prophecies received in the dream (oraculum), (...) the foretelling of a future event (visio), (...) and the symbolic dream, which requires interpretation (somnium) (Freud, 1900).

To understand the evolution of sleep in mammals, one must first note that we lack the kind of direct evidence that lends plausibility to so many evolutionary narratives in biology. We completely lack neurophysiological and neuroanatomical records of ancestor species, and fossilized skulls tell little or nothing about the deep brain nuclei that generate and maintain sleep. At present, the most important data with regard to sleep and dream evolution come from the comparative neurobiological investigation of sleep in extant vertebrate species (Figure 1a). Reptiles, birds, and mammals experience at least two very distinct neural states. During waking (WK), amniotes interact with the environment, carving their ecological niches. During slow-wave sleep (SWS), a periodic state in which brainwaves and neurons slow down and synchronize, amniotes undergo behavioral quiescence and broad sensorymotor shutdown. Crocodilians, birds, and

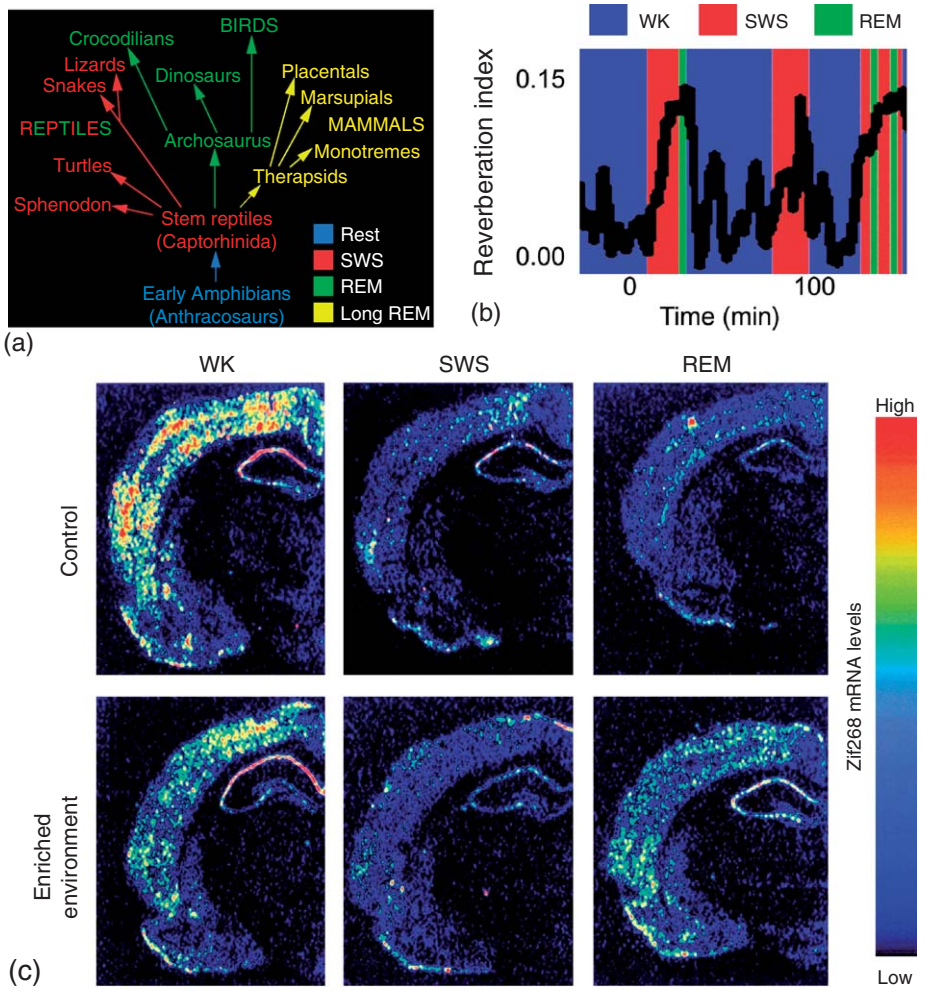

Figure 1 Functional evolution of sleep states. a, Sleep states in extant amniote species and presumed distribution in extinct ancestors; b, memory reverberation measured by neuronal ensemble correlation is strongest during SWS. Shown is a superimposition of successive neuronal ensemble correlations and concurrent behavioral states for neocortical neurons. Correlation peaks correspond to SWS or REM, while troughs match WK epochs. Adapted from Ribeiro, S., Gervasoni, D., Soares, E. S., *et al.* 2004a. Long-lasting novelty-induced neuronal reverberation during SWS in multiple forebrain areas. *PLoS Biol.* 2, 126–137. c, Experience-dependent upregulation of zif268 gene expression in the rat brain during REM sleep. Shown are autoradiograms of representative brain sections hybridized with a zif268 radioactive riboprobe. In controls kept in their familiar home cages before the experiment (top panels), zif268 expression decreased from WK to SWS and REM sleep. In animals exposed to an enriched environment for 3h before the experiment (bottom panels), zif268 levels decreased from WK to SWS, but increased from the latter to REM sleep. This effect was particularly noticeable in the cerebral cortex and the hippocampus. Adapted from Ribeiro, S., Goyal, V., Mello, C. V., and Pavlides, C. 1999. Brain gene expression during REM sleep depends on prior waking experience. *Learn. Mem.* 6, 500–508.

mammals also display another state called rapid-eye-movement sleep (REM), in which behavioral quiescence is concomitant with an increased activation of specific brain areas. Birds and crocodilians have very short REM episodes, lasting a few seconds each. In contrast, mammals have long REM episodes that may last more than 1h. In humans, REM is highly correlated with the occurrence of dreaming. What were the selection pressures that shaped SWS and REM? What are the extant functions of sleep and dreams, and how did they evolve?

After more than a century of anatomical and physiological investigation of the brain, the biological mechanisms underlying the generation and maintenance of sleep are reasonably understood (Aserinsky and Kleitman, 1953; Dement, 1958; Dement and Kleitman, 1957a, 1957b; Grastyan and Karmos, 1961; Jouvet, 1967; Jouvet *et al.*, 1959; Lee and Jones, 2004; Luppi *et al.*, 2004; Roffwarg *et al.*, 1962; Rechtschaffen and Kales, 1968; Siegel, 1990; Steriade, 1992; Steriade *et al.*, 1993; Sutcliffe and de Lecea, 2002; Timo-Iaria *et al.*, 1970), but the functions of sleep and dreams

remain controversial (Dave and Margoliash, 2000; Frank *et al.*, 2001; Gutwein *et al.*, 1980; Hennevin *et al.*, 1989; Hirase *et al.*, 2001; Hoffman and McNaughton, 2002; Lee and Wilson, 2002; Louie and Wilson, 2001; Maquet *et al.*, 2000; Nadasdy *et al.*, 1999; Pavlides and Winson, 1989; Peigneux *et al.*, 2003; Pompeiano *et al.*, 1994; Qin *et al.*, 1997; Ribeiro *et al.*, 1999, 2002, 2004a; Siegel, 2001; Skaggs and McNaughton, 1996; Winson, 1985; Wilson and Naughton, 1994). Deeper rifts exist within psychology. More than a century after the publication of *The Interpretation of Dreams* (Freud, 1900), a consensus about dreams and their relationship to human consciousness is yet to be achieved. While depth psychology focuses on dream meaning and wish fulfillment (Fosshage and Loew, 1978; Freud, 1900; Jung, 1953a, 1953b, 1974; Jung *et al.*, 1969; Solms, 2004), experimental psychology scrutinizes sleep-dependent learning in search of less subjective dream purposes (Bryson and Schacher, 1969; Fishbein, 1971; Jenkins and Dallenbach, 1924; Karni *et al.*, 1994; Lucero, 1970; Leconte and Bloch, 1970; Leconte and Hennevin, 1971; Maquet *et al.*, 2003; Mednick *et al.*, 2003; Pearlman, 1969; Smith and Butler, 1982; Smith and Lapp, 1986; Smith and Rose, 1996; Stickgold *et al.*, 2000; Walker *et al.*, 2002b; Wetzel *et al.*, 2003). Due to their different methods, each of these fields of investigation has, to a large extent, established a sleep and dream phenomenology of their own.

The persistent gap separating the neural processes underlying sleep from a comprehensive account of subjective dreaming has produced dream models that are outright incompatible with fundamental observations available to any introspective dreamer. One example of such dissociation is the notion that the bizarreness and hyperassociativeness of dreams can be trivially explained by random neuronal activation of the neocortex during REM (see Neuronal Migration, A Tale of Two CPGs: Phylogenetically Polymorphic Networks; Crick and Mitchison, 1983, 1995). According to this anti-Freudian theory, dreams arise from stochastic deep brain inputs to the neocortex, evoking a succession of neuronal firing patterns that correspond to randomly assembled memory fragments. The model proposes that such process has the function of erasing irrelevant memory traces, cleaning up storage space to allow the formation of new memories. A corollary of this theory is that dream content is intrinsically meaningless. This conclusion undermines the very significance of dream interpretation as a relevant window into human consciousness (Freud, 1900).

The 'random cortical activation' theory does not survive confrontation with the fact that dreams can be remarkably repetitive, especially when major trauma has occurred. Indeed, recursive nightmares are an important symptom of post-traumatic stress disorder, which is characterized by disturbed, hyperaroused REM (Ross *et al.*, 1994, 1999). For instance, war veterans dream about battle events for decades after the end of combat (Esposito *et al.*, 1999; Neylan *et al.*, 1998; Schreuder *et al.*, 1998). Given the colossal number of neurons and synapses in the human neocortex, it is clearly impossible to explain the activation of nearly identical neuronal firing patterns over several consecutive dreams by way of random neocortical activation.

If it is true that some neurobiological theories of dreaming lack introspection and conflict hopelessly with the basic dream phenomena, the humanities often err due to unwarranted anthropocentric or ethnocentric perspectives. The anti-Freudian philosopher Owen Flanagan, for instance, argues that dreams cannot possibly be a biological adaptation, based on his failure to recognize fitness-enhancing elements on his own dreams. Owen concludes that dreaming is meaningless and serves no function at all: "dreams are the spandrels of sleep" (Flanagan, 2000). On the other side of the road, the psychoanalytical tradition has been faulted and even ridiculed for insisting that dreams are always an attempt to fulfill a wish (Freud, 1900), and for considering censorship of scandalous thoughts a universal function of dreams (Freud, 1915, 1920) instead of a specific cultural mark of the conservative Viennese society in which Freud lived and produced his work (Gay, 1989).

It is time for a synthesis. A satisfactory theory of sleep and dreaming must first account for all the related phenomenology, and not selected parts of it. Second, it must distinguish the several functions of the different sleep and dream states. Third, it must produce a plausible evolutionary narrative of how these states enhanced fitness over phylogenetic time, evolving into a layered set of functions that can only be peeled apart in the appropriate chronological order.

3.32.2 The Evolution of SWS

When, why, and how did sleep evolve? With perhaps a few exceptions, all animals display intermittent rest in alternation with periods of activity, for energy conservation, metabolite replenishment, and predator avoidance (Berger, 1993; Berger and Phillips, 1990, 1995; Inoue *et al.*, 1995; Rattenborg *et al.*, 1999). While rest is unpredictable and mostly determined by external variables, sleep is an endogenous state of deep quiescence tightly regulated by mechanisms tuned to circadian

variations in ambient light. Flies and crabs possess a quiescent state similar to SWS in amniotes (Nitz *et al.*, 2002; Ramon *et al.*, 2004; Shaw *et al.*, 2000). It is not clear whether sleep is a conserved trait in vertebrates and invertebrates, or just a convergent adaptation driven by similar selection pressures such as circadian light changes. On one hand, the neurobiology of sleep in nonarthropod invertebrates is a vast unexplored frontier. On the other hand, it is still debatable whether fish (Shapiro and Hepburn, 1976; Tobler and Borbely, 1985) and amphibians (Hobson *et al.*, 1968; Lazarev, 1978; Monnier, 1980; Vataev, 1989) truly sleep, or rather display intermittent rest periods that occur whenever permitted by the lack of environmental opportunities or threats (Campbell and Tobler, 1984; Kavanau, 1998). The aquatic medium is only superficially affected by circadian light changes, being opaque most of the time due to depth or suspended particles. As a consequence, fish rely heavily on electroception, magnetoception, and olfaction to survive, but vision is usually of reduced importance (Butler and Hodos, 1996; Grier, 1984).

Amniotes are a monophyletic group derived from Anthracosaurs, a group of Paleozoic amphibians (Gauthier, 1994) (Figure 1a). Given the presence of SWS in all extant reptile, avian, and mammalian species investigated to date (Campbell and Tobler, 1984; De Vera *et al.*, 1994; Dewasmes *et al.*, 1985; Flanigan, 1973; Flanigan *et al.*, 1974; Hess *et al.*, 1953; Hobson, 1989; Huntley, 1987; Kavanau, 1997; Kleitman, 1963; Ookawa, 1972; Rojas-Ramirez and Tauber, 1970; Szymczak, 1987; Timo-Iaria *et al.*, 1970; Van Twyver and Allison, 1970; Wayne, 1950), one must conclude that SWS was favorably selected early on in the amniote lineage, during the ecological pilgrimage from water to dry lands of the Carboniferous period (354–290 Mya) (Futuyma, 1986). Conquest of the terrestrial environment involved a drastic change of the vertebrate sensory environment, as land dwellers in general depend heavily on visual and auditory information to survive. It is fair to assume that the absence of light during nighttime, by forcing protoreptiles to hide from predators in burrows, caves, or nests (Grier, 1984), was a strong constraint on the evolution of early SWS. The first sleepers were probably the ancestors of turtles (Broom, 1924; deBraga and Rieppel, 1997), large and morose Captorhinids (Figure 1a) that thrived in a vast Eden populated by relatively harmless protein-rich invertebrates and abundant edible vegetation, but eventually evolved heavy armor and discrete habits to avoid the predation of other evolving amniotes (Carroll, 1988). Sleep co-evolved with massive reciprocal connections between thalamus and cortex (Butler, 1994; Karten, 1991, 1997; Karten and Shimizu, 1989; Northcutt, 1981; Reiner, 1991). During SWS, thalamocortical interactions give rise to neuronal bursting and slow local field potential oscillations that maintain the sensory disconnection typical of SWS (Steriade, 1992; Steriade *et al.*, 1993).

How relevant was this step? SWS more than fulfills the primary rest functions related to energy conservation and metabolite turnover, but it also happens to enhance an additional biological function of immense behavioral impact – learning (Bryson and Schacher, 1969; Fishbein, 1971; Jenkins and Dallenbach, 1924; Karni *et al.*, 1994; Lucero, 1970; Leconte and Bloch, 1970; Leconte and Hennevin, 1971; Maquet *et al.*, 2003; Mednick *et al.*, 2003; Pearlman, 1969; Smith and Butler, 1982; Smith and Lapp, 1986; Smith and Rose, 1996; Stickgold *et al.*, 2000; Walker *et al.*, 2002b; Wetzel *et al.*, 2003). A 1991 random survey of 1000 Americans by the National Sleep Foundation and the Gallup Organization verified the devastating effects of insomnia on many aspects of the WK function, including impaired memory and decreased ability to accomplish daily tasks. During SWS, waking patterns of neuronal activity reverberate in several forebrain areas including the hippocampus and the neocortex (Dave and Margoliash, 2000; Hirase *et al.*, 2001; Hoffman and McNaughton, 2002; Lee and Wilson, 2002; Louie and Wilson, 2001; Maquet *et al.*, 2000; Nadasdy *et al.*, 1999; Pavlides and Winson, 1989; Peigneux *et al.*, 2003; Qin *et al.*, 1997; Ribeiro *et al.*, 2004a; Skaggs and McNaughton, 1996; Wilson and McNaughton, 1994), reflecting the absence of sensory interference (Pavlides and Ribeiro, 2003; Ribeiro *et al.*, 2004a). Such reverberation seems to promote the pretranscriptional amplification and consolidation of memories by way of calcium-dependent mechanisms (Buzsaki, 1998; Destexhe and Sejnowski, 2003; Massimini and Amzica, 2001; Sejnowski and Destexhe, 2000; Steriade *et al.*, 1993). By focusing endogenous brain activity on recently utilized neuronal networks, SWS increases the contrast between what will or will not be remembered so as to effectively amplify selected memories (Figure 1b). While the body saves energy, SWS promotes learning by repetition. It is conceivable that the cognitive function of SWS evolved as a mere epiphenomenon of the adaptation to a marked circadian regulation of rest behavior. Still, it is tempting to speculate that the evolution of an offline brain state able to enhance memory consolidation played a significant role in the reptile radiation that led to the long Mesozoic age of saurian supremacy (~250–65 Mya).

3.32.3 The Evolution of REM

REM is characterized by eye-closure, increased cerebral activation, and total absence of muscle movements, except for occasional localized muscle twitches (Aserinsky and Kleitman, 1953; Dement, 1958; Dement and Kleitman, 1957a, 1957b; Grastyan and Karmos, 1961; Jouvet, 1967; Jouvet *et al.*, 1959; Rechtschaffen and Kales, 1968; Roffwarg *et al.*, 1962; Timo-Iaria *et al.*, 1970). The mass extinction at the end of the Cretaceous (65 Mya) allowed for the ecological spawn of birds and mammals, warm-blooded vertebrates characterized by having REM (Siegel, 1995) and a superior capacity for learning (Balaban, 1988; Kaminski *et al.*, 2004; Kroodsma, 1974; Pepperberg and Brezinsky, 1991; Savage-Rumbaugh *et al.*, 1980; Thompson and Herman, 1977; Thorpe, 1958; Whiten *et al.*, 1999) (Figure 1a). Several pontine, mesencephalic, and forebrain nuclei co-evolved with REM for its generation and maintenance (Lu *et al.*, 2000; Luppi *et al.*, 2004; Nelson *et al.*, 1983). REM upregulates the cortical expression of the plasticity-related genes arc and zif268, (Ribeiro *et al.*, 1999, 2002, in preparation; Ulloor and Datta, 2005), which trigger the strengthening and remodeling of selected synaptic connections (Lemaire *et al.*, 1990; Richardson *et al.*, 1992; Sukhatme *et al.*, 1988; Wallace *et al.*, 1995). The same genes are downregulated during SWS, so that a single night of sleep witnesses several stop-and-go gene upregulation cycles (Ribeiro *et al.*, 1999) (Figure 1c). Both arc and zif268 are necessary for the formation of long-term memories (Bozon *et al.*, 2003; Jones *et al.*, 2001; Guzowski *et al.*, 2000). Arc interacts with the cytoskeleton and calcium-dependent enzymes at the presynaptic terminal (Donai *et al.*, 2003; Guzowski *et al.*, 2000; Lyford *et al.*, 1995), while zif268 promotes changes on the postsynaptic terminal in response to presynaptic excitation (Petersohn *et al.*, 1995; Takeuchi *et al.*, 2002; Thiel *et al.*, 1994). Furthermore, zif268 upregulation inhibits proteasome activity (James *et al.*, 2006), shifting the neuronal metabolism toward protein synthesis and synaptic plasticity (DiAntonio and Hicke, 2004). As a consequence, the SWS–REM cycle produces an anterograde propagation of memory traces across brain areas (Ribeiro *et al.*, 2002), increasing the reach and strength of memories over time (Ribeiro and Nicolelis, 2004). A single cycle involving both phases suffices to consolidate certain short-term memories (Mednick *et al.*, 2003), but it is the repetition of several cycles during a night that promotes deep transformations, gradually propagating memories through several brain areas (Pavlides and Ribeiro, 2003). This process likely anchors memories ever more solidly in the neuronal matrix (Cermak and Craik, 1979; Craik and Lockhart, 1972), causing a cumulative increment of learning at each night of sleep (Walker *et al.*, 2003). In mammals, anterograde propagation of gene expression during sleep seems to promote the continuous exodus of memories from the hippocampus to the cerebral cortex (Ribeiro *et al.*, 2002, in preparation), periodically freeing new coding space at the entry gate for episodic memories (Izquierdo and Medina, 1997; Bontempi *et al.*, 1999; Frankland and Bontempi, 2005; Frankland *et al.*, 2001; Scoville and Milner, 1957; Squire, 1992). It may be hard to determine today how important was the acquisition of REM for the ecological success of birds and mammals, but certainly its addition to SWS made for much faster, stronger, and durable learning.

Although ancestral reptiles were the first to evolve proper sleep, modern reptiles sleep less than most mammals, and (except for crocodiles) lack REM (Hobson, 1989). Why did turtles, lizards, and snakes fail to develop REM? Ectothermy is the most likely reason (Kavanau, 1997, 2002), because mammals and birds can only enter REM within a narrow range of relatively high temperatures (Amini-Sereshki and Morrison, 1982; Azzaroni and Parmeggiani, 1993; Glotzbach and Heller, 1994; Satinoff, 1988; Szymusiak *et al.*, 1999). Another possible key may be found in the temporal order of the two sleep states, and their different susceptibilities to fear. Under normal circumstances, in men and mice alike, REM can only follow SWS but the opposite never occurs (Aserinsky and Kleitman, 1953; Jouvet, 1967; Rechtschaffen and Kales, 1968; Timo-Iaria *et al.*, 1970). Furthermore, REM can only occur after a fair amount of SWS has taken place (Aserinsky and Kleitman, 1953; Jouvet, 1967; Rechtschaffen and Kales, 1968; Timo-Iaria *et al.*, 1970). Importantly, rats exposed to fear conditioning have a strong suppression of subsequent REM, but not SWS (Sanford *et al.*, 2001). The appearance of ever more implacable predator dinosaurs over the Mesozoic turned life on the dry land increasingly threatening. Could predation have prevented the ancestors of modern reptiles to sleep well? Maybe the myth of the dragon that sleeps with one open eye has its ethologic foundation in the fact that some reptiles display unihemispheric SWS, a strange split-brain state also present in birds and aquatic mammals (Ayala-Guerrero *et al.*, 1988; Ball *et al.*, 1986; Flanigan *et al.*, 1974; Lilly, 1964; Lyamin *et al.*, 2000; Mukhametov *et al.*, 1992; Rattenborg *et al.*, 2000; Szymczak *et al.*, 1996), shown to be positively correlated with predation risk (Rattenborg *et al.*, 1999). Or perhaps, quite to

the contrary, the cognitive advantage provided by reptilian SWS far exceeded the need for more animal intelligence in the rich and stable Mesozoic environment. Whether the most intelligent of the mighty Jurassic dinosaurs ever evolved REM is a mystery, but their closest relatives on the Earth, crocodiles (Flanigan *et al*., 1973) and birds (Dewasmes *et al*., 1985; Flanigan, 1973; Ookawa, 1972; Szymczak, 1987), do have REM (Figure 1a). Given the enormous evolutionary distance between the mammalian and saurian lineages, it is possible that REM is a convergent adaptation that evolved twice in vertebrates. Further neuroanatomical and neurophysiological investigation of the REM-related neural circuitry in birds and crocodilians is in order to resolve the issue.

3.32.4 The Evolution of Extended REM

When it comes to REM differences between mammals and birds, time is of essence. Whereas most mammals display a few long REM episodes per 24 h cycle, with episode duration of up to hundreds of minutes, birds exhibit hundreds of ultrashort REM episodes in a single night, with duration smaller than 20 s (Dewasmes *et al*., 1985; Flanigan, 1973; Ookawa, 1972; Szymczak, 1987) (Figure 1a). Why did birds fail to develop long REM? A clue may be found in the fact that, during REM, despite the lack of sensory inputs, great portions of the forebrain become as active as during waking. Such high levels of activity fail to become overt behavior (and therefore interrupt sleep) because inhibitory glycinergic neurons in the pons effectively block most of the muscle activity during REM (Jouvet, 1994). Thus, it is conceivable that the need to keep residual muscle tonus for perching was the ecological constraint for the small duration of avian REM episodes (Dewasmes *et al*., 1985; Flanigan, 1973; Ookawa, 1972; Szymczak, 1987). The current evidence indicates that plasticity-related gene expression, possibly the primary cognitive function of REM, is uncorrelated with time spent in REM (Ribeiro, 2000; Ribeiro *et al*., 1999, 2002), and reaches maximum levels even when REM episodes are cut very short (Shi *et al*., 2004). What sort of selection pressures drove the elongation of individual REM episodes in mammals? Do extended REM episodes contribute to learning in any special way?

The molecular and cellular sleep-dependent mechanisms described so far are involved in the stabilization and strengthening of already-acquired memories. There is however another form of learning, more dramatic and puzzling. The insight (Kohler, 1947), also known as abduction (Peirce, 1958), corresponds to the creation of new memories and ideas not trivially derived from pre-existing memories. Although insights may occur during waking (Jung-Beeman *et al*., 2004), they are greatly facilitated by sleep (Wagner *et al*., 2004). Several notorious examples of sleep-dependent insight can be drawn from both science and art (Barrett, 2001). Kekulé dreamt a snake eating its own tail and thus discovered the circular structure of benzene. Mendeleev, the discoverer of the periodic table, visualized his breakthrough concept in a dream. Intense dreams very much inspired artists like Dürer, Blake, Dalí, Frida Kahlo, and many others (Barrett, 2001). Although the biological mechanisms underlying sleep-dependent insight still remain unknown, the available subjective reports of the phenomenon point to an important role of dreams (Barrett, 2001).

In support of this hypothesis, mounting evidence indicates that extended REM harbors nonstationary neuronal reverberation, in contrast with highly stationary reverberation during SWS (Pavlides and Winson, 1989; Ribeiro *et al*., 2004a, 2004b; Winson and Abzug, 1977). Such 'noisy' reverberation during REM, long postulated by psychology (Hartmann, 1967, 1998) , should promote memory restructuring instead of memory strengthening, assembling 'new memories' from fragments of pre-existing ones. In other words, insights may derive from the shuffling or recombination of relevant memory traces during REM. Evidence in favor of this hypothesis comes from psychological experiments in which subjects were woken up from either SWS or REM and immediately asked to resolve anagrams. The results indicate that REM promotes more flexible cognitive processing than SWS, suggesting that memory restructuring is indeed facilitated by REM (Walker *et al*., 2002a). The evolution of extended REM may be related to the positive selection of insightful behavior, a feature of great importance in unstable changing environments such as the Earth after the Cretaceous–Tertiary cataclysm. All known species of monotremates, marsupials, and placentals possess relatively long REM episodes (Siegel, 2004), ranging from a few to several minutes. The echidna, long suspected to be the only exception among mammals (Allison *et al*., 1972), actually has a REM-like state (Nicol *et al*., 2000; Siegel *et al*., 1996).

3.32.5 The Evolution of Dreams as Emotional Simulations of Past and Future

In humans, REM is nearly always accompanied by dreaming (Dement and Kleitman, 1957a, 1957b;

Roffwarg *et al.*, 1962). Although the strengthening and restructuring of memories are at the roots of the cognitive functions of sleep and dreams, these concepts do not account entirely for the symbolic complexity that characterizes the oneiric narrative in adults. After all, it is not common to dream about the repetition of hard tasks, nor about static and isolated images, nor with the resolution of riddles. Dreams may have first evolved as a collateral effect of extended REM, and are likely present in all the mammals that possess such trait. Any pet owner knows that cats and dogs seem to act out dreams during sleep. More controlled evidence of dreaming in nonhuman mammals was obtained by lesions of the brainstem nuclei that promote muscle atonia during REM (Jouvet and Delorme, 1965). Cats with such lesions sleep quietly through SWS, but upon entering REM become suddenly agitated by vigorous species-specific behaviors, such as meowing and pouncing. What do cats dream about? What are the neural substrates of dreaming, what is the purpose of dreams as narratives, and which selection pressures shaped their evolution?

During human REM, a selected set of forebrain areas gets activated, including portions of the hypothalamus, amygdala, septum, and ventral striatum, as well as the anterior cingulate, orbitofrontal, entorhinal, and insular cortices (Braun *et al.*, 1997; Maquet *et al.*, 1996; Nofzinger *et al.*, 1997). Furthermore, it has been shown that dreaming ceases upon lesion of mesolimbic pathways connecting reward centers with the thalamus, striatum, and cortex (Solms, 1997, 2000). This suggests that dreams promote the "integration of neocortical function [...] with motivational and reward mechanisms" (Nofzinger *et al.*, 1997).

Human dreams are subjective narratives composed of familiar and unfamiliar beings, things, and places, interacting around a self-representation of the dreamer that mostly observes an unfolding plot. Dreams vary in intensity, ranging from confused and faint impressions to complex time-evolving narratives with vivid imagery and surprising turns. Although dreams tend to be dominated by visual images, they can also involve combinations of auditory, olfactory, tactile, gustatory, motor, vestibular, and linguistic modalities. Dreams can sometimes be extremely pleasant or just the opposite, but are usually characterized by a mix of emotions. Dreams are also hyperassociative, linking characters, places, and actions in bizarre ways. While normal dreams usually lack in logical coherence, remarkably meaningful dreams do occur to most people at least a few times in life. As noted by Freud, dreams often involve elements of the experience of the preceding day(s), the 'day residue' (Freud, 1900). Dreams can also anticipate events of the coming day(s), particularly when subjects undergo extreme anxiety and expectation. A good example is provided by the dreams of students before difficult exams, which often contain detailed anticipatory simulations of the expected challenges, either in content and/or context. Freud also observed that dream narratives fulfill wishes (or anti-wishes) of the awakened subject, simulating the realization (or frustration) of specific desires (Freud, 1920). Although the prevalence of overt wish fulfillment in the dreams of normal adults is low, it is common in young children (Foulkes, 1982).

Dreams are caused by intense and nonstationary memory reverberation during extended REM, with the neocortex lightened up at high neuronal firing rates and oscillatory frequencies predominantly above 30 Hz (Cantero *et al.*, 2004). The qualitative feeling of 'quasireality' in dreams derives from the fact that memory reverberation during REM occurs at an intensity and oscillatory spectrum comparable to those of WK (Steriade *et al.*, 1993). Such high levels of excitation cause the reverberating memories that comprise dreams to appear bright and vivid to the self-representation, and variations in neural activity levels explain the dynamic range of vividness that characterizes normal dreaming. Similarly, the simultaneous occurrence of neuronal reverberation in multiple forebrain sites during REM (Maquet *et al.*, 2000; Pennartz *et al.*, 2004; Ribeiro *et al.*, 2004a) explains the wide variation in dream modality. There is also a compelling relation between the high variability of neuronal reverberation during REM and the fragmentation, condensation, and bizarreness of dreams. Far from being random (Crick and Mitchison, 1983, 1995), dream narratives highlight waking events according to how recent, novel, and behaviorally significant they were, that is, dreams are directed by the anxieties and preoccupations of the dreamer (Winson, 1985). Dreams seldom occur during SWS (Kales *et al.*, 1967; Nielsen, 2000; Roffwarg *et al.*, 1962). Instead, the subjective experience of SWS consists of low-intensity but coherent thoughts resembling waking reasoning (Fosse *et al.*, 2004). The coherence of such 'mentation' likely reflects the high stability of neuronal reverberation during SWS (Pavlides and Winson, 1989; Ribeiro *et al.*, 2004a; Winson and Abzug, 1977). By the same token, the lack of intense imagery during SWS probably reflects the decreased cortical activity and slow oscillations below 4 Hz that characterize memory reverberation in this state (Steriade *et al.*, 1993; Timo-Iaria *et al.*, 1970).

If dreams first arose as a byproduct of neuronal reverberation during extended REM, what (if any) the functions of dreams are? So far, we discussed how sleep states co-evolved with learning-related neural mechanisms. Dreams, just like sleep states, were also selected for their adaptive value for learning. This may not be apparent in most people's dreams (Flanagan, 2000; Germain *et al.*, 2000), but normal human dreaming is arguably a good model of normal mammalian dreaming. Humans organized in complex societies face minimal behavioral challenges, in comparison with the constant game of life and death experienced by freely ranging animals. In nature, vital resources are scarce, competition is relentless, and populations of nearly all species suffer predation. Contrary to humans, most animals face the perspective of being eaten alive on a daily basis. Animal behavior in the wilderness is surely selected for fast and effective learning. The evolution of dreams in higher vertebrates was certainly shaped by a tough environment in which uncertainty was the rule, and REM-dependent insight was positively selected. Given this set of constraints, one should expect freely ranging mammals to possess a very limited dream repertoire, consisting of actions to avoid predation, spatial maps to orient foraging, mating, and a few other species-specific behaviors of high adaptive value.

To conceive the selection pressures that shaped dream evolution in nonhuman mammals, we must look toward the kind of dreams that humans have when confronted with primeval challenges. Two types of dream are of special interest, those following or preceding highly significant events (either good or bad). For instance, major psychological or physical trauma usually triggers vivid and repetitive nightmares (e.g., wartime dreams) (Kanzer, 1949; Ross *et al.*, 1994, 1999). The excessive reverberation of traumatic memories during sleep stems from the overwhelming emotional strength of those memories at the time of encoding. It is as if the brain was stuck with a problem that has no answer, which is often the case in seriously injured human patients. As to the second case, dreams that predict possible predation in the future are usually rare in humans, except during wartime. A more familiar example to most people can be found in the anticipatory dreams preceding school exams, which simulate potentially dangerous events from the point of view of the dreamer.

Dreaming co-evolved with a hypertrophy of emotion-related brain structures, such as the amygdala, the ventral striatum, and the hypothalamus (Adolphs *et al.*, 1995; Braun *et al.*, 1997; Cahill *et al.*, 1995; Davis, 1994; Maquet *et al.*, 1996, 1997; Nofzinger *et al.*, 1997). The data and concepts discussed so far suggest that mammalian dreams are probabilistic simulations of past events and future expectations. The main function of these simulations would be to test specific novel behaviors against a memory replica of the world, rather than the real world itself, leading to learning without risk. This hypothesis is a generalization of the threat simulation theory of dreaming (Revonsuo, 2000), that is, dreams may either simulate actions that lead to undesirable consequences and therefore should be avoided in the real world (e.g., being predated upon), or actions that lead to a desirable outcome and therefore should be performed in the real world (e.g., finding mates and food). An investigation of REM mentation found that over 70% of the reports included emotions, with a balanced proportion of positive and negative emotions (Fosse *et al.*, 2001). The notion that nightmares evolved as a way to negatively modulate particularly dangerous behavior simulations, while blissful dreams correspond to the association of pleasure (reward) with dream simulations of especially adaptive behaviors, is analogous to the concepts of eros and thanatos proposed by Freud as life and death drives (Freud, 1920).

In all species studied so far, REM is much more prominent in juveniles than in adults (Feinberg *et al.*, 1967; Hobson, 1989). Likewise, play is a behavior that evolved exclusively in birds and mammals and that is much more pronounced in youngsters than in adults (Grier, 1984; Hutt, 1966; Poole, 1966; Schenkel, 1966). These observations reflect the fact that naïve individuals rely heavily on simulation-based learning processes to survive in unpredictable and dangerous environments, online as well as offline (Chapman and Underwood, 2000; Cheyne, 2000; Humphrey, 2000). It is conceivable that daydreaming evolved as an invasion of dream-like mnemonic reverberation on top of waking sensory inputs. Playing, imagining, remembering, planning, scheming, practicing, and training skills are all examples of an extension of the self – an entity of the present – through past and future, a process that may be at the roots of our very kind of consciousness.

3.32.6 Human Dreams, Present and Future

According to the view presented here, the function of dreams is to trim and shape the memories acquired during waking, in a cyclic process of creation, selection, and generalization of conjectures about the world. Dreams are neither isolated pieces of a puzzle nor linear strings of memories, but rather a

concatenation of sensory and motor representations according to the dominant emotions of the dreamer. Dreams function as blind oracles, biological machines that create future scenarios based solely on the past experience, guiding the waking actions so as to maximize fitness. This aspect of future prediction, or more exactly future guessing, is probably the explanation for the widespread belief in dream premonition among ancient societies. Despite their stochasticity, dreams sometimes yield very accurate predictions of future events. This is a very rare phenomenon in modern human society, but dream soothsayers prospered during all of the antiquity (Jung *et al.*, 1969; Miller, 1997), and dream interpretation continues to play an important role in many so-called 'primitive' cultures (Kilton, 1951; Lincoln, 2003; Cawte, 1984; Shulman *et al.*, 1999).

As the challenges faced by humans became gradually easier and complex, dreams lost much of their predictive power but acquired a diversified symbolic repertoire. In comparison with other wild mammals in nature, modern middle-class humans experience much less serious anxieties. Nonhuman predators are very rare, law enforcement restricts conspecific predation, and, however stressful school and workplace examinations may be, they do not involve physical pain and possible mutilation. Nutritious food can be acquired in large quantities at grocery stores, health care is provided quickly after accidental injuries, and we inhabit permanent, safe, and solid shelters. In modern humans, dreams are no longer under the influence of life-or-death events, but are rather dominated by a myriad of minor frustrations, challenges, and expectations. In the absence of highly eventful recent experiences, it is not surprising that normal human dreams tend to mix recent though somewhat trivial elements of waking life with old, but strongly encoded, memories of childhood (Freud, 1900).

One remarkable feature of dreams is that they are almost never observed by dream characters other than the self-representation. Under normal circumstances, dreamers have limited control of their dream actions, and no control whatsoever of other dream characters and scenes, which display a large degree of autonomy. It seems obvious from these facts that the self-representation is just one among the many memories activated during REM, woven into dream narrative by the idiosyncratic probabilities of memory association in each individual. Dreams, conceived by Freud as "a conglomerate of psychic formations" (Freud, 1900), seem to reflect the fragmented activation of the very stuff the unconscious is made of, that is, latent memories (Freud, 1915). The limited volitional power of the self-representation during dreams likely reflects the deactivation during REM of the dorsolateral prefrontal cortex (Maquet *et al.*, 1996; Muzur *et al.*, 2002), a brain region essential for the planning, execution, and evaluation of goal-directed behaviors (Schultz, 2002; Tanji and Hoshi, 2001).

It is interesting to speculate on how human dreams will be in the future, as selection pressures continue to change. Although most people report no awareness of being dreaming while dreams takes place, it is possible for one to be aware of dreaming without waking up, a state called lucid dreaming (LaBerge and Dement, 1982; LaBerge *et al.*, 1981, 1986; Tart, 1965, 1972). Lucid dreams occur during REM episodes of greatly increased metabolism, characterized by increased eye movement density, heart rate, and respiration rate (Brylowski *et al.*, 1989). During lucid dreams it is possible for the dreamer to assume partial or total control of the unfolding dream narrative (LaBerge and Dement, 1982; LaBerge *et al.*, 1981), which indicates that the self-representation is more dominant in lucid dreams than in regular ones. This suggests that activity in the prefrontal cortex is enhanced during lucid dreams by mechanisms yet unknown but probably involving hyperdopaminergia and/or hypercholinergia. Despite the abundance of subjective reports on the use of lucid dreams to improve performance on a variety of real life skills (Brooks and Vogelsong, 2000; Green and McCreery, 1995; LaBerge, 1991; Wangyal *et al.*, 1998), the cognitive potential of such dreams also remains to be investigated by science. The use of lucid dreams for conscious simulation-based learning, if confirmed and made accessible to the general public, may represent a breakthrough for the future evolution of human consciousness.

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3.33 Evolution of the Hippocampus

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Glossary

amnesia	Memory impairment characterized by profound forgetfulness.
consolidation	Process by which memory becomes independent of hippocampal region over time.
declarative memory	Memory for facts and events.
episodic memory	Memory for events.
familiarity	Component of recognition memory characterized by a memory in the absence of recollected details.
hippocampal region	Cornu ammonis fields of the hippocampus proper, dentate gyrus, and subiculum.
macrosomatic	Describes an animal with a well-developed olfactory system
medial temporal lobe	Portion of brain, present in larger-brained mammals such as primates, that contains the hippocampal and parahippocampal regions.
microsomatic	Describes an animal with a poorly developed olfactory system
parahippocampal region	Entorhinal, perirhinal, and postrhinal (parahippocampal in primates) cortices.
place cell	Neuron whose firing rate correlates strongly with animal's location in an environment.
recognition memory	Capacity to judge an item as having been previously encountered.
recollection	Component of recognition memory characterized by retrieval of specific details relating to the incident in which the item to be remembered was encountered.
retrograde amnesia	Loss of information acquired prior to onset of brain damage.
semantic memory	Memory for facts.

3.33.1 Introduction

The hippocampus is a brain area that has received considerable attention because of its distinctive anatomy and its important role in memory. A particularly productive approach to studying the hippocampus has been to examine either its form or function across mammalian species (Brown and Aggleton, 2001; Burwell *et al*., 1995; Cohen and Eichenbaum, 1993; Insausti, 1993; Squire, 1992) or to consider whether homologous structures exist in other vertebrates (Aboitiz *et al*., 2002; Bingman *et al*., 2003; Day, 2003; Jacobs, 2003; Salas *et al*., 2003; Sherry and Schacter, 1987). The present article builds on these previous efforts and considers the evolution of the mammalian hippocampus from both anatomical and functional viewpoints. Based on this dual approach, we make two main points.

First, the anatomy of the hippocampal region is largely conserved across mammals. The connectivity and cytoarchitectural features of the hippocampus, dentate gyrus, and subiculum are

remarkably similar for all mammalian species for which information is available (see Sex and Species Differences in Hippocampal Volume). Further, the surrounding cortices in the parahippocampal region also show a substantial degree of conservation across the mammalian taxon. The anatomical details of the hippocampal and parahippocampal regions are not identical from species to species, but these differences are overshadowed by the substantial divergence in the organization of the neocortex. Moreover, structures homologous to the mammalian hippocampus appear in birds and reptiles, yet these structures do not directly parallel the distinct subdivisions of the hippocampal region. Accordingly, the first half of the article considers the anatomical homology of the mammalian hippocampus and details its features along with those of the adjacent parahippocampal region. This half ends by looking to birds and reptiles for evidence of how the hippocampus may have appeared in the earliest mammals.

Second, the hippocampus serves the same fundamental mnemonic function across mammals, and homologous structures in other vertebrates may support a similar capacity. Today, we know that the distinctive anatomy and physiology of the hippocampus anchors only one of several memory systems of the mammalian brain. Together with the parahippocampal region, the human hippocampal region enables a record of our experiences that can be subsequently brought back to mind as facts and events (Manns and Squire, 2002; Poldrack and Gabrieli, 1997; Schacter *et al*., 1998). This notion of memory as conscious recollection is difficult to extend to experimental animals, yet other operating characteristics of hippocampus-dependent memory apply equally well across mammals, including rapid learning, complex associative organization, and flexibility in retrieval (Eichenbaum and Cohen, 2001). The second half of the article tracks the progression of thought regarding the function of the mammalian hippocampus, starting with a point around 50 years ago at which the function of the hippocampus was uncertain and was presumed to be different for humans and experimental animals.

Current research focuses on identifying the fundamental principles that define hippocampus-dependent memory across species. We argue that this effort will be best served by taking an evolutionary approach. The divergence of mammals has provided a natural experiment in which a largely conserved hippocampal system can be explored among neocortical conditions that differ across species. The goal of this approach is to highlight the conserved function of the hippocampus and downplay most species-specific distinctions as a byproduct of differing neocortical inputs.

It is worth pointing out that the reader will find articles Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications, Sex and Species Differences in Hippocampal Volume, Evolution of the Nervous System in Reptiles, The Evolution of Vocal Learning Systems in Birds and Humans, The Hippocampal Formation in Food-Storing Birds, and Evolution of Vertebrate Olfactory Subsystems in the present work as very relevant and informative.

3.33.2 Anatomical Homology

3.33.2.1 Anatomy of the Mammalian Hippocampal and Parahippocampal Regions

3.33.2.1.1 Terminology The hippocampus is highly interconnected with several neighboring and closely interconnected structures, and its anatomy is best understood in combination with these structures (see Amaral and Witter (1995) for a more extensive anatomical review). Indeed, the term hippocampus is often used to refer to not only the cornu ammonis (CA; or hippocampus proper) but also to the dentate gyrus. The hippocampus proper is contiguous with the subiculum, and here these regions together with the dentate gyrus are called the hippocampal region. The adjacent parahippocampal region includes the entorhinal, perirhinal, and postrhinal (parahippocampal in primates) cortices and provides the majority of cortical input to the hippocampal region and is the recipient of its major cortical outputs (Burwell *et al*., 1995).

3.33.2.1.2 Gross morphology The hippocampus proper and dentate gyrus are both curled sheets of cortex that are rolled together to form a tube-like structure. In many small-brained mammals, the long axis of the tube begins at a medial, dorsal locus just posterior to the septum, and curves to a ventral and caudal apex before bending and heading rostrally and slightly laterally within the temporal region (see Figure 1a for the position of the hippocampal region in the rat brain). In mammals with larger brains, the general shape of the elongated tube has been conserved, but the structure has slid down along the same septotemporal axis described above so that in primates, the hippocampus is contained within the medial aspect of the temporal lobe (Figure 1). The parahippocampal region surrounds the hippocampal region and therefore takes on a different position

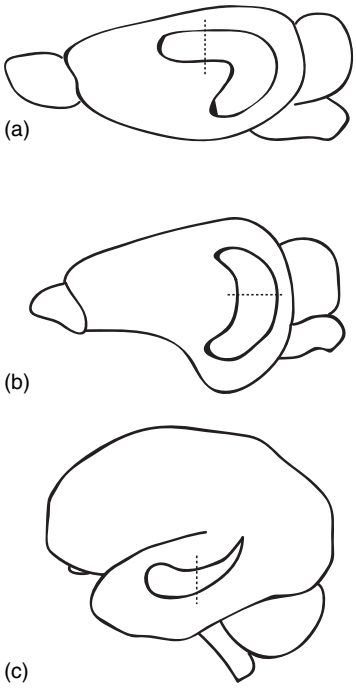

Figure 1 Position of the hippocampus in the brain of a rat (a), tree shrew (b), and human (c). The difference in position between small, medium, and big-brained mammals suggests that the hippocampus has slid through evolution along its long axis to the point that, in humans, the hippocampus is contained entirely within medial portion of the temporal lobe. The dashed lines in each drawing indicate cross sections perpendicular to the long axis of the hippocampus, similar to those depicted in Figure 2.

in the brain according to the position of the hippocampal region (Burwell, 2000).

3.33.2.1.3 Cytoarchitecture In all mammals, a cross section of the hippocampal region taken perpendicular to the long axis reveals the densely packed cell layers of the hippocampus proper and dentate gyrus, which at most septotemporal levels appear to fit together like interlocking arcs. Figure 2 shows examples from four species. The hippocampus proper consists of pyramidal cells bounded above and below by cell-sparse layers and can be divided into three subfields: CA1, CA2, and CA3. The pyramidal cells in CA1 were originally distinguished from those in CA3 on the basis of size; CA1 pyramidal cells are slightly smaller (Ramon y Cajal, 1911). The CA2 subregion is a small transitional area between CA1 and CA3. The dentate gyrus is also a curved sheet of three-layered cortex, although its principal cells are granule cells. Immediately adjacent to CA1 is the subiculum, another three-layered section of cortex whose principal cells are pyramidal neurons. Although the laminar organization of each subregion represents a 'simple' cortical organization with only one layer of principle cells, the principle cells in each subregion are wrapped in a plexus woven from interneurons.

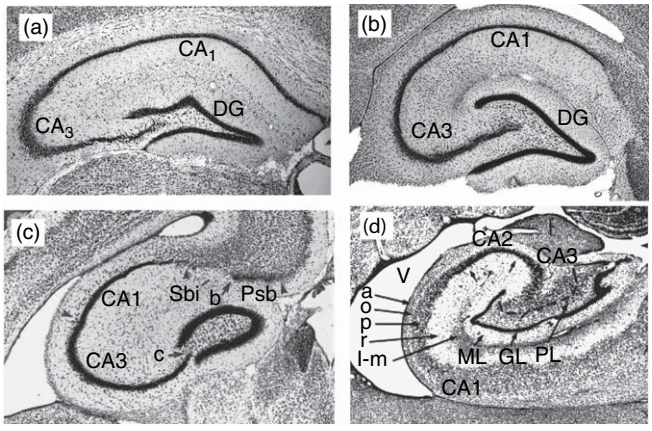

Figure 2 Cross sections taken perpendicular to the long axis of the hippocampal region in a mouse (a), tree shrew (b), tenrec (c), and human (d). The cell fields (CA1, CA3) of the hippocampus proper and the dentate gyrus (DG) appear similar in all four mammals. Note that CA1 appears below CA3 in the human hippocampus due to the evolutionary slide illustrated in Figure 1. See original sources for additional information. a, Reproduced from Van Groen, T., Kadish, I., and Wyss, J. M. 2002. Species differences in the projections from the entorhinal cortex to the hippocampus. *Brain Res. Bull.* 57(3–4), 553–556, with permission from Elsevier. b, Keuker, J. I., Rochford, C. D., Witter, M. P., and Fuchs, E. 2003. A cytoarchitectonic study of the hippocampal formation of the tree shrew (*Tupaia belangeri*). *J. Chem. Neuroanat.* 26(1), 1–15, with permission from Elsevier. c, Kunzle, H. and Radtke-Schuller, S. 2001. Hippocampal fields in the hedgehog tenrec. Their architecture and major intrinsic connections. *Neurosci. Res.* 41(3), 267–291, with permission from Elsevier. d, Reprinted from Amaral, D. G. 1999. Introduction: What is where in the medial temporal lobe? *Hippocampus* 9(1), 1–6.

The adjacent entorhinal, perirhinal, and postrhinal/parahippocampal cortices have a more complicated laminar organization than the three-layered areas in the hippocampal region (for detailed descriptions, see Burwell, 2000; Suzuki and Amaral, 2003). Although the laminar profile of these regions does not directly correspond to the six-layered neocortex, each area of the parahippocampal region is typically partitioned into six layers. The cellular composition of these layers differs between the three regions and, in combination with differing patterns of connectivity, defines their borders. However, the cytoarchitectural details differ even within a region, and no single criterion can be used to define all the borders of any region. Further, the entorhinal, perirhinal, and postrhinal/parahippocampal cortices are typically divided into several subregions based on local differences in cytoarchitecture and connectivity. A full account of the cytoarchitecture in these regions is beyond the scope of the present article.

3.33.2.1.4 Intrinsic circuitry of the hippocampal region The major intrahippocampal connections are serial and unidirectional (Amaral and Witter, 1995). This intrinsic circuit traces a path from the dentate gyrus to CA3 before continuing to CA1 and finally to the subiculum. In addition, the pyramidal cells in CA3 send a substantial number of axons to other pyramidal cells in CA3. The connections between subregions are organized in different patterns, suggesting that information is being transformed in distinct ways at each step in the serial circuit. This organization is depicted in Figure 3 and is described next.

The projection from the dentate gyrus to CA3 involves a lamellar organization such that, at successive septotemporal levels, dentate cells project to the entire transverse extent of CA3 at about the same level (Gaarskjaer, 1986; Swanson *et al.*, 1978). In contrast to this transverse dispersion of connectivity, the projection from CA3 to CA1 involves an organization in which CA3 cells from a given septotemporal level project to about two-thirds of the septotemporal extent of CA1 (Ishizuka *et al.*, 1990; Li *et al.*, 1994). Moreover, the CA3 projections do extend in the transverse plane, although a gradient applies such that CA3 pyramidal cells close to the CA1 border project to the adjacent CA1 cells and CA3 pyramidal cells closest to the dentate gyrus project to CA1 cells closest to the subiculum. The projection from CA1 to subiculum is perhaps the most structured of the intrahippocampal projections and is organized in at least two dimensions: along the transverse axis and along the septotemporal axis (Amaral *et al.*, 1991). Three nearly discrete transverse columns project to the subiculum such that the third of CA1 cells closest to the subiculum project to the third of the subiculum that is immediately adjacent to CA1. The middle third of CA1 projects to the middle third of the subiculum, and the third of CA1 closest to CA2 (farthest from the subiculum) projects to the third of the subiculum farthest from CA1. Like the CA3 to CA1 projection, the CA1 to subiculum projection is also distributed along the septotemporal axis such that CA1 cells at any septotemporal level project to about one third of the septotemporal extent of the subiculum.

The anatomy of the hippocampal region offers insight into the potential role of its subregions. The

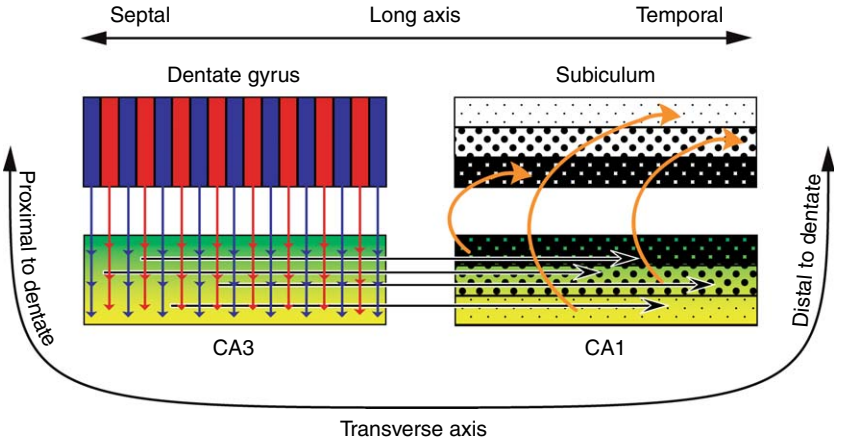

Figure 3 Connections between subregions of mammalian hippocampal region. The dentate gyrus projects to CA3 in a lamellar fashion (red and blue slices), whereas CA3 projections to CA1 are dispersed along the long axis. A gradient also applies to this projection (green to yellow) such that CA3 cells proximal to the dentate gyrus tend to project to CA1 cells distal to the dentate gyrus. The projections from CA1 to subiculum are partitioned into three transverse columns (indicated by dot patterns). Further, projections from CA1 reach about a third of the septotemporal extent of the subiculum. See text for more details.

orthogonal dispersion gradients of dentate gyrus to CA3 projections (transverse) and CA3 to CA1 projections (septotemporal) suggest that these connections might be involved in the final stage of reformatting sensory information in the service of enabling arbitrary associations within and across modalities. The recurrent CA3 to CA3 connections might also participate in this process. In comparison, the more organized CA1 to subiculum projection could represent the first step in repackaging new associations in a format compatible with the topography of the neocortex. In any case, it is clear that the serial circuit through the hippocampal region is not simply a passive relay of information.

3.33.2.1.5 Connections with the entorhinal cortex In addition to the prominent serial organization of intrahippocampal connectivity, there are parallel direct connections between each of the hippocampal subregions and the entorhinal cortex in all mammals (Figure 4). Thus, although some information traveling through the hippocampal region might proceed serially, there are also 'shortcuts' into and out of each subregion. The projections from the entorhinal cortex are collectively called the perforant path, but the trajectory of this path differs for CA1 and subiculum on one hand and dentate gyrus and CA3 on the other (Witter *et al.*, 2000b). The projections to CA3 and dentate gyrus originate mostly in layer II of the entorhinal cortex, whereas the projections to CA1 and subiculum originate mostly in layer III of the entorhinal cortex. The layer III projection is mirrored by direct return projections from the subiculum and CA1 to the entorhinal cortex.

The projections from entorhinal cortex to the hippocampal subregions can be distinguished further on the basis of the areas within entorhinal cortex from which the connections originate (Witter, 1993). Specifically, there are two parallel pathways, one arising from the lateral entorhinal area (LEA) and the other arising from the medial entorhinal area (MEA). The line bisecting LEA and MEA is not actually perpendicular to the lateral/

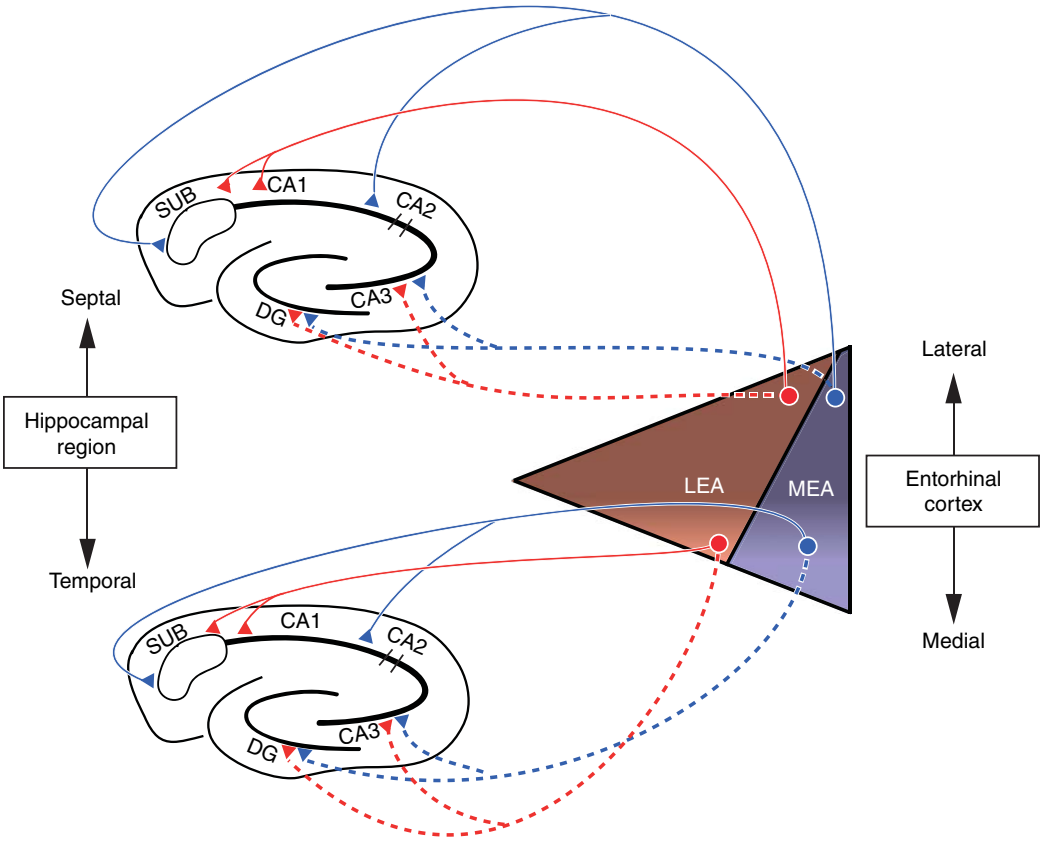

Figure 4 Organization of the mammalian perforant path. Three trends are apparent in the entorhinal projections to the hippocampal region. First, the lateral–medial axis of entorhinal cells corresponds to a septotemporal termination gradient in the hippocampal region. Second, separate branches of the perforant path originate in LEA and MEA. These projections are combined in the dentate gyrus and CA3 but are kept separate in the subiculum and CA1. Third, projections to the dentate gyrus and CA3 originate primarily in layer II of the entorhinal cortex (dashed lines), but projections to the subiculum and CA1 originate primarily in layer III (solid lines).

medial cardinal axis in any species, such that LEA and MEA are defined by the origins of the two pathways rather than by their cardinal positions. Both pathways contain projections from layers II and III and project to each of the four hippocampal subregions. However, the pattern in which the fibers from both pathways terminate in hippocampal targets differs between CA3 and dentate gyrus on one hand and CA1 and subiculum on the other (see Figure 4). The projections from LEA and MEA target the same subsets of dentate and CA3 cells. In contrast, the projections from LEA target portions of CA1 and subiculum that differ from the portions targeted by the projections arising in MEA. In particular, projections arising in LEA target CA1 and subiculum cells near the subiculum/CA1 border, whereas projections arising in MEA target CA1 and subiculum cells farthest from the subiculum/CA1 border. Thus, information passing through LEA and MEA appears to be combined in the dentate gyrus and CA3 but kept separate in the subiculum and CA1. Of course, both CA1 and the subiculum also receive intermixed LEA and MEA information via the serial input from dentate gyrus and CA3.

One additional feature characterizes the overall topography of the entorhinal inputs into the hippocampal region. Projections originating in the lateral aspect of the entorhinal cortex (including the lateral aspects of both the LEA and MEA) terminate largely in the septal end of the hippocampal subregions. Conversely, projections originating in the medial aspect of the entorhinal cortex (including the medial aspects of both the LEA and MEA) terminate largely in the temporal end of the hippocampal subregions.

3.33.2.1.6 Intrinsic circuitry of the parahippocampal region The LEA and MEA can be further distinguished on the basis of their inputs originating in the rest of the parahippocampal region. LEA receives more cortical projections from the perirhinal cortex, whereas MEA receives more cortical projections from the parahippocampal/postrhinal cortex (Witter *et al*., 2000a). This difference is distinguished even further by the fact that perirhinal cortex appears to receive a different subset of olfactory and neocortical inputs as compared to parahippocampal/postrhinal cortex (Burwell and Amaral, 1998a; Suzuki and Amaral, 1994). The postrhinal/parahippocampal cortex receives more inputs from cortical areas important for allocentric spatial information. The perirhinal cortex receives more inputs from olfactory areas and neocortical areas important for nonspatial information. Based on these observations, one view of the connectivity of the parahippocampal region is that information reaching the perirhinal cortex follows a path through the LEA to the hippocampal region that runs parallel to the path taken by information reaching the postrhinal cortex and continuing through the MEA (Witter *et al*., 2000a). These pathways appear to be largely combined in dentate gyrus and CA3 but kept at least somewhat separate in CA1 and the subiculum. However, the notion of parallel, functionally distinct input streams is tempered by the presence of a substantial projection from parahippocampal/postrhinal cortex to perirhinal cortex (and a smaller return projection) in addition to connections between LEA and MEA.

3.33.2.2 Summary of Anatomy of the Hippocampal and Parahippocampal Regions

The animals of the mammalian taxon represent a great diversity of habitats, means of locomotion, preferred diets, and social structure. They also represent a great diversity of neuroanatomy. For example, tenrecs and hedgehogs are both small-brained insectivores whose neocortex is predominately composed of primary sensory areas (Catania *et al*., 2000; Krubitzer *et al*., 1997). In contrast, the brains of primates contain numerous neocortical areas that are devoted to integrating information across modalities. Further, the amount of tissue devoted to a particular sensory modality also varies substantially between species (Krubitzer and Kaas, 2005). In macrosomatic animals such as rodents, large portions of the brain are involved in processing odors. In other animals, vision (e.g., primates), audition (e.g., bats), or somatosensation (e.g., star-nosed moles) have become disproportionately represented in the brain. Further, the anatomy of the hippocampal and parahippocampal regions includes many complex and highly organized patterns of interconnectivity. This complexity would seem to provide many opportunities for divergence throughout evolution, especially when considering that many other features differ substantially between species. Thus, it is surprising that the predominant trend with respect to the anatomy of the hippocampal and parahippocampal regions is one of conservation rather than divergence. Figure 5 shows an evolutionary tree of some of the animals discussed in this article and is meant illustrate how the diversity of these selected mammals contrast with the conservation of the anatomy of the hippocampal and parahippocampal regions. Nevertheless, there are differences between species with respect to the anatomy of the hippocampal region and to a somewhat greater extent the

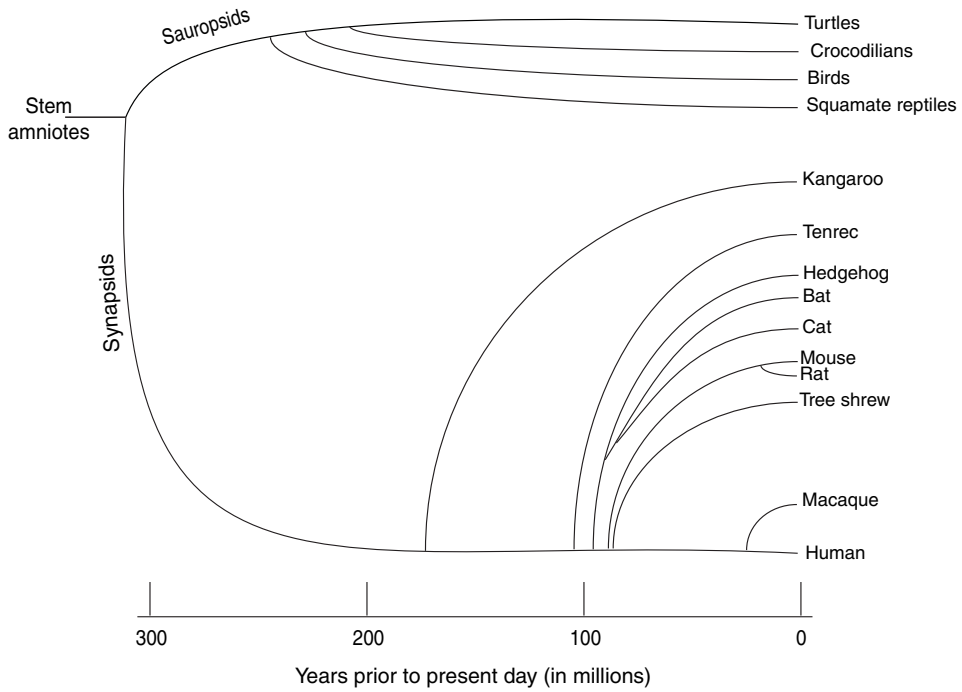

Figure 5 Phylogeny of birds, reptiles, and selected mammals. The dates are estimates based on recent molecular techniques (Arnason *et al.*, 2002; Springer *et al.*, 2003).

parahippocampal region. These differences are considered next.

3.33.2.3 Anatomical Differences between Species

3.33.2.3.1 Hippocampal region Species differences in the hippocampal region are best characterized as refinement rather than reorganization. The intermediate subregion CA2 can be clearly distinguished in primates (Bakst and Amaral, 1984; Green and Mesulam, 1988), but its identification is more difficult in smaller-brained mammals such as hedgehogs and tenrecs (Kunzle and Radtke-Schuller, 2001; West *et al.*, 1984). Also, the border that demarcates the transition from CA1 to subiculum is less clearly defined in small-brained mammals (Kunzle and Radtke-Schuller, 2001; West *et al.*, 1984). Furthermore, in hedgehogs, but not in tenrecs or in larger-brained mammals, the mossy fiber projection from dentate gyrus to CA3 invades CA1 to some degree (Kunzle and Radtke-Schuller, 2001; West *et al.*, 1984). Taken together, the overall similarities in cytoarchitectural plan and connectivity described above far outweigh these minor differences, especially when considering the complexity of the region's circuitry. Nevertheless, the trend for the hippocampal region appears to be one of increasing distinction between subregions as a refinement that often comes with evolution.

3.33.2.3.2 Entorhinal cortex The entorhinal cortex also follows an evolutionary trend of increasing diversification. In most mammals studied, including the hedgehog, LEA is clearly distinguished from MEA (Insausti, 1993; West *et al.*, 1984). One possible exception is the tenrec, in which the entorhinal cortex was poorly differentiated from the piriform cortex and LEA and MEA were not distinguished from one another (Kunzle and Radtke-Schuller, 2001). Nevertheless, in other mammals, the tendency is for entorhinal cortex to increase in diversity with brain size, such that six, seven, and eight subdivisions have been identified in the rat, monkey (macaque), and human, respectively (Insausti, 1993).

Despite the increasing complexity of the entorhinal cortex and refinement of the hippocampal region, the highly structured organization of connectivity between the areas is quite similar across mammals. In particular, the specific topography of entorhinal inputs to the hippocampal formation (Figure 4) is similar in all species for which detailed anatomical information is available, including bats (Buhl and Dann, 1991), mice (Van Groen *et al.*, 2002), rats (Witter, 1993), cats (Witter and Groenewegen, 1984), and monkeys (Witter and Amaral, 1991). However, there are several exceptions that are worth mentioning. In mice, the entorhinal projection to CA3 originates predominately in layer III rather than in layer II as

described in larger-brained mammals, including rats (Van Groen *et al*., 2002). Further, several species differences have been noted in the laterality of projections between regions. For example, projections from the subiculum to entorhinal cortex are solely ipsilateral in rats but are bilateral in cats and monkeys (Amaral and Witter, 1995). It is not always the case that bigger-brained mammals show more anatomical refinement than smaller-brained mammals. For example, LEA and MEA projections to dentate gyrus terminate at the same portions of the granule cell dendrites in monkeys (Witter and Amaral, 1991). In contrast, in the other mammals studied (bats, mice, cats, and rodents), projections from the LEA terminate in the superficial third of the granule cell dendrites, whereas projections from MEA terminate in the middle third of the granule cell dendrites (Buhl and Dann, 1991; Van Groen *et al*., 2002; Witter, 1993; Witter and Groenewegen, 1984). Thus, whereas there is a trend of increasing diversification and refinement over the course of evolution, there does not appear to be a clear shift in the organization of the connections between the hippocampal region and entorhinal cortex. Indeed, in comparison with the dramatic differences in neocortex between mammals (Krubitzer and Kaas, 2005), the hippocampal region and entorhinal cortex are surprisingly similar.

One potentially important distinction between macrosomatic and microsomatic mammals involves the prominence of olfactory input to the entorhinal cortex (Insausti *et al*, 2002). In particular, a direct projection exists in rats from the olfactory bulb to almost the entire extent of the entorhinal cortex (Price, 1973). In contrast, in the macaque, the olfactory bulb projects to only one of the seven subdivisions of the monkey entorhinal cortex, which was estimated to comprise 15% of the region's total area (Witter *et al*., 1989). The corresponding entorhinal subdivision in humans comprises less than 5% of the total human entorhinal cortex (Insausti *et al*., 1995). To the extent that the projection from the olfactory bulb to entorhinal cortex represents the prominence of olfactory processing in the hippocampal region, there is a clear trend toward the reduction of olfactory input to the hippocampus in microsomatic mammals.

3.33.2.3.3 Perirhinal and postrhinal/parahippocampal cortices The entorhinal cortex appears to have undergone more changes over the course of evolution than the hippocampal region, and the perirhinal and postrhinal/parahippocampal cortices may have undergone even more changes than the entorhinal cortex (Burwell, 2000). However, this observation is limited by the fact that detailed anatomy of the perirhinal and postrhinal/parahippocampal cortices regarding the neocortical afferents, interconnectivity, and projections to the entorhinal cortex and hippocampal region are available for only the rat (Burwell and Amaral, 1998a, 1998b) and the macaque (Lavenex *et al*., 2002, 2004; Suzuki and Amaral, 1994, 2003). Although these animals represent only one branch on the mammalian evolutionary tree (see Figure 5), they differ substantially in that the rat is a nocturnal, macrosomatic, and relatively small-brained mammal, whereas the macaque is a diurnal, microsomatic, and big-brained mammal. Thus, the commonalities in anatomy between the two mammals can highlight fundamental organizational principles of the perirhinal and postrhinal/parahippocampal cortices, and the differences can illustrate one path taken by the parahippocampal region in the evolution of big-brained mammals.

In both the rat and the macaque, the perirhinal and postrhinal/parahippocampal cortices represent major routes of entry into the entorhinal cortex and hippocampal region (Burwell and Amaral, 1998b; Suzuki and Amaral, 1994). Many unimodal and polymodal cortical regions project to the perirhinal cortex or to the postrhinal/parahippocampal cortex. The incoming information is presumably processed and passed on to the entorhinal cortex, where it is likely further processed before being relayed to the hippocampal region. In both the rat and the macaque, the perirhinal cortex tends to project more to the LEA of the entorhinal cortex, and the postrhinal/parahippocampal cortex tends to project more to the MEA (Witter *et al*., 2000a). Also, in both mammals, a strong projection from the postrhinal/parahippocampal cortex to the perirhinal cortex is met with a more modest return projection (Burwell and Amaral, 1998a; Lavenex *et al*., 2004). Thus, the hippocampal and parahippocampal regions can be described as a hierarchy of connectivity in which the perirhinal and postrhinal/parahippocampal cortices are positioned near the top and funnel information into the LEA and MEA, information which is then combined in the hippocampal region (Lavenex and Amaral, 2000; Witter *et al*., 2000a).

However, compared to the macaque, the patterns of connectivity in the rat less clearly conform to the idea of a hierarchy. In the macaque, more than two-thirds of the cortical input to the entorhinal cortex originates in the either the perirhinal or postrhinal/parahippocampal cortices (Suzuki and Amaral, 1994). In comparison, less than one-fourth of cortical afferents of the entorhinal cortex in rats

originate in these regions (Burwell and Amaral, 1998b). Some, but not all, of this difference can be accounted for by considering the prominent olfactory input in the rat that bypasses the perirhinal cortex and projects directly to the entorhinal cortex. Thus, the more rigidly serialized hierarchy in the monkey suggests that the primate entorhinal cortex (and therefore the hippocampal region) receives information that is on average even more highly processed than it is in the rodent. Further, the macaque entorhinal cortex reciprocates its strong perirhinal and postrhinal/parahippocampal input with equivalently strong return projections. In the rat, the projections into the entorhinal cortex are stronger than the return projections (Burwell and Amaral, 1998a).

3.33.2.3.4 Neocortical input to the parahippocampal region The most notable difference between the parahippocampal regions in rats and monkeys relates to the makeup of cortical information projecting into the perirhinal and postrhinal/parahippocampal cortices. Indeed, there are numerous dissimilarities that relate generally to the differences between a small-brained and a big-brained mammal (Krubitzer and Kaas, 2005). Compared to the rat neocortex, the macaque neocortex is substantially more invaginated and shows a more defined laminar organization. The macaque also has disproportionately enlarged frontal lobes and has more unimodal and polymodal association cortical areas. Further, the macaque displays a much more elaborate visual system. However, olfactory and somatosensory cortex is disproportionately larger in rats and thus may provide more detailed odor and tactile information to the parahippocampal region. Thus, even if the parahippocampal region was identical between rats and monkeys, differences between the species in terms of the kinds of information processed by the perirhinal and postrhinal/parahippocampal cortices would be virtually assured by the substantial neocortical differences. Furthermore, because the inputs to the parahippocampal region largely determine the input to the hippocampal region, one might also expect to observe differences in the content of information that is available to the rat and the monkey hippocampus. Thus, although the rat and macaque parahippocampal regions may share a separation of spatial and nonspatial inputs between postrhinal/parahippocampal cortex and perirhinal cortex, respectively, the details of the spatial and nonspatial information reaching these structures likely differs markedly between the species.

3.33.2.4 Summary of Anatomical Homology

The organization of the neocortex differs substantially between mammals, and because neocortical organization determines the organization of inputs to the parahippocampal region, the information fed into the parahippocampal and hippocampal regions might differ considerably across the taxon. Nevertheless, the internal anatomical details of the hippocampal and parahippocampal regions are quite similar across mammals. Indeed, the appearance of the hippocampus is so similar – even in mammals such as the tenrec whose brain is thought to resemble those of the earliest mammals – that one might expect to find brain structures resembling the hippocampus in animals who shared a common ancestor with mammals. Accordingly, we next envision the earliest mammals and consider whether birds and reptiles have a brain structure that is homologous to the mammalian hippocampus.

3.33.3 Ancestral Homologue of the Hippocampal Region

The earliest mammals appeared during the Triassic period, more than 200 Mya. They were likely small, nocturnal animals who relied on their well-developed olfactory abilities to capture their insect meals (Allman, 1999). The ongoing diversification of mammals increased around 65 Mya, coincident with the Cretaceous/Cenozoic boundary that marks the extinction of dinosaurs (Springer *et al.*, 2003). The increased diversification was likely due at least in part to the decrease in competition from dinosaurs, which opened many diurnal habitats, but was probably also due to the increasing separation of land masses (Hedges *et al.*, 1996). In any case, early mammals shared a recent ancestor with sauropsids, a group of animals that today includes birds and reptiles (Kumar and Hedges, 1998; see Figure 5). The brains of modern mammals appear quite different from those of birds and reptiles, but there are also many similarities between the taxa. These commonalities can suggest how the brains of the early mammals might have appeared. Indeed, both reptiles and birds have brain regions that are thought to be homologous to the mammalian hippocampus (Colombo and Broadbent, 2000; Aboitiz *et al.*, 2002).

3.33.3.1 A Hippocampal Homologue in Birds and Reptiles

3.33.3.1.1 Anatomical similarities Regions within the medial cortex in reptiles (possibly including

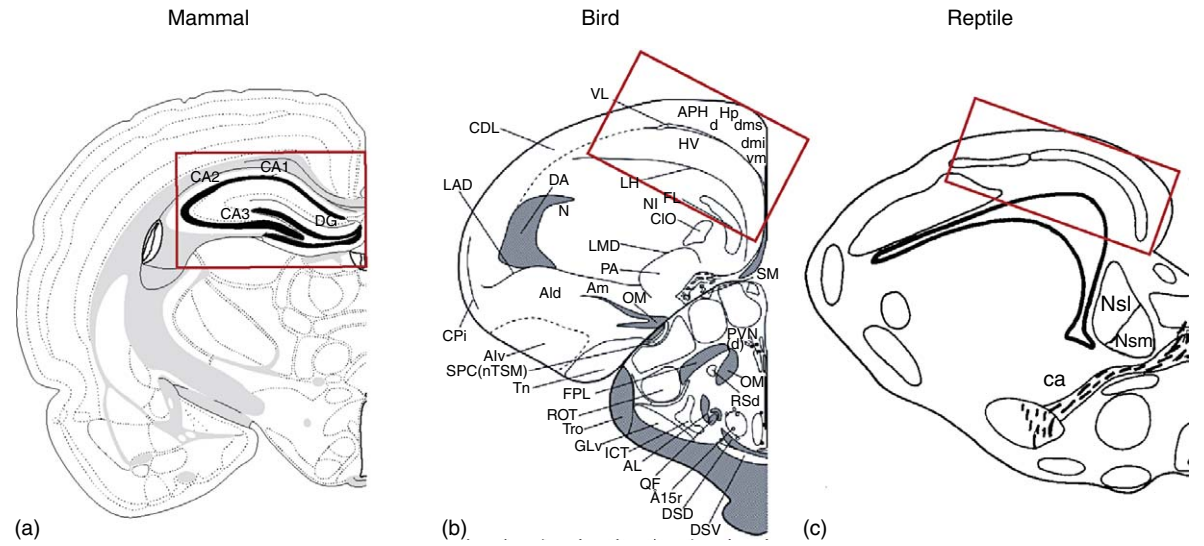

Figure 6 Brain regions thought to be homologous to the mammalian hippocampus in birds and reptiles (indicated by rectangles). One hemisphere from a rat, chicken, and lizard (gecko) is shown. See original sources for additional information. a, Reproduced from Swanson, L. W. 1998. Brain Maps: Structure of the Rat Brain, 2nd edn., with permission from Elsevier. b, Courtesy of Wayne Kuenzle. c, Reproduced from Hoogland, P. V., Martinez-Garcia, F., Geneser, F. A., and Vermeulen-VanderZee, E. 1998. Convergence of thalamic and cholinergic projections in the 'dentate area' of lizards. *Brain Behav. Evol.* 51(2), 113–122, with permission from S. Karger AG, Basel.

dorsomedial cortex) and medial pallium in birds (area hippocampus and area parahippocampalis) share several anatomical features with the mammalian hippocampus (see The Hippocampal Formation in Food-Storing Birds). For example, in all three cases the hippocampal homologue is a three-layered section of cortex that develops from the pallial telencephalon and is situated medially in the brain, adjacent to a main ventricle (Figure 6). In all three groups, the hippocampal homologue receives prominent projections from visual and olfactory cortices (Atoji *et al.*, 2002; Hoogland and Vermeulen-Vanderzee, 1993; Lavenex and Amaral, 2000). Also, the hippocampal homologue in all three groups shows physiological evidence of synaptic plasticity, suggesting an ability to associate incoming information (Bliss and Lomo, 1973; Muñoz *et al.*, 1998; Shapiro and Wieraszko, 1996). Thus, as in mammals, the hippocampal homologue in birds and reptiles may serve as a site of integration from already processed information.

3.33.3.1.2 Anatomical differences At the same time, there are notable dissimilarities between the mammalian hippocampus and the homologous regions in reptiles and birds. Superficially, neither the reptilian medial cortex nor the avian hippocampus have distinguishable subfields equivalent to those of the hippocampus proper, the dentate gyrus, and subiculum. Nor does the architecture of the reptilian or avian hippocampus resemble the characteristic shape of interlocking arcs formed by the densely packed cell layers of the hippocampus and dentate gyrus in mammals (Figure 6). Furthermore, dissimilarities in the distribution of sensory pathways between mammals and sauropsids suggest substantial differences in the kind of information processed by hippocampal homologues. In mammals, the hippocampus enjoys a confluence of highly processed unimodal and polymodal information (Lavenex and Amaral, 2000). Through its connections with the adjacent entorhinal, perirhinal, and parahippocampal/postrhinal cortices, the hippocampus receives input from widespread cortical areas. These connections suggest that the hippocampus serves as a central associative node in the cortical network that supports integrative processing in the mammalian brain. Likewise, in birds and reptiles, the hippocampal homologue also enjoys prominent connections with cortical/pallial regions. However, in comparison with the mammalian forebrain, the sauropsid cortex appears to play a disproportionately smaller role in cognitive functioning. Instead, higher-order functions that are mediated by the neocortex in mammals are supported by the dorsal ventricular region in reptiles and by the archistriatum/arcopallium in birds (Aboitiz *et al.*, 2002). Moreover, the hippocampal homologue in birds and reptiles enjoys few direct or indirect connections with these regions (Atoji *et al.*, 2002; Dubbeldam, 1998; Hoogland and Vermeulen-Vanderzee, 1993; Ten Donkelaar, 1998). Thus, the hippocampal homologue in sauropsids receives a

more limited range of connections and may support a more specific subset of associative abilities as compared to the mammalian hippocampus.

3.33.3.1.3 Functional similarities A growing number of studies suggest that the reptilian medial cortex and the avian hippocampus share some functional similarities with the mammalian hippocampus (Bingman, 1992; Day, 2003; Jacobs, 2003; Salas *et al*., 2003). Among the most commonly studied functions of the hippocampus in mammals is spatial memory (reviewed more comprehensively in the second part of this article). Ablation studies have indicated that the hippocampal homologue in reptiles and birds is important for spatial learning functions that are known to rely on hippocampal function in mammals. These experiments show that damage to the putative hippocampal homologue disrupts performance when the animal must learn relationships between distant environmental cues to identify important places in the external world but not when learning can be supported by approaching a specific landmark at the site of a reward (Bingman *et al*., 2003; Salas *et al*., 2003). For example, turtles with damage to the medial cortex were impaired at learning the location of an unmarked goal in an open field water maze surrounded by visual cues but were unimpaired when the target was marked by a consistent visual stimulus (Lopez *et al*., 2003). Similarly, homing pigeons with damage to the hippocampal homologue were able to orient themselves using the sun and familiar local landmarks at the release point but appeared deficient at navigating by using cues they saw along the route home (Gagliardo *et al*., 1999). Consistent with these findings on hippocampal damage, studies on the firing properties of hippocampal neurons also suggest a possible similarity in spatial representations by the hippocampus. Many studies in rodents and other mammals have identified hippocampal neurons that fire selectively when the animal is in a particular place within its environment (O'Keefe and Nadel, 1978). Recently, cells with the same property have also been identified in the avian hippocampus (Siegel *et al*., 2005). Thus, one commonality between mammals, reptiles, and birds is that the hippocampal homologue appears to be important for associating spatial relationships between environmental cues and learning places where important events occur.

3.33.3.1.4 Functional differences? The comparative anatomy of the hippocampus is consistent with the notion of a more limited scope of information processing in sauropsids than in mammals, and the results of several ablation studies are consistent with the idea as well. Mammals with damage to the hippocampal region have been found to be impaired on a variety of nonspatial as well as spatial memory tasks (Eichenbaum *et al*., 1999; also discussed in more detail below). For example, in one study (Bunsey and Eichenbaum, 1996), rats were trained on a transitive inference task in which they learned a series of overlapping paired odor associations. When presented with odor A, rats were rewarded for selecting odor B, and when presented with odor B, rats were rewarded for selecting odor C. Having learned the A–B and B–C pairs, normal rats also responded to C when presented with A, demonstrating they had linked the two paired associates and could infer the relationship between the indirectly related items A and C. Rats with damage to the hippocampus could gradually acquire the paired associates but did not show the transitive inference for A–C. In contrast to the findings on rats, a similar study found that birds with hippocampal lesions performed just as well as intact birds on the transitive inference task (Strasser, *et al*., 2004). Similarly, several studies have shown that mammals with damage to the hippocampal region are impaired in learning sensory discrimination reversals (e.g., Murray and Ridley, 1999), whereas turtles with lesions to the medial cortex are unimpaired at discrimination reversal learning (Grisham and Powers, 1990). These findings suggest that the hippocampal homologue in birds and reptiles might be more selectively involved in associations that involve a spatial component.

3.33.3.2 Summary of the Ancestral Homologue

The earliest hippocampus was likely a medial portion of a simple cortical mantle, and this medial portion was likely connected with dorsal and lateral portions of the mantle that processed visual and olfactory information, respectively. The visual and olfactory connections might have been brought together to form stimulus–stimulus associations to support spatial learning abilities in early mammals. However, it is unclear if this spatial learning ability was the sole ancestral function of the hippocampal homologue. It remains possible that further studies in birds and reptiles will uncover additional nonspatial associative abilities supported by the hippocampal homologue in these animals. Furthermore, even if the sauropsid hippocampal homologue is determined to be involved solely in forming associations that involve a spatial component, it cannot be assumed that this spatial specificity represents the ancestral condition. Indeed, several recent studies in goldfish have

indicated that the presumed hippocampal homologue in these vertebrates is important for nonspatial as well as spatial memory (Broglio *et al*., 2005). For example, goldfish with lesions to the hippocampal homologue were impaired in classical conditioning of the eye retraction response when a trace interval intervened between the conditioned and unconditioned stimuli but learned at the normal rate when those stimuli were contiguous (Álvarez *et al*., 2003), a pattern of results similar to those observed in several species of mammals (Clark *et al*., 2002). Thus, it is possible that the earliest hippocampal homologue supported a general (spatial and nonspatial) associative function and that this ability became specialized in birds and reptiles. Further studies using the classical conditioning paradigm, for example, might provide the critical evidence.

How might the ancestral hippocampus have supported the integration of information derived from its cortical inputs? Assuming that, as in extant mammals and sauropsids, the inputs to the ancestral hippocampus included prominent olfactory and visual afferents, one fascinating possibility concerns the mismatch in neural topography between olfaction and other sensory modalities. Visual stimuli are typically encoded in a retinotopic fashion that organizes information in terms of the spatial locations of external cues in the environment. By contrast, odors contain no inherent directionality, and accordingly there exists no topographic map for odor location in the olfactory cortex. Thus, associations between odors and other types of stimuli require reformatting in at least one modality to provide a common scheme of organization. For a comparison, consider how visual and auditory information is associated by birds. Both types of information are organized by a spatial topography, and an efficient overlapping map of the sight and sound from a noise-emitting object, such as a rustling mouse, has been identified in the barn owl tectum (Knudsen, 1987). It is difficult to imagine how this topographic organization could efficiently include the odor of the mouse. Further, it is important to consider that the olfactory bulb increased in size in early mammals (Aboitiz *et al*., 2002). Thus, the increasing adaptive value of olfactory abilities in catching prey may have driven the evolution of the hippocampus to support nontopographic associations between reformatted sensory information. Although olfaction may have made the greatest demand for nontopographic associations, the advantage of such a memory scheme would not have been limited to olfactory associations. Indeed, the hippocampus remains a crucial memory structure in microsomatic mammals including humans, so we might properly assume there is adaptive advantage for a nontopographic associative memory over a broad range of materials and modalities. The next half of the article considers whether this associative ability of the hippocampus is conserved across mammals.

3.33.4 Functional Homology of the Hippocampal and Parahippocampal Regions Across Mammals

3.33.4.1 Early Evidence on Hippocampal Function

The mammalian hippocampus appeared over 200 Mya, but the study of its function began in earnest only 50 years ago. The earliest and still compelling insights about hippocampal function in memory began with the dramatic characterization of the patient HM (Scoville and Milner, 1957). In an attempt to alleviate debilitating seizures, an experimental surgical procedure was performed in which large portions of both his right and left medial temporal lobes were resected. The ablation included large portions of the hippocampal region, the entorhinal cortex, and the perirhinal cortex (Corkin *et al*., 1997). The surgery reduced the intensity of the seizures but also had the unexpected and profound effect of leaving HM virtually incapable of acquiring new memories across a broad range of modalities of information. In striking contrast, his perceptual and motor capacities and other cognitive abilities, including language and attention, appeared normal. In addition, HM's capacity to acquire and retain information in mind for a brief period was also intact, although the new information was lost as soon as his attention was directed away from it. Although the majority of his childhood memories survived the surgery, there was a retrograde loss of memories acquired for several years prior to the surgery. The main interpretation of the findings was that the hippocampus was important for the consolidation of short-term memories into lasting long-term memories.

Immediately following the reports on HM, there were several attempts to determine whether the hippocampus was also involved in memory in monkeys and rats. Results from a large number of studies in rats of operant conditioning, sensory discrimination, maze learning, and avoidance learning were mixed and inconclusive (reviewed in Cohen and Eichenbaum, 1993; Eichenbaum and Cohen, 2001; O'Keefe and Nadel, 1978). The results from rats ranged from severe performance deficits to normal performance. Some studies even observed facilitation of learning following damage to the hippocampus. Thus, based on these early results, one possibility was that the hippocampus served

memory in humans but some other nonmemory cognitive function, such as response inhibition, in experimental animals.

The 1970s saw several important ideas emerge regarding the function of the hippocampus in experimental animals. Hirsh (1974) suggested the hippocampus was critical for context-dependent retrieval but not for modifications of behavior "along the performance line." Olton *et al.* (1979) suggested that the hippocampus was critical for what he called working memory (memory for single events) but not for reference memory (learning that can be applied across many events). O'Keefe and Nadel (1978) put forth the idea that the hippocampus is selectively involved in map-like spatial memory but not in learning guided by nonspatial cues. The ideas were far from consensual as to what specific function might be supported by the hippocampus in experimental animals, but all the views shared in common two features. First, all agreed that the hippocampus was involved in some aspect of memory. Second, all agreed that memory was not a single ability but was instead capable of being separated into multiple forms of memory, one that depended on the hippocampus and others that did not.

The spatial memory view of the hippocampus was the most successful of these early ideas. The idea of map-like spatial memory appeared well-suited to rats, for whom a memory of the area's geographical layout would be advantageous in supporting nighttime foraging. Further, the spatial map theory was supported by compelling results from studies in which action potentials of single hippocampal neurons were recorded while rats performed spatial tasks or merely explored an open field. The main finding of these studies was that many of the principal cells recorded from CA1 and CA3, which are noted for their very low baseline activity, increased their firing rate dramatically when the rat was in a particular location within the chamber (Muller *et al.*, 1987; O'Keefe, 1979; O'Keefe and Dostrovsky, 1971). O'Keefe and Nadel's (1978) interpretation of these findings was that the firing of hippocampal neurons signaled occupancy of a particular coordinate locus, a place field, within a cognitive map established in the hippocampus.

At the same time that these ideas emerged from work in experimental animals, the concept of multiple memory systems was being refined in parallel by work in human amnesic patients. Work with patient HM had already demonstrated that the hippocampus was not needed to acquire new motor skills (Milner, 1962). Yet these findings were typically set aside as motor-based exceptions to the general view that all memory depended on the hippocampus. A key finding came when Cohen and Squire (1980) observed intact learning outside the domain of motor skills in amnesic patients. Patients and age-matched volunteers were asked to read mirror-reversed words over the course of several days. Patients and control participants both improved their reading speed with practice, but only the control participants were able to describe subsequently the details of the testing situation. These results helped make the point that the human hippocampus was important for memory in the everyday sense of the word but was not important for other examples of procedural memory, such as the acquisition of cognitive skills, that are expressed through performance rather than recollection.

The observation of hippocampal cells with place fields in rats was compelling, yet for those who worked with amnesic patients, the idea of a special role for the hippocampus in spatial memory appeared to ignore a large body of data on HM and other amnesic patients indicating a critical role for the hippocampus in nonspatial memory, including verbal memory of recently encountered events that were not prominently spatial in nature. This disjuncture in the findings on rodents and humans exacerbated the already widely held view that the hippocampus supported distinct functions in humans and animals.

3.33.4.2 Convergence of Ideas on Hippocampal Function in Humans and Experimental Animals

A resolution of the contrasting findings from humans and experimental animals became available only through the systematic administration of similar tasks to humans and experimental animals. Points of contact between humans and experimental animals have now been made with numerous tasks, including the transitive inference task described in the first half of the article (Bunsey and Eichenbaum, 1996; Heckers *et al.*, 2004; Nagode and Pardo, 2002; Preston *et al.*, 2004). Here, we focus on three tasks that have been especially informative, either for the volume of data available or for the clarity of the results across species. The first is a task in which multiple pairwise discriminations are learned concurrently. The second is a recognition memory task called delayed nonmatch to sample (DNMS). The third is classical conditioning of the eyeblink response. A fourth point of contact exists, not in the form of a specific task, but as a pattern of results from several tasks related to premorbid memory in amnesic patients and experimental animals with damage to the hippocampus. That is, there is a similar pattern of temporally graded retrograde amnesia across mammals.

3.33.4.2.1 Concurrent discrimination One task that has been given to both amnesic patients and monkeys with medial temporal lobe damage is a task in which multiple sets of pairwise object discrimination problems must be learned concurrently. Both healthy humans and intact monkeys learn to identify the correct item for each of up to 20 pairs of randomly assigned junk objects over the course of multiple testing sessions. Monkeys with damage limited mostly to the medial temporal lobe perform as well as intact animals (Buffalo *et al.*, 1998; Malamut *et al.*, 1984; Teng *et al.*, 2000). In contrast, amnesic patients typically attain levels of performance much lower that that shown by healthy individuals (Hood *et al.*, 1999; Squire *et al.*, 1988). Based on these findings, one possibility was that the hippocampal and parahippocampal regions performed a different function in humans and monkeys, one that was important for the concurrent discrimination task and one that was not. Another possibility was that the hippocampal and parahippocampal regions served similar functions in humans and monkeys, both potentially contributing to the concurrent discrimination task. From this viewpoint, monkeys with medial temporal lobe damage performed normally because their memory impairment was masked by a capacity for habit learning that was underdeveloped in humans (Hood *et al.*, 1999).

A third possibility is that the line that divides declarative memory and nondeclarative memory is drawn similarly for monkeys, humans, and possibly all mammals (Squire *et al.*, 1988). From this viewpoint, the hippocampal and parahippocampal regions serve the same function in humans and experimental animals, and the capacity for hippocampus-independent procedural learning is also similar for all mammals. The difference is that humans enjoy cognitive skills such as verbal labeling and elaborations, which are presumably absent or less well developed in other mammals, leading them to adopt a memorization strategy. When this strategy is unavailable, as in the case of profoundly amnesic patients, humans appear to be able to fall back on habit learning and show a rate of learning on the concurrent discrimination task that is similar to that shown by monkeys (Bayley *et al.*, 2005a).

3.33.4.2.2 Delayed nonmatch-to-sample After HM's profound amnesia was described (Scoville and Milner, 1957), many studies in experimental animals were performed in attempting to duplicate his damage and memory impairment. One early line of studies focused on a test of recognition memory in which animals were first presented with one of two colored sample stimuli, then following a variable delay, were required to chose the sample stimulus over the other stimulus, that is, to match the sample. The same two color pattern stimuli were used on each trial, with either selected randomly to be the sample on that trial. The expectation was that damage to the medial temporal lobe would reproduce the observation of delay-dependent memory impairment in HM. However, monkeys with medial temporal lobe lesions performed surprisingly well on this and other delayed response tests, even at memory delay intervals of several seconds (Correll and Scoville, 1965, 1967; Drachman and Ommaya, 1964). These findings contributed to the early view that hippocampal function differs in animals and humans.

However, a breakthrough occurred when the task was modified to use different three-dimensional objects on each trial (Gaffan, 1974; Mishkin and Delacour, 1975). Also, because monkeys showed a natural preference for manipulating novel objects, experiments began using a nonmatch rule (i.e., the unstudied object was rewarded) for efficiency. Thus, the procedure was changed to become the now widely used trial-unique DNMS task. The results on the DNMS task were very different than those in the early studies. Monkeys with damage similar to that produced in HM could learn the nonmatching rule and performed normally at very brief delays, but rapidly declined in performance as the delay was increased, thus reproducing the pattern of delay-dependent memory impairment in human amnesia (Squire and Zola, 1996). The DNMS task was also soon adapted for use in rats (Mumby *et al.*, 1992). Similar to the results from humans and monkeys, damage that included both the hippocampal region and parahippocampal region resulted in a delay-dependent impairment in recognition memory performance (Mumby and Pinel, 1994). Although researchers have more recently debated whether the hippocampal region itself is important for recognition memory, the use of the DNMS task across species helped identify that the mammalian hippocampal and parahippocampal regions enable long-term memory for items encountered only once. Based on these and other observations, it is now appreciated that rapid, single-exposure learning is a hallmark of hippocampal-based learning.

3.33.4.2.3 Classical conditioning of the eyeblink response Another example of a success in demonstrating similarities in the profile of hippocampus-dependent learning across species shows an impressive distinction generated by a simple procedural parameter. In classical conditioning of the eyeblink response, a tone is repeatedly followed by

a mild puff of air to one's eye, causing a reflexive blink. After several tone–air puff pairings, subjects (across several mammalian species) begin to blink following tone onset and prior to the air puff, demonstrating the conditioned response. In the standard version (called delay eyeblink conditioning), the onset of the tone precedes the onset of the air puff and continues such that the stimuli then overlap and co-terminate. This simple form of associative learning is supported by a carefully described circuit that includes the brainstem and cerebellum (Christian and Thompson, 2003). In a slightly modified version (called trace eyeblink conditioning), the tone ends before the air puff and a brief ($\leq$1s) silent interval separates the two stimuli. This small gap in time necessitates the recruitment of additional brain structures, including the hippocampus, to support the association of the tone and the puff in the cerebellum. The distinction between trace and delay eyeblink classical conditioning is particularly compelling when one considers that the dependence of trace eyeblink conditioning on the integrity of the hippocampus has been established for mice, rats, rabbits, and humans (Clark and Squire, 2000). Thus, the difference between delay and trace eyeblink classical conditioning suggests something fundamental about the function of the hippocampus that has been conserved though evolution.

Classical conditioning was once viewed as a form of procedural learning, typically outside the domain of critical hippocampal involvement. However, the findings of additional experiments in humans have indicated that acquisition of trace, but not delay, eyeblink conditioning correlates with participants' ability to report information about the relationship between the tone and the air puff (Clark *et al.*, 2002). These results suggest two points. First, the close similarity of the experimental parameters for delay and trace eyeblink conditioning illustrate how subtle the change can be that disposes performance to rely on hippocampus-dependent memory available for verbal report. Second, the relationship in humans between trace eyeblink conditioning and awareness of the stimulus contingencies suggests that something similar might be occurring in experimental animals who successfully acquire trace eyeblink conditioning.

3.33.4.2.4 Retrograde amnesia In addition to cross-species similarities in the cognitive demands dependent on the hippocampus and in the nature of events represented by the hippocampus, there is also considerable evidence indicating conservation of a time-limited role of the hippocampus in memory consolidation. In numerous studies in experimental animals who were given lesions to the hippocampal region at various intervals after acquiring new information, the result emerged that the hippocampus is needed to retrieve information for a finite period of time (for a review, see Squire *et al.*, 2001). The typical finding is that animals in which the hippocampus was ablated immediately after a training session subsequently displayed impaired performance, whereas animals in which the hippocampus was removed one week to one month after a training session subsequently displayed normal performance. The interpretation of these studies was that the hippocampus was needed for acquisition and initial retrieval of the new memory, but that over time the memory eventually became independent of the hippocampus through a process of consolidation (Squire *et al.*, 2001). Although the critical training–lesion interval varied somewhat from study to study, the retrograde amnesia suggested that the process of consolidation lasts from a few days to a month.

These findings are qualitatively very similar to the pattern of temporally graded retrograde amnesia in HM and other amnesic patients. For example, one study assessed amnesic patients' memory for newsworthy incidents that occurred at various years prior to the patients sustaining damage thought to be limited to the hippocampal region (Manns *et al.*, 2003b). These results suggested that the impact of the hippocampal damage extended to memory from 5 to 10 years prior to onset of damage. Thus, although the results suggest that the process of consolidation is substantially longer in humans as compared to experimental animals, the findings indicate that the hippocampus plays a time-limited role in memory across species.

Taken together, the results of these three specific tasks and the common observation of temporally graded retrograde amnesia strongly suggest that the hippocampus serves a similar role in memory in both humans and in experimental animals. However, much debate surrounds the question of how to best characterize this role. For example, still under debate is whether the hippocampus itself is crucial for simple recognition memory or whether structures in the parahippocampal region support this ability (Manns *et al.*, 2003a; Mumby, 2001; Clark *et al.*, 2001). However, this uncertainty applies equally to the findings on humans, monkeys, and rats. In any case, it is clear that the extended hippocampal memory system, including the parahippocampal region, is important for examples of single-trial learning, including recognition memory judgments, for spatial memory, and for forming arbitrary relationships between stimuli, as in the transitive inference task. The example of trace eyeblink conditioning also illustrates that the

hippocampus is crucial for learning under circumstances in which the capabilities of extrahippocampal structures are unable to support the learning. In these instances, the hippocampal contribution becomes indispensable.

3.33.4.3 Remaining Points of Disconnect between Humans and Experimental Animals

With the greater understanding of multiple memory systems and the assurance of consistency of function between humans and experimental animals, researchers could explore hippocampal function in a variety of mammalian species and expect with some confidence that the results would be relevant to the entire taxon. This exploration has led to an understanding that the original features of memory that distinguished hippocampal function in humans and animals are more compatible with a cross-species approach than once was believed.

3.33.4.3.1 Understanding how place cells relate to the human hippocampus A remaining point of potential discontinuity between species is the difficulty in resolving the prominence of spatial correlates in the firing of rodent hippocampal cells with the observation that the human hippocampus is important for all examples of declarative memory, both spatial and nonspatial. The conclusion that hippocampal neurons fire primarily in association with an animal's location in its environment, whereas the human hippocampus is required for and engaged by a broad variety of nonspatial memories would seem to present a major exception to cross-species similarity of hippocampal function.

However, recent parallel studies in both rats and humans have demonstrated a much broader scope of information encoded by hippocampal neurons in both rats and humans. In rats, a direct comparison of spatial and nonspatial coding by hippocampal neurons was investigated by recording from hippocampal cells as rats sampled nonspatial cues at many locations in an environment (Wood *et al.*, 1999). The rats performed a task in which they had to recognize any of nine olfactory cues that were placed in any of nine locations. Because the locations of the odors were varied systematically, cellular activity related to the odors and to memory performance could be dissociated from activity related to the animal's location. The study found that similar proportions of hippocampal cells fired in association with a particular odor, a particular place, or whether the stimulus was recognized. In addition, a large subset of hippocampal neurons fired in association with only a particular combination of the odor, the place where it was sampled, and the match/nonmatch status of the odor. In a remarkably similar study on humans, Ekstrom *et al.* (2003) recorded the activity of hippocampal neurons in human subjects as they played a taxi driver game, searching for passengers to be picked up and dropped off at various locations in a virtual reality town. Similar to the findings with rats, equivalent proportions of the cells fired in association with particular landmarks, views of the environment, or places occupied in the virtual town. Also, many of these cells fired selectively in association with specific combinations of a place and the view of a particular scene or a particular goal.

Other studies have also reported a remarkable similarity of hippocampal neuron firing patterns in monkeys and humans associated with nonspatial stimulus analysis. Hampson *et al.* (2004) trained monkeys on matching-to-sample problems, then probed the nature of the representation of stimuli by recording from hippocampal cells when the animals were shown novel stimuli that shared features with the trained cues. They found many hippocampal neurons that encoded meaningful categories of stimulus features and appeared to employ these representations to recognize the same features across many situations. Kreiman *et al.* (2000a) characterized hippocampal firing patterns in humans during presentations of a variety of visual stimuli. They reported a substantial number of hippocampal neurons that fired when the subject viewed specific categories of material (e.g., faces, famous people, animals, scenes, houses) across many exemplars of each. A subsequent study showed that these neurons are activated when a subject simply imagines its optimal stimulus, supporting a role for hippocampal networks in recollection of specific memories (Kreiman *et al.*, 2000b). This combination of findings across species provides compelling evidence for the notion that some hippocampal cells represent abstract features of nonspatial stimuli that appear in different experiences.

Studies across species also emphasize the presentation of objects in relation to their location in the environment. Hippocampal cells that represent specific salient objects in the context of a particular environment have also been observed in studies of rats engaged in foraging (Gothard *et al.*, 1996; Rivard *et al.*, 2004) and escape behavior (Hollup *et al.*, 2001) in open fields. In addition, two recent studies highlight the associative coding of events and places by hippocampal neurons in rats and monkeys. In one study, rats were trained on an auditory fear conditioning task (Moita *et al.*, 2003). Prior to fear conditioning, few hippocampal

cells were activated by an auditory stimulus. Following pairings of tone presentations and shocks, many cells fired briskly to the tone when the animal was in a particular place where the cell fired above baseline. Another recent study examined the firing properties of hippocampal neurons in monkeys performing a task where they rapidly learned new scene–location associations (Wirth *et al*., 2003). Just as the monkeys acquired a new response to a location in the scene, neurons in the hippocampus changed their firing patterns to become selective to particular scenes. These scene–location associations persist even long after learning is completed (Yanike *et al*., 2004). These findings are entirely consistent with the findings of prevalent hippocampal neuronal activity associated with conjunctions of events and locations in Wood *et al*. (1999) study on rats and Ekstrom *et al*. (2003) study on humans. Collectively, these findings indicate that a prevalent property of hippocampal firing patterns in rats, monkey, and humans involves the representation of unique associations of stimuli, their significance, specific behaviors, and the places where these events occur.

3.33.4.3.2 Understanding how episodic memory relates to hippocampal function in experimental animals Another point of potential discontinuity involves a form of declarative memory called episodic memory. Episodic memory is characterized as the ability to replay in mind a particular episode in one's life, and this capacity has been closely identified with hippocampal function in humans (Tulving, 2002). Defined in these terms, episodic memory is considered by some to be a uniquely human ability (Tulving, 1983). If so, then the human capacity for episodic memory represents a break in the continuity of hippocampal research between humans and experimental animals. The difficulty in addressing this issue is that experimental animals are unable to report on their subjective experience.

A less mentalistic definition of episodic memory, and one that is experimentally tractable in experimental animals as well as in humans, characterizes episodic memory as including details about the time and place in which an episode occurred. That is, episodic memory includes information about the 'what', 'where', and 'when' of an event (Clayton and Dickenson, 1998). Experimental animals, including rodents and birds, have demonstrated evidence of the ability to remember where and when unique events occurred (Clayton *et al*., 2003; Dere *et al*., 2005; Eacott *et al*., 2005; Morris, 2001). Further, damage to the hippocampus impairs this capacity (Ergorul and Eichenbaum, 2004). By this definition then, it appears that animals other than humans are capable of episodic-like memory and that this ability depends on the hippocampus. However, the definition of episodic memory as the combination of 'what', 'where', and 'when' may be overly strict and may exclude examples of hippocampus-dependent memory. In particular, tasks that require memory for either temporal order (Fortin *et al*., 2002; Kesner *et al*., 2002) or spatial location (Day *et al*., 2003; O'Keefe, 1993) alone depend on the integrity of the hippocampus. Thus, although the hippocampus in humans and experimental animals appears to be crucial for combining temporal and spatial elements of a particular incident, this capacity does not represent the totality of its function.

Another approach to studying episodic memory in humans and experimental animals focuses on the notion that recognition memory can be supported by two processes: an episodic-like recollection of specific details and a feeling of familiarity with a previously experienced item (Atkinson and Juola, 1974; Mandler, 1980; Yonelinas, 1994). Although the status of familiarity-based judgments is currently under debate (Brown and Aggleton, 2001; Squire *et al*., 2004), it is generally accepted that the human hippocampus is important for recognition memory based on recollection. One approach to quantifying the relative contribution of recollection and familiarity to recognition memory judgments has adopted signal detection theory to characterize recognition memory in terms accuracy (proportion of hits to correct rejections) as either a function of confidence levels or tendency to endorse and item as having been repeated. These measures result in a receiver-operating characteristic (ROC) curve that, by one model, contains the signatures of distinct recollection and familiarity contributions to recognition (Yonelinas, 2001). From this viewpoint, a typical ROC curve can be thought of as composite of a familiarity curve that is symmetric to the diagonal and a recollection line that is asymmetric. Mathematical decomposition of these plots can thus provide numerical estimates of recollection and familiarity. One appeal of this technique is that the definition of episodic-like memory, the numerical estimate of recollection, can be used in both humans and experimental animals.

A recent study reported that the ROC curve in human amnesic patients with hippocampal damage is curvilinear and symmetric, indicating a loss of recollection (Yonelinas *et al*., 2002). Consistent with that finding, a recent study of rats found that the ROC curve of normal rats was both asymmetric and curvilinear, similar to the composite recollection and familiarity ROC curve observed in normal

human subjects (Fortin *et al.*, 2004). In contrast, performance of rats with damage to the hippocampus was best fit by a symmetric curve, suggesting a loss of episodic-like recollection. Although the measure of recollection in these studies is necessarily indirect, the closely parallel results suggest that the hippocampus contributes to episodic memory, or something closely resembling episodic memory, in both humans and experimental animals.

However, it should be noted that the view of the hippocampal function based on the distinction between recollection and familiarity is not consensual. Some argue that the shape of ROC curves can be better explained by factors such as differences in the variability in the perceived memory strength between studied and unstudied items (Donaldson, 1996). Others charge that the idea is based on a psychological dichotomy that is incompatible with the anatomical view of the hippocampal and parahippocampal regions as a hierarchical network of interconnectivity (Squire *et al.*, 2004). Further, the notions of recollection and familiarity are based on terminology and concepts tailored to psychological findings in humans. Although contact has been made between humans and rats with respect to the importance of the hippocampus for recollection, one could argue that the distinction is not one that is ideally suited to an evolutionary approach to the study of memory and the hippocampus.

3.33.4.4 Possible Divergence between Species

Although the bulk of the anatomical data from mammals regarding the hippocampal and parahippocampal regions indicates that the similarities outweigh the differences, several prominent anatomical differences were noted between rodents and primates. Perhaps the most striking difference is the prominence of olfactory input that reaches the entorhinal cortex in the rat. Based on this observation, one possibility is that the rat entorhinal cortex exhibits specialization in olfactory memory that is not exhibited by the primate entorhinal cortex. Indeed, in one study, rats with hippocampal lesions were nevertheless able to identify a novel odor from among 24 recently encountered odors (Dudchenko *et al.*, 2000). Their performance was presumably supported in part by the entorhinal cortex and was as good as that as shown by healthy rats. The basic procedure was adapted and given to humans with damage to the hippocampal region (Levy *et al.*, 2003). In contrast to the findings in rats, humans with damage to the hippocampal region performed poorly and performed worse than healthy individuals. Although it is difficult to rule out other variables such as the extent of the hippocampal damage, these studies hint that the rat entorhinal cortex might be able to support more odor-related memory abilities as compared to the human entorhinal cortex.

Another difference observed between humans and experimental animals concerns the apparent duration of memory consolidation. In experimental animals, the process apparently requires around a month when damage is restricted to the hippocampal region (Squire *et al.*, 2001). In humans, the process can last several years when damage is thought to be limited to the hippocampal region and can last even longer when damage also includes structures in the parahippocampal region (Bayley *et al.*, 2005b; Kapur and Brooks, 1999; Manns *et al.*, 2003b; Reed and Squire, 1998; Rempel-Clower *et al.*, 1996). Several factors likely contribute to the different timescales observed between humans and experimental animals. First, anatomical studies indicate that the organization of the hippocampal and parahippocampal regions in rats is less hierarchical than it is in the monkey. In the rat, the hippocampal region and the entorhinal cortex show more direct connections with olfactory and neocortical areas than are observed in the monkey (Burwell and Amaral, 1998b; Suzuki and Amaral, 1994). If the trend toward a more strict hierarchy was continued in humans, the decrease in direct cortical connections with the hippocampus and entorhinal cortex might suggest why the process of consolidation takes longer in humans. Second, the type of information being assessed in human studies of consolidation is often very different from the type of information being assessed in experimental animals. Studies in humans typically examine factual information about the world. In comparison, studies in experimental animals typically examine presumably simpler associations. For example, one study in rabbits identified that lesions one day after training, but not 30 days after training, impaired subsequent performance on a trace eyeblink conditioning experiment (Kim *et al.*, 1995). One possibility then is that some of the discrepancy between humans and experimental animals could be accounted for by differences in the complexity of the memories assessed.

A related topic concerns whether or not spatial memory holds a special status in terms of memory consolidation in the rat. In humans, it appears that spatial memory eventually becomes independent of the hippocampus, just as other examples of memory that are acquired by the hippocampus are thought to become. In one study, a profoundly amnesic patient who had virtually complete damage to the hippocampal region was nevertheless able to pretend he

was standing in his childhood neighborhood and point accurately to several town landmarks (Teng and Squire, 1999). In rats, the results are somewhat different. Indeed, most studies in rats have found that the hippocampus remains important for spatial memory for as long as the memory remains measurable (Moscovitch *et al*., 2005). In one study, healthy rats were able to demonstrate memory for a spatial location learned more than 14 weeks previously. However, rats with damage to the hippocampus performed at chance even when the training–surgery interval was 14 weeks (Clark *et al*., 2005). That is, there was no evidence for consolidation of spatial memory in these rats, despite the fact that the training–surgery interval was longer than that typically reveals consolidation of nonspatial memory in experimental animals. It is possible that the rat experienced environmental pressures that caused spatial memory to acquire a status in which its persistence became tied to the function of the hippocampus. However, it is also possible that further study will identify a process of consolidation for spatial memory in the rat. Indeed, one recent study in rats found that post-training lesions to the hippocampus spared memory for locations of different rewards, but only when the animals were exposed to the locations of the rewards very early in life (Winocur *et al*., 2005).

3.33.4.5 A Species-General Mechanistic Account of the Hippocampus

A challenge in memory research has been to provide a mechanistic account that could connect the anatomy of the hippocampal and parahippocampal regions to the examples of memory that depend on these structures. That is, it is now clear that the hippocampal and parahippocampal regions are important for the initial acquisition and temporary maintenance of declarative memory, but it is unclear exactly how these regions support this capacity.

One promising approach has been the development of anatomically plausible computational models of the hippocampal and parahippocampal regions. One particularly influential model built on the characterization for the hippocampal and parahippocampal regions as a hierarchical network and proposed that the hippocampus served as a central node of synaptic change (McClelland *et al*., 1995). The hierarchical network proposed by the model offered the brain several mnemonic advantages. First, incoming information that would be processed by widespread neocortical sites could be condensed through the process of funneling information through the parahippocampal region on its way to the hippocampal region. Accordingly, long-lasting associations between very different types of information could be made very quickly through a limited number of synaptic changes in the hippocampus. Thus, the model described how the anatomy of the hippocampus enabled rapid acquisition of arbitrary associations. Second, the binding of disparate neocortical sites by the hippocampus might allow the neocortical sites to develop more direct interconnectivity over time. That is, the model described how the process of consolidation might occur. This second point was based on the idea that several neocortical nodes would be joined by the hippocampus into a subnetwork whose co-activity could be propelled by repetition, rehearsal, or spontaneous reinstantiation during sleep. An important idea that emerged from the model was that the hippocampus allowed the brain to acquire new information rapidly and then to gradually interleave that information in existing neocortical networks. That is, according to the model, the hippocampus solves the problem of how to acquire new information quickly without disturbing the delicate network of existing knowledge.

The advantage to models like the one described above is that the concepts should apply equally well to any mammal, provided that the anatomical constraints included in the model are not violated by any species-specific anatomical idiosyncrasies. Indeed, additional computational models will be the most useful to an evolutionary approach when they are based on facets of the anatomy that are shared across mammals. In particular, accumulating evidence suggests that the information arriving at the hippocampal region through perirhinal cortex and LEA differs in content as compared to the information arriving at the hippocampal region through postrhinal/parahippocampal cortex and MEA. Further, the anatomy suggests that the dentate gyrus and CA3 may be important for combining this information and incorporating it with as yet uncombined information in CA1 and subiculum. Computational models that take advantage of this pattern of connectivity, which is shared by at least rats and monkeys, could contribute significantly to understanding principles of hippocampal function that apply across species.

3.33.5 Conclusions

The anatomy of the hippocampal and parahippocampal regions represents an elegant solution to a difficult memory problem. Natural selection shaped a network of structures that could quickly form stable associations between pieces of information

that bore no topographic similarity to one another. Evidently, a rough approximation of an answer had been sketched out early in the evolution of vertebrates, prior to the emergence of mammals. The modern blueprint appeared in the earliest mammalian hippocampus, and the solution was repeated again and again throughout the taxon. The occasional variations in anatomy between mammals with regard to the parahippocampal region might then represent flourishes added to what was already an anatomical masterpiece.

If the anatomy of the mammalian hippocampus is a finished product, then the study of its function might be best described as a work in progress. On one hand, much is already known about the function of the hippocampal and parahippocampal regions. These structures together support only one kind of memory, a type of memory referred to as declarative memory that is unambiguously important for recollecting episodic details, encoding spatial locations, and forming abstract or arbitrary associations. On the other hand, much is left to be discovered. A question at the forefront of memory research is how the individual components of the hippocampal and parahippocampal regions might each contribute to declarative memory. Accordingly, there is debate as to whether the hippocampus itself is important for aspects of declarative memory such as nonspatial memory and judgments of familiarity or if these abilities are supported by areas within the parahippocampal region. Thus, a clear set of principles that account for all examples of hippocampus-dependent memory has not been agreed upon. The hope is that the psychological, anatomical, physiological, and computational approaches will be combined to produce a view of hippocampal function that makes sense at all levels.

As researchers move toward a consensual view regarding the roles of the hippocampal and parahippocampal regions in memory, one challenge will be the struggle to identify ideas that attain a satisfactory level of psychological specificity for humans while maintaining enough contact with the anatomical and mechanistic details to generalize to all mammals. The goal is to identify the unifying principles that apply to all examples of hippocampus-dependent memory, both in humans and experimental animals. To date, many ideas that have been proposed that contain elements of species-specific psychology, such as spatial memory in rats and episodic memory in humans. The difficulty is that often the elements of psychology vary dramatically between species – despite the fact that the anatomical details of the hippocampal and parahippocampal regions are remarkably similar. For example, humans enjoy certain cognitive skills such as chunking and elaboration that may steer them toward memorization strategies. These abilities are presumably less advanced in experimental animals and therefore might lead animals to rely more on trial and error. In turn, rats may have evolved specialized skill sets that allow them in their nighttime foraging to be highly attuned to spatial layout and their position within it. For neither rats nor humans is it likely that these cognitive skills are fundamentally derived from the hippocampus. Instead, these skills are likely supported by areas outside the hippocampal and parahippocampal regions such as prefrontal cortex and posterior parietal cortex. That is, it is possible that any apparent differences in terms of the psychological properties of hippocampus-dependent memory might be more related to the differences in neocortical input to the hippocampal and parahippocampal regions rather than due to any differences between species for these areas themselves.

An evolutionary approach can help us understand the functional machinery of the mammalian hippocampus. At the same time, an enormous asterisk must follow any statement about our current understanding of the hippocampus, for the vast amount of data come from rodents and primates. Most of the mammalian taxon is unexplored, and every mammal presents an opportunity to probe the function of the hippocampal and parahippocampal regions with a unique neocortical instrument. For example, one might explore how auditory information in the bat echolocation system arrives in the hippocampal region. One might also take advantage of the simple cortex of the hedgehog to pare down the inputs to the parahippocampal and hippocampal regions. Thus, an evolutionary perspective can not only reveal the conservation of hippocampal form but can also offer many opportunities for exploring the function of this form.

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http://www.avianbrain.org – Newly updated terminology for avian neuroanatomy.

http://www.brainmuseum.org – Whole brain and sectioned images for many mammals.

3.34 Evolution of the Elephant Brain: A Paradox between Brain Size and Cognitive Behavior

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Glossary

areas or modules	Large, functionally significant subdivisions of the cerebral cortex.
clan	A group of elephants made up of families which periodically come together and then separate but maintain communication with each other.
matriarch	The female leader of an elephant family that is generally related to other females in the family and who is the oldest and still capable of leading.
seismic signals	Vibrations transmitted across the top layers of the earth.

3.34.1 Introduction

Of all large-brained species, elephants have received the least attention from neurobiologists in terms of understanding neocortical cytoarchitecture and cerebral information processing. Other large-brained species, in particular chimpanzees and humans, have attracted considerable interest and much has been learned about the constraints that large brains impose on the nature and speed of information processing (Krubitzer, 1995; Kaas, 2000; Hofman, 2003; Changizi and Shimojo, 2005; see Mosaic Evolution of Brain Structure in Mammals, Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications, Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals, The Evolution of Crossed and Uncrossed Retinal Pathways in Mammals, Do All Mammals Have a Prefrontal Cortex?, The Evolution of the Basal Ganglia in Mammals and Other Vertebrates, The Evolution of the Dorsal Thalamus in Mammals). Elephants present a challenge in understanding the evolution of large brains because they have the largest brains of all terrestrial animals with the greatest volume of cerebral cortex, and even the greatest volume of nonsensorimotor cortex, which is that part of the cerebral cortex presumably involved in higher-order brain functions. The presence of such a massive cerebral cortex in the elephant poses a dilemma because several studies on elephant cognitive behavior, including tool use, mirror-based self-recognition, visual discrimination learning, and so-called insight behavior, reveal that their performance is unimpressive in comparison to chimpanzees, let alone humans. In this article we suggest that the paradigm of relating cognitive behavior to the amount of neocortex, as conventionally applied to large-brained primates, is not appropriate for elephants and point to some anatomical and cytoarchitectural features of the brains of elephants that would appear to explain why elephants fall short on their performance on primate-like cognitive behavior but excel in other areas of higher-order brain functions, namely long-term, extensive information storage.

There are currently three recognized species of elephants: (1) the African savannah elephant (*Loxodonta africana*), which is the largest species; (2) the small forest elephant, *Loxodonta cyclotis*; and (3) the Asian elephant (*Elephas maximus*). Because information on the behavior and brain structure of the forest elephant is virtually nonexistent, we discuss only the African and Asian species in the article.

3.34.2 Behavioral Considerations

Here we briefly review relevant behavioral studies on elephants to provide a background for discussion of elephant brain anatomy, cytoarchitecture, and information processing.

3.34.2.1 Comparative Aspects of Cognitive Behavior

For this topic it is appropriate to compare elephants with chimpanzees (*Pan troglodytes*), the great ape most commonly studied with regard to cognitive behavior. Using sticks to fish termites out of underground nests or to reach inside bones for marrow (van Schaik *et al.*, 1999; Matsuzawa, 2003) is a classic example of tool use in great apes. Perhaps the best example of complex tool use by chimpanzees is cracking nuts open by holding a nut against an anvil stone and hitting it with a smaller hammer stone (Humle and Matsuzawa, 2001).

Elephants engage in several types of tool use. Fly switching with branches, while not particularly complex, would appear, in fact, to be the first documented example of tool use among nonhuman animals, dating back to when a wildlife adventurer wrote in 1838 of seeing elephants emerging into an open glen, "bearing in their trunks the branches of trees with which they indolently protected themselves from flies" (Harris, 1838, p. 169). Elephant fly switching was even mentioned by Darwin (1871) in discussing the intelligence of beasts. We have documented the efficacy of fly switching in repelling flies (Hart and Hart, 1994) and the modification of branches to use as switches (Hart *et al.*, 2001). Asian and African elephants engage in other types of tool use, including scratching with a stick and throwing stones at rodents competing for fruit (Hart and Hart, 1994; Kurt and Hartl, 1995; Wickler and Seibt, 1997). Despite the historical significance, the complexity of tool use of elephants pales in comparison with the rich repertoire of fast-action, highly coordinated tool use described for chimpanzees such as the hammer–anvil style of cracking nuts.

Simultaneous visual discrimination learning is another area of cognitive behavior in which elephants have been compared to standards set by other mammals. Under seminatural conditions, Asian elephants may learn a black/white or large/small discrimination but the performance of even the fastest-learning elephants is unremarkable compared with other mammals (Nissani *et al.*, 2005). Tests of insight behavior, exemplified by pulling a cord to obtain a desirable object, also reveal disappointing behavior by elephants which do not perform in a manner consistent with that of chimpanzees, rhesus monkeys, and even several species of birds (Nissani, 2004).

Finally, in the way of classical tests of cognitive behavior, there is the test of self-recognition, as studied in the well-known mirror experiment in which the self-recognizing animal touches a mark on its head in front of a mirror. In contrast to chimpanzees that perform well (Povinelli *et al.*, 1997), Asian elephants fail in self-recognition (Povinelli, 1989; Nissani, 2004).

3.34.2.2 Elephants and Long-Term, Extensive Memory

Now we turn to an aspect of behavior for which elephants have legendary abilities ("memory like an elephant"; "an elephant never forgets"). Quantitative field studies actually support this popular perspective. One would expect long-term memory about details of the environment regarding food and water resources to be essential for survival of herbivores like elephants, whose digestive system is adapted to handle large volumes of low-quality forage. Among the studies where movements are documented by individual animal recognition and/or radio-tagged individuals are those of desert elephants that occupy a huge home range during the dry season and visit water holes spaced more than 60 km apart (Viljoen, 1989). One cannot help but be impressed by the ability of an elephant family, led by a matriarch of some 30-plus years, to head unerringly toward an isolated water hole after a stressful 4 days without water; especially when particular water holes may only be visited every 8 months or so. The same desert elephants travel annually from their home ranges to new forage grounds in response to localized rainfall almost 200 km away (Viljoen, 1989). The fact that elephants may arrive at such distant locations as soon as 3 days after the start of rainfall, and without prevailing winds to carry chemosensory cues (Viljoen, 1989), suggests they are responding to one or more sensory cues of distant rainfall. One possibility is that seismic waves, which are detectable by elephants (O'Connell-Rodwell *et al.*, 2005), and which are produced by lightning strikes and the accompanying thunder vibrations (O'Connell-Rodwell *et al.*, 2001), may be sufficient for elephants to detect over long distances. Thus, travel to distant foraging grounds could be triggered by seismic signals from distant storms with direction coordinated by long-term memory of where vegetation is likely to appear.

The fitness value of the long-term spatial–temporal information retained by long-living matriarchs was vividly illustrated in a study conducted during a prolonged drought in Tarangire National Park in Tanzania (Foley, 2002). Clans in which families

were led by older matriarchs left the park to forage in nonpark areas. However, a clan in which families were led by only young matriarchs (due to poaching) stayed in the park and sustained severe losses from insufficient water and forage: infant mortality and all-age mortality were more than double that of the clans with older matriarchs. To have experienced the most recent severe drought, where matriarchs could have remembered where to go, they would have had to be at least 35 years old and the matriarchs of the clan not leaving the park were apparently not this old.

Social memory is another area in which elephants show exceptional ability. An aspect of social behavior involves chemosensory communication and urine could theoretically allow an animal to identify particular conspecifics even decades after the last encounter. During sexual encounters, urine is typically orally investigated by adult male elephants, as in other ungulates, through the process of flehmen behavior that involves the transport of fluid materials from the mouth to the vomeronasal organ (Hart *et al.*, 1989). Because long-lived male elephants leave their natal group, an ability to identify the urine of mothers would be important in avoiding inbreeding. This appears to be the case, as revealed in a controlled laboratory study in which adult males identified maternal urine decades after being separated from their mothers (Rasmussen and Krishnamurthy, 2000).

Acoustic stimuli represent another area in which elephants may recognize individuals. Elephants can recognize individual calls of 100 or more elephants at 1 km or more. Because there is uneven attenuation of the various frequencies of the contact calls at 1 km away, this means that such extensive recognition can occur with just a fraction of the acoustic signature that is otherwise present at close range (McComb *et al.*, 2000, 2003). Discriminating between familiar and unfamiliar elephants is important in coordinating interactions at water holes and avoiding conflicts. One study revealed that families with older matriarchs are better at discriminating between familiar and unfamiliar individuals than families with younger matriarchs, and the age of the matriarch was a significant predictor of the number of calves successfully produced in the family per female (McComb *et al.*, 2001).

3.34.3 Information Processing in Large Brains

The foregoing discussion presents a sampling of the behavioral information available and suggests that the performance of elephants on fast-action, and/or fine-grained cognitive tasks is noticeably inferior to that of great apes (and humans) but that long-term or extensive memory ability would appear to exceed that of great apes and possibly even humans. We now turn to a discussion of some special anatomical features, as well as aspects of cerebral cortical cytoarchitecture and neuronal interactions that logically relate to these major behavioral differences.

3.34.3.1 The Elephant Hippocampus

As an initial consideration, an emphasis on long-term memory performance brings up the question of whether the hippocampal complex, long associated with memory consolidation, might be enhanced in elephants. Of relevance is that recent studies emphasize that the hippocampus is more crucial for the formation and retention of cognitive maps that code for unfamiliar spatial–temporal relationships that are viewpoint-independent representations of the environment than for memory consolidation involving familiar representations (Sweatt, 2004). Given that elephants appear to excel in the retention of infrequently used distant geographical areas, where their treks to the distant areas are independent of where they start out from (viewpoint-independent), the hippocampus would be particularly important to evaluate. Consistent with an expectation of a large hippocampus, recent magnetic resonance imaging of an African elephant brain reveals that the hippocampus is unusually large and convoluted, compared even with human brains (Hakeem *et al.*, 2005).

3.34.3.2 The Elephant Cerebral Cortex and Encephalization Quotient

As pointed out, the cerebral cortex of the elephant brain is the largest of all terrestrial animals and about three times larger than that of the human (Figure 1). The conventional approach to examining the relationship between brain size and higher-order brain functions is through calculation of the encephalization quotient (EQ), which is a representation of the ratio of brain volume to body mass (Jerison, 1973). Accordingly, the EQ of humans is 7.5, chimpanzees 2.5, Asian elephants 2.3, and the African elephant 1.3 (Jerison, 1973; Cutler, 1979). The markedly lower EQ of the African elephant reflects the fact that the brain is only slightly larger than that of the Asian but body mass is much greater.

3.34.3.3 Information Processing: Neurons of Elephants and Large-Brained Primates

Although going against the grain of conventionally profiling the relationship between EQ and cognitive ability, one could argue that more relevant for

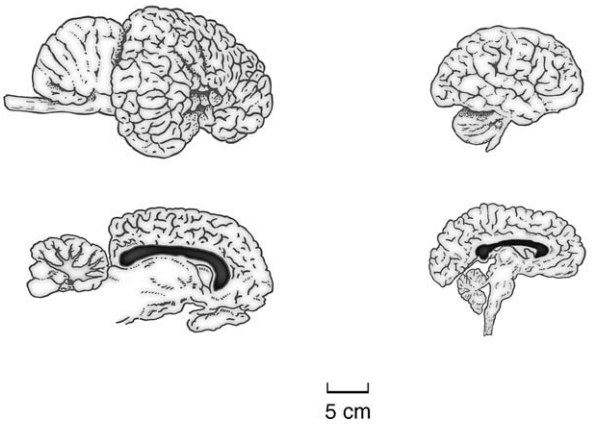

Figure 1 Lateral and midsagittal views of the elephant (left) and human (right) brain showing the relative sizes of cerebral hemispheres (top) and corpus callosum (solid black) in the midsagittal sections (bottom). The lateral views are drawn after images provided from the Comparative Mammalian Brain Collections with support from the National Science Foundation and the mid-sagittal views are drawn from magnetic resonance images from Hakeem *et al.* (2005). The scale is from Hakeem *et al.* (2005).

understanding behavioral capacities is the total amount of cerebral cortex which is available for higher-order brain functions. The cerebral cortex available for such functions is roughly represented by the volume of nonsensorimotor cortex, which may be calculated using a model of scaling cerebral cortical volume to body mass in primitive (marsupial) mammals, where almost all of the cerebral cortex is assumed to represent sensorimotor cortex (Hofman, 1982). One can then estimate the amount of sensorimotor cortex needed to support a given body mass in a large-brained species and subtract this from the total volume of cerebral cortex to arrive at the volume of nonsensorimotor cortex (Hofman, 1982). Accordingly, the chimpanzee is estimated to have 160 cm^3 of nonsensorimotor cortex, the human 660 cm^3, the Asian elephant 1800 cm^3, and the African elephant 1400 cm^3.

A greater volume of cerebral cortex does not necessarily translate into a greater number of information-processing neurons. The classical analyses by Tower (1954) introduced the concept of declining densities of neurons as cerebral cortex volume increases across species. Tower estimated neuron densities (rounded to nearest 1000 neurons per mm^3) of chimpanzees at 15000, humans at 9000, and Asian elephants at 7000. The more recent, and frequently cited, comparative study by Haug (1987), based on samples from multiple areas of the cerebral cortex, revealed much higher neuron densities for primates. Haug placed the approximate densities for chimpanzees at 41000, humans at 26000, and African elephants at 7000 per mm^3. What is noteworthy is that the very low density of neurons in the African elephant cortex in the Haug study was the same as that of the Asian elephant in the Tower study, perhaps reflecting the expectation that neuron density counts with various methodologies would be more similar with less dense neurons such as in the elephant.

Taking into account neuronal density based on data from Haug (1987), and cortical volumes based on data from Hofman (1982), we estimate the human brain to have about 17.4 billion neurons in the cerebral cortex compared with 12.6 billion for the elephant and 6.7 billion for the chimpanzee. A more updated technique of neuron counting reveals the human cortex to have about 20 billion neurons (range 19–23 billion; Pakkenberg and Gundersen, 1997). Because comparative data with the newer technique are not available for the elephant and chimpanzee, our comparisons use the Haug data, assuming the general picture would not change. One can roughly estimate the number of cortical neurons available for cognitive and long-term memory behavior by breaking down cortical volume into sensorimotor and nonsensorimotor volumes, so that the human brain is estimated to have about 17 billion neurons in the nonsensorimotor cortex, the elephant about 11 billion, and the chimpanzee about 6.5 billion.

With reduced neuron density, there is generally an increase in average neuron size. As reported by Haug (1987), human and chimpanzee cortical neurons were estimated to have a mean size of 1200 μm^3 with a high proportion of small neurons (granular cells), while the elephant has a mean neuron size of 4200 μm^3, and a size distribution toward large and very large neurons (pyramidal cells). Although not measured, one could assume that the cortical neurons of elephants have a greater number of synapses and interconnecting axonal ramifications than those of large-brained primates (Harrison *et al.*, 2002).

Cortical neurons interact with distant neurons of the cortex through the white matter which mostly comprises such transcortical axons (Zhang and Sejnowski, 2000). Several authorities have pointed out that across species cortical white matter increases disproportionately with cortical volume (Hofman, 1989, 2001; Allman, 1998; Zhang and Sejnowski, 2000; Bush and Allman, 2003). As the cerebral cortex increases in volume, a certain point is reached where interconnectivity is adversely impacted (Kaas, 2000; Hofman, 2001, 2003; Changizi and Shimojo, 2005). Also, because

transmission time along axons accounts for almost all of the time delay in information-processing time (Harrison *et al.*, 2002), another concern with large brains with extensive white matter is that information processing becomes prolonged.

3.34.3.4 Neuronal Interconnectivity and Information-Processing Efficiency: Primates versus Elephants

Ostensibly, to maintain an optimal level of interconnectivity, and a minimal information-processing time, cortical neurons of great apes and humans have evolved to become less global in transcortical connections and increasingly compartmentalized with more local circuit or modular connections (Kaas, 2000; Changizi and Shimojo, 2005). The manifestation of this is an increase in multiple cortical areas (Krubitzer, 1995), where the efficiency of interconnectivity and information processing within a subpopulation of neurons is maintained. One way of portraying this phenomenon across species is by calculating the proportion of neurons within a module that are local circuit neurons (LCNs), connecting only to other neurons in the same or adjacent modules (Hofman, 1985). Thus, in going from the chimpanzee to the human brain, where there is a threefold increase in cortical volume, LCNs increase from 93% to 98%.

In contrast to this continuum toward increasing compartmentalization and reduced global interconnections, which characterizes the evolution of large brains in primates, in elephants there appears to be a neural cytoarchitectural bias toward maintaining long-distance global connections and a reduced participation in local cortical areas or modules. Evidence for this comes from the above-mentioned study by Hofman (1985) on LCNs where, in going from the human to the elephant cerebral cortex, which is three times greater, LCNs actually decrease from 98% to 91% (Hofman, 1985).

With reduced axonal projections to local areas and a bias toward global connections in elephants, one would expect the disproportionate scaling of white- to gray-matter volume to be continued. Recent magnetic resonance imaging of an elephant brain reveals that the ratio of white to gray matter is in keeping with the disproportionate scaling principle (Hakeem *et al.*, 2005). In elephants, the corpus callosum, representing contralateral white-matter projections, is about twice the cross-sectional area of humans (Figure 1). Because the density of elephant cortical neurons is less than one-third that of humans, one interpretation of this finding is that a much larger proportion of cortical neurons in the elephant brain send axons through the white matter to distant cortical neurons than in the brains of comparison primates. Axons of large diameter conduct impulses much more rapidly than axons of small diameter, and would reduce information-processing time, but large axons occupy much more space (Swadlow, 2000) and this would reduce interconnectivity. If the axons were small, allowing for more interconnectivity, this would increase information-processing time. The size of axons comprising the elephant white matter is not yet known, but whether primarily large or small, there would appear to be a cost to information-processing efficiency.

3.34.4 Relating Brain Information Processing to Behavior

The large number of nonsensorimotor cortical neurons of elephants, second only to humans among terrestrial mammals, coupled with a bias toward maintaining global connections throughout the cerebral cortex, would appear to be related to their extraordinary capacity to acquire and retain long-term memory from a wide variety of social and spatial–temporal domains. The costs of maintaining transcortical global connections are prolonged information-processing time and less local circuit interconnectivity. These costs would appear to be related to the fact that elephants do not compare favorably even with great apes in the area of fast-action, fine-grained feats of cognitive behavior, even though great apes have about one-tenth the nonsensorimotor cortical volume of elephants.

While one may wonder if elephants have long-term social or spatial–temporal memory ability that exceeds that of humans, an interpretation along this line is difficult. Humans have at least 60% more cortical neurons available for higher-order brain functions. One could argue that humans have sufficient cortical white-matter projections for involvement of a large number of cortical neurons in transcortical communication, still leaving a high proportion for local circuit processing. Of course, humans cannot be compared with elephants in areas such as memory of acoustic or chemosensory ones where sensory capacities are vastly different. While examples of feats of long-term memory in humans comparable to that of elephants living in nature are generally not available, one cannot help but be intrigued by a rough parallel between the reports on the savant syndrome seen in a very small percentage of autistic patients who have extraordinary long-term and detailed memory abilities. Such exceptional skills are seen in the context of general, and sometimes severe, intellectual impairment (Miller, 1999).

Savants, previously referred to as *idiot savants*, have been of interest since the early 1700s (Heaton and Wallace, 2004). Savants are typically reported to have suffered some type of brain damage (Heaton and Wallace, 2004). One could conjecture that on occasion a certain type of brain damage may lead to neuronal regeneration and cortical reorganization of information-processing capacities along the lines typical of the normal patterns of elephants. Indeed, if one wanted to carry this conjecture further, one could say that, in comparison to great apes, large-brained elephants are intellectually impaired or 'idiots', but have skills in memory ability far exceeding that of 'normal' great apes.

Finally, the cytoarchitectural model of neural information-processing characteristic of large-brained primates should not be expected to apply to large brains that arise through a different evolutionary path. It is now clear that the evolution of elephants proceeded from aquatic rather than terrestrial mammalian lines (Glickman *et al.*, 2005). Conventional paleontological evidence, reinforced by mitochondrial DNA analyses (De Jong, 1998) and histological studies on the kidneys and testes, show that elephants and manatees (*Sirenia*) have a common aquatic ancestor unrelated to the ancestor of cetaceans. Thus, one can point to not only the influence of anatomy, physiology, and lifestyle to explain the evolution of differences in neural cytoarchitecture and information processing between elephants and primates, but a discontinuity in evolutionary development as well.

The perspective presented here is tentative and intended to bring together species differences in behavior of elephants and large-brained primates with recent information on the neural bases of the behavior. Clearly missing is more quantitative information on the three species of elephants, especially with regard to compartmentalization of the cerebral cortex and interconnectivity between adjacent areas versus distant cortical areas. For the time being, the available information offers a resolution to the paradox presented by the large, complex brain of elephants and the emerging picture of limitations in several aspects of cognitive behavior coupled with extraordinary ability in the area of long-term and extensive memory.

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http://brainmuseum.org – Comparative Mammalian Brain Collections.

3.35 The Evolution of the Dorsal Thalamus in Mammals

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Glossary

layers and subnuclei	Parts of nuclei sometimes differ somewhat in histological characteristics, connections, and neuron response characteristics to the extent that they are recognized as subnuclei or layers, while having enough features in common to include them in a nucleus. Sometimes subnuclei are called nuclei.
nuclear complex	Adjoining nuclei of related functions are sometimes grouped into a complex such as the pulvinar complex. In some instances, the nuclei of the complex may have differentiated from a single ancestral nucleus.
nucleus	A collection of neurons and other cells in the thalamus that are united by a common function. Nuclei have been historically identified in brain sections as groups of neurons that differ from surrounding thalamus in the packing of neurons, cell types, and other histological characteristics. Nuclei should also differ in connections and, of course, the response properties of their neurons.
thalamus	A part of the forebrain between the cerebral cortex and midbrain. This review concerns the dorsal thalamus, the division that is largest in mammals, and projects to neocortex.

3.35.1 Introduction

While the region of the diencephalon called the thalamus includes the ventral thalamus, the hypothalamus, and the epithalamus, authors commonly use the term to refer to the dorsal thalamus only, the topic of this review. The dorsal thalamus of mammals is a collection of nuclei in the diencephalon with neurons that project to neocortex. If the neocortex is removed, the projection neurons die, leaving the nuclei of the dorsal thalamus severely degenerated, while the nuclei of the ventral thalamus, hypothalamus, and epithalamus remain intact or slowly respond to changes in the dorsal thalamus (Rose and Woolsey, 1943). In this way, the dorsal thalamus can be experimentally distinguished from other parts of thalamus. This is not to say that all of the neurons of the dorsal thalamus project to neocortex, as there are many intrinsic neurons as well, and a number of neurons project to the striatum (Jones, 1985), a major target of some of the nuclei of the dorsal thalamus of the reptilian ancestors of mammals. The major steps in the evolution of the thalamus in vertebrates, and the transition from the thalamus of reptiles to that of mammals, have been discussed in this series and elsewhere (Butler, 1994; Puelles, 2001; see The Dual Elaboration Hypothesis of the Evolution of the Dorsal Thalamus, Field Homologies, Evolution of the Nervous System in Reptiles). Therefore, this review focuses on the specializations of nuclei of the mammalian thalamus as the various branches of the mammalian radiation lead to the over 4500 extant species (Wilson and Reeder, 1993). Of course, there have been few or no observations on the thalamus of most of these species, so the concentration is necessarily on the thalamic nuclei of the few well-studied taxa.

Jones (1985) defined a thalamus nucleus as "a circumscribed region of cytoarchitecture receiving a particular set of afferent connections and projecting

within the borders of a particular field or fields." To elaborate on this definition, a nucleus is a collection of neurons and other cells that are unified by participating in a common function or set of functions. In order to do this, neurons within a nucleus require a unique set of inputs and outputs, and a great number of other specializations are possible as well, including neurons with distinctive morphological and histochemical properties. Nissl stains may reveal nuclei distinguished by neurons of distinctive sizes and staining properties, and this has been the traditional approach toward defining and identifying nuclei. In current investigations, nuclei are often identified with more assurance by differences in the expression of neurotransmitters and other components of neurons. Nevertheless, a major problem in comparative studies of thalamic organization is in reliably distinguishing nuclei. Identifying nuclei that are poorly or differently differentiated in various taxa can be difficult and result in errors. Regions of the thalamus can be misidentified as nuclei or misnamed, a problem confounded by the lack of a standard nomenclature. Homologous nuclei are not only given different names in the thalamus of birds, reptiles, and mammals, but different names in different mammals, or even in the same mammal by investigators that favor either one or another name. Another problem is distinguishing parts of nuclei from subnuclei. For example, the ventroposterior medial (VPM) nucleus is histologically distinguishable from the ventroposterior lateral (VPL) nucleus, but both are parts of the same functional unit, the ventral posterior nucleus, that contains a systematic representation of the cutaneous receptors of the contralateral half of the body. The ventroposterior (VP) nucleus also goes by several other names, including the ventrobasal nucleus. Finally, there is the problem of identifying nuclei that are present in some mammals and absent in others. There is generally a reluctance to identify any brain structure as new (Striedter, 2005), because it is difficult to determine if an easily identified structure in some taxa is not present in some cryptic form in other taxa. A similar dilemma exists with regard to the evolution of cortical fields, but it has gradually become clear that some areas of primate neocortex, for example, have no apparent homologues in other mammals (e.g., middle temporal (MT) visual area of primates; see Kaas and Preuss, 1993). It seems likely that some thalamic nuclei have evolved in some branches of the mammalian radiation but not in others. The evidence has become very strong that early mammals had few cortical areas, and the number of areas has increased independently in several lines of mammalian evolution by adding new areas. A comparable pattern of evolution must have occurred for the dorsal thalamus of mammals, with the thalamus adding new nuclei as the cortex added areas, although not necessarily in a matching matter. But less is certain about the thalamus, as the organization of the mammalian thalamus has been less intensively investigated. Thus, this review starts by considering the well-defined thalamic nuclei and how they vary across taxa, and then addresses the issue of increased complexity and new nuclei. We start with the visual relay nucleus, the dorsal lateral geniculate nucleus (LGN) (called dorsal to distinguish it from the ventral lateral (VL) geniculate nucleus of the ventral thalamus), often simply identified as the LGN. As the neuroanatomist Rose (1971) noted, "In the dorsal thalamus itself our anatomical and functional knowledge is at its best when it concerns the projection nuclei of the great afferent systems." The situation remains much the same today (see Mosaic Evolution of Brain Structure in Mammals, The Evolution of Neuron Classes in the Neocortex of Mammals, Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals, Do All Mammals Have a Prefrontal Cortex?, The Evolution of the Basal Ganglia in Mammals and Other Vertebrates, The Dual Elaboration Hypothesis of the Evolution of the Dorsal Thalamus).

3.35.2 The Lateral Geniculate Nucleus

The dorsal LGN is a thalamic structure common to all mammals. The LGN receives inputs from the ganglion cells of the retinas of both eyes, and has neurons that project to primary visual cortex (area 17 or striate cortex). This is a pattern that has been retained from the reptilian ancestors of mammals: extant reptiles such as turtles have a small but distinct dorsal LGN with retinal input and projections to dorsal cortex, the homologue of mammalian neocortex (Hall and Ebner, 1970b; Hall *et al.*, 1977; Ulinski, 1986; Zhu *et al.*, 2005). The LGN is located on the lateral margin of the thalamus, where it is innervated by axons coursing in the optic tract as other axons and collaterals of axons continue on to the superior colliculus of the midbrain. Across mammals, the LGN differs greatly in histological appearance, from a scattered group of neurons that is only marginally distinct from the adjoining thalamus, to a well-segregated and variously laminated structure.

A common form of the LGN, found in some members of most of the major branches of the mammalian radiation, is a rather undifferentiated nucleus with no obvious substructure, such as the LGN of a hedgehog (Figure 1). This nucleus is characterized by a nearly uniform distribution of

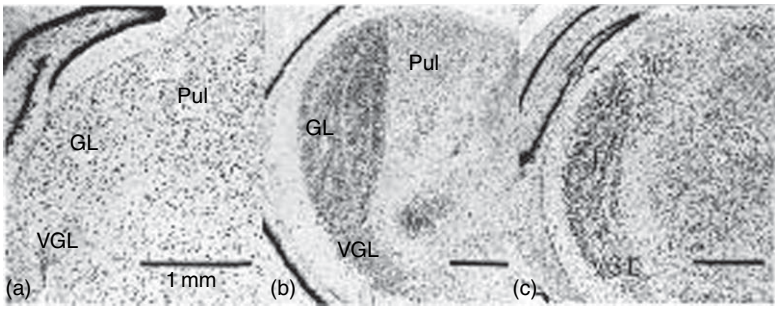

Figure 1 A coronal brain section stained for cells (Nissl preparation) through the dorsal lateral geniculate nucleus (GL), the ventral lateral geniculate nucleus (VGL), and the pulvinar (Pul) of, a, hedgehog, a small insectivore; b, a tree squirrel; and c, a tree shrew. Note that the nuclei are poorly differentiated from each other in the hedgehog, but well differentiated from each other in the squirrel and the tree shrew. Note also that the dorsal lateral geniculate nucleus has visible but different types of layers in the squirrel and tree shrew, and that no visible layers are seen in the nucleus in the hedgehog. The pulvinar is also more differentiated in squirrels and tree shrews. Scale bar: 1 mm.

neurons that are not very different in appearance and distribution from those in adjoining parts of the thalamus. Nevertheless, there is a concealed lamination, as retinal projections from the ipsilateral eye occupy a dorsocentral oval in the nucleus surrounded by terminations from the contralateral eye (Hall and Ebner, 1970a). Thus, the LGN of hedgehogs appears to have a middle layer with inputs from the ipsilateral eye, and adjoining layers with inputs from the contralateral eye. The layer for the ipsilateral eye does not extend into the most ventral third of the nucleus because that is where the monocular visual field of peripheral vision of the contralateral eye is represented (Kaas *et al.*, 1972). A similar LGN is found in Afrotherian tenrecs (Künzle, 1988), North American opossums of the marsupial radiation (Royce *et al.*, 1976), some rodents such as rats (Reese, 1988), rabbits (Holcombe and Guillery, 1984), pangolins (Lee *et al.*, 1991), and echidnas and platypuses of the monotreme radiation (Campbell and Hayhow, 1971, 1972). Such a distribution of a poorly differentiated LGN with perhaps three cryptic layers argues that this type of LGN was present in early mammals and was retained in many branches of the mammalian radiation. As most early mammals were small and likely nocturnal (Kielan-Jaworowska *et al.*, 2004; Rose and Archibald, 2005; however, see Martin, 2006), a large, highly differentiated visual thalamus would not be expected, and the present-day mammals with a poorly differentiated visual thalamus are those mammals that are not highly visual.

In contrast to the mammals noted above, the LGNs of the highly visual mammals that have been studied have an architectonic appearance that is distinct from the adjoining thalamus, and typically have several layers of two or more types. Thus, squirrels, a highly visual rodent, have a large LGN of darkly staining neurons that has three visible layers. These layers are separated from each other by cell-poor septa (Figure 1), with one of these layers subdivided by a segregation of retinal inputs into a middle layer of ipsilateral retinal inputs, and two adjoining layers of contralateral retinal inputs, making five layers in all (Kaas *et al.*, 1972). As another visually dominated mammal, tree shrews, are small, squirrel-like mammals that are closely related to primates (Kaas, 2002), tree shrews have a clearly laminated LGN, but in a different pattern of six layers that are separated by cell-poor septa (Figure 1). The layers are further distinguished by inputs from either the ipsilateral or the contralateral eye, histochemical characteristics, and innervation by different types of retinal ganglion cells, including the ON and OFF and W-cell pathways (Conway and Schiller, 1983; Conley *et al.*, 1984; Diamond *et al.*, 1993). ON ganglion cells are those that respond to an increase of light in the excitatory receptive field (light onset), while OFF ganglion cells respond to the dimming of light (light offset). The W-cell pathway includes ganglion cells with thin axons and slow conduction, possibly homologous with the K pathway of primates (see below).

Other visual mammals also have complexly differentiated LGNs but of various types. Sanderson *et al.* (1984, 1987) have described the LGN of some of the diprotodont marsupials, an advanced order including kangaroos, wombats, koalas, and varieties of possums. The LGN of these marsupials includes a visibly laminated segment with three cytoarchitectonic regions, some of which subdivide further, and a segment without visible lamination, which is subdivided by regions of inputs from either the ipsilateral or contralateral eye. Across species, the number of eye-specific layers varies from 8 to

11. The LGN of different ungulates has been described as consisting of three to five layers (see for review, Sanderson *et al.*, 1984; Clarke *et al.*, 1988). Carnivores are generally described as having two dorsal layers: layer A for the contralateral eye, layer A1 for the ipsilateral eye, and three ventral C (smaller cell) layers (Kaas *et al.*, 1972, 1973). In addition, mink and weasels of Mustelidae taxon of carnivores have duplicated their A and A1 layers with one A and one A1 layer for ON retinal ganglion cells (those responding to light onset) and one A and one A1 layer for OFF ganglion cells (those responding to light offset) (Sanderson, 1974; LeVay and McConnell, 1982). The projections from the ON and OFF ganglion cells are mixed in the A and A1 layers of other carnivores. The echolocating bats typically have poorly differentiated visual systems with a simple LGN, while the crepuscular fruit-eating megabats have large eyes and an LGN of five or six layers (see Kaas and Preuss, 1993, for review). The LGN of gliding lemurs, considered close relatives of primates, appears to have six layers (Kaas *et al.*, 1978; Kaas and Preuss, 1993).

Primates are highly visual mammals, and this is reflected in the LGN (Kaas *et al.*, 1978). The basic lamination pattern (Figure 2) consists of two parvocellular layers (one for each eye), with inputs from a class of retinal ganglion cells (P cells) that are specialized for detailed object vision and color, and two magnocellular layers (one for each eye), with inputs from the M-cell class of ganglion cells that are important for motion detection and vision in dim light. In addition, small koniocellular neurons are sometimes recognized as scattered within the septal zones between layers or as forming distinct layers. In prosimian primates, two thick koniocellular layers are generally recognized (Figure 3), while thinner distributions of K cells are only sometimes recognized as layers. In addition to the layers noted above, the parvocellular layers subdivide to form four or more parvocellular layers or sublayers in some taxa of anthropoid primates (Kaas *et al.*, 1978). In general, the magnocellular and koniocellular layers are well developed in nocturnal primates, while the parvocellular layers are well developed in diurnal primates. Thus, the laminar pattern is complex and variable across primate taxa.

Other features of the LGN also vary across mammals. In general, primary visual cortex is the main target of LGN projection neurons, with very few projections to other visual areas. But this too is one of the variable features. In cats and at least some other carnivores, a major projection of one class of LGN neurons is to the second visual area,

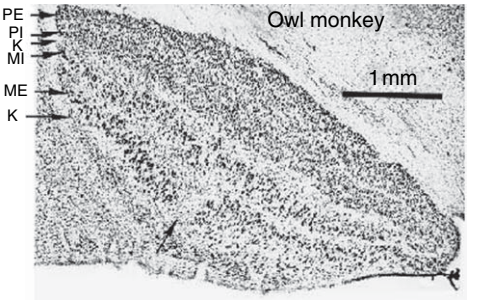

Figure 2 A parasagittal brain section through the dorsal LGN of an owl monkey. Owl monkeys are the only nocturnal monkeys, and this is reflected in the proportionately small parvocellular layers. Note that the external parvocellular layer (PE) is only marginally segregated by a cell-poor septum from the internal parvocellular layer (PI). Nevertheless, it is apparent that PE extends to the rostral pole of the nucleus (right) while PI does not, as PE represents the complete contralateral visual hemifield via the contralateral eye, while PI represents the slightly smaller binocular hemifield via the ipsilateral eye. The neurons of the magnocellular layers, MI (internal) and ME (external), are noticeably larger. ME, with input from the contralateral eye, also extends to the rostral pole of the nucleus, while MI, with input from the ipsilateral eye, does not. Koniocellular (K) layers of the smallest neurons are not commonly reorganized in anthropoid primates, although two K layers are widely recognized in nocturnal prosimian primates. In anthropoid primates, the two K layers noted here are well differentiated, and expanded in owl monkeys, suggesting that K layers are especially important for vision in dim light. The arrow pointing to the middle of ME indicates a small discontinuity in the layer that corresponds to the receptor-free oval of the optic disk (nerve head) of the contralateral eye (a discontinuity also occurs in PE of nearby brain sections). Such discontinuities are only apparent in mammals with good visual acuity. Modified from Kaas, J. H., Guillery, R. W., and Allman, J. M. 1973. The representation of the optic disc in the dorsal lateral geniculate nucleus: A comparative study. *J. Comp. Neurol.* 147, 163–180.

V2, and projections from another class of LGN cells extend across four visual areas (Stone, 1983). Some of the LGN neurons in primates also project to nonprimary visual areas in primates (Stepniewska *et al.*, 1999), including to visual areas such as the MT visual area that appear to be unique to primates. Thus, as new visual areas emerged in early primates or the immediate ancestors of primates, LGN projection patterns were altered to include these visual areas.

In summary, the poorly differentiated LGN with little substructure of early mammals appears to have differentiated in several ways in a number of branches of the mammalian radiation. There have been independent increases in numbers of cell layers. These layers appear to be segregating inputs from functionally different classes of retinal ganglion cells, inputs from the superior colliculus, and inputs from the ipsilateral and contralateral eyes. Some of the resulting segregations are similar,

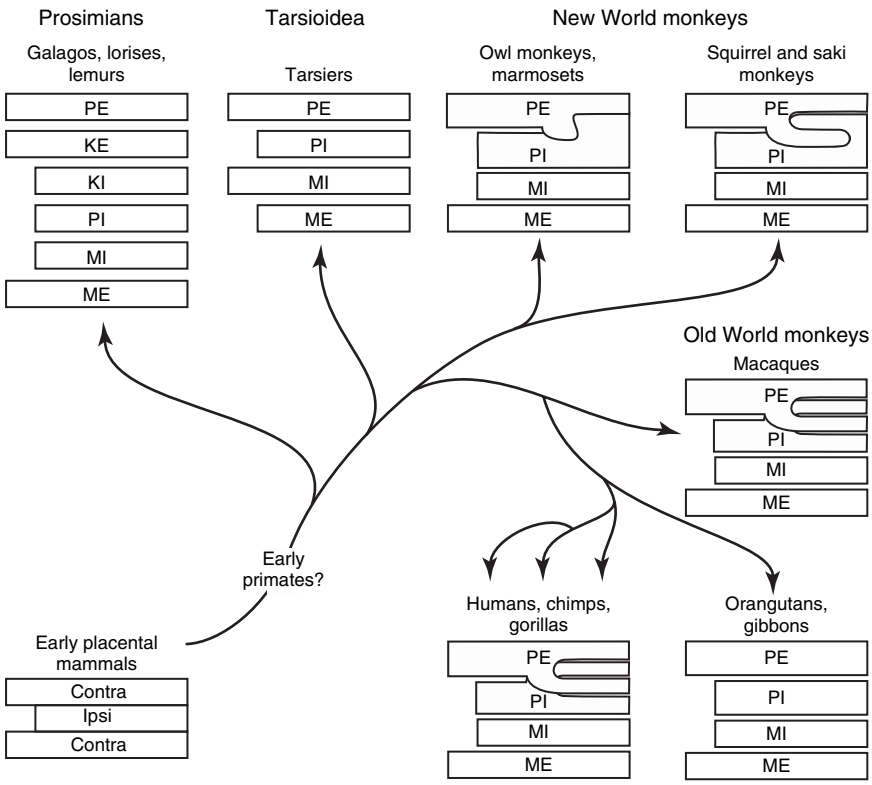

Figure 3 A schematic of the lamination pattern of the dorsal LGNs of primate taxa. Layers are labeled by the type of neurons that they contain (P, parvocellular layers; M, magnocellular layers; K, koniocellular layers) and their internal (I) or external (E) position in the nucleus. Short layers receive inputs from the ipsilateral eye and long layers from the contralateral eye. In some taxa, P layers subdivide and interdigitate, while in owl monkeys and marmosets, there is only a hint of this. Spaces between layers mean that the layers are well separated by septa. Note that the lamination pattern varies across primate taxa. Modified from Kaas, J. H., Huerta, M. F., Weber, J. T., and Harting, J. K. 1978. Patterns of retinal terminations and laminar organization of the lateral geniculate nucleus of primates. *J. Comp. Neurol.* 182, 517–554.

although independently derived, such as layers for ON and OFF ganglion cells in some carnivores and in tree shrews. Other types of segregations of neurons into layers may be unique, as there is much structural diversity. What this means in terms of acquired functions is not completely clear, but highly visual mammals have more distinct layers and more layers, and nocturnal mammals emphasize different types of layers than do diurnal mammals. It is also important to remember that changes were not always in the direction of increased differentiation. The LGNs of the microphthalmic blind mole rats, for example, have greatly regressed (Cooper *et al.*, 1993).

3.35.3 The Visual Pulvinar

The pulvinar in primates is a complex of nuclei that are largely, but not completely, visual in function. Because this part of the thalamus was so prominent in primates, most early comparative neuroscientists considered the pulvinar to be unique to primates, and a homologous region was not recognized in other mammals. Instead, this part of the visual thalamus was thought to be the homologue of another part of the primate thalamus, the lateral posterior nucleus, which in primates is generally associated with the somatosensory system. Le Gros Clark (1932) was one of the first to recognize that "the pars posterior of the lateral nucleus is the homologue of at least part of what, in higher primates, is termed the pulvinar." Although evidence accumulated in support of considering parts of the primate pulvinar complex homologous with parts of the lateral posterior complex in other mammals, the use of the term lateral posterior to refer to the pulvinar in nonprimate mammals has usually, but not always, continued. To add to the confusion, both terms have been used in the same mammal to refer to different parts of the pulvinar complex, as is currently done in cats. This confusion of terms has undoubtedly hindered comparative studies of the organization, and studies of the visual thalamus in mammals. Here the term visual

pulvinar is used in all mammals to designate those parts of the thalamus that receive inputs from the superior colliculus and/or are reciprocally connected with visual areas of cortex.

The reptilian homologue of the mammalian pulvinar complex, if any, is somewhat uncertain. In both reptiles and birds, the optic tectum (the homologue of the superior colliculus) projects to a well-defined nucleus rotundus of the dorsal thalamus (see Belekhova *et al.*, 2003; for review). For some time, a homology of the mammalian pulvinar complex with nucleus rotundus has been widely accepted, but recently nucleus rotundus has been compared to the intralaminar complex of mammals (see Belekhova *et al.*, 2003, for review). While the issue remains unresolved, the bulk of the evidence appears to favor the long-standing view of a rotundus–pulvinar homology. In support of this view, Major *et al.* (2000) have provided evidence that the tectorotundal and tectopulvinar pathways are homologous to the level of even involving the same cell subtypes.

In early mammals, the pulvinar complex appears to have consisted of a rather poorly differentiated group of cells in the posterior thalamus, just medial to the LGN, much as in present-day hedgehogs (Figure 1a). In such mammals, this poorly differentiated group of neurons receives inputs from the superior colliculus and projects to visual cortex, including primary visual cortex and the second area, V2. Quite possibly two or three regions or nuclei can be distinguished based on differences in cortical and superior colliculus connections (Crain and Hall, 1980). Overall, this caudal portion of the thalamus receives superior colliculus inputs and projects to visual cortex in all mammals studied, but the size and histological differentiation of the region differ considerably. Thus, a large pulvinar nucleus is easily identified histologically in both squirrels and tree shrews (Figure 1), and has expanded and become more differentiated than in most mammals. Moreover, in both tree shrews and squirrels, the pulvinar is not a single nucleus, but a complex of several nuclei differing histochemically and in connections. Thus, in tree shrews the pulvinar complex consists of four nuclei (Figure 4a) (Lyon *et al.*, 2003a, 2003b). A large central nucleus (Pc) receives a retinotopic pattern of projections from the superior colliculus and projects to primary visual cortex and adjoining visual areas. A smaller dorsal nucleus (Pd) receives diffuse projections from the superior colliculus, stains darkly for acetylcholinesterase, and projects to higher-order visual areas of the temporal lobe. A small ventral nucleus (Pv) expresses high levels of the antigen for the Cat-301 antibody that identify large neurons with rapidly conducting axons, is interconnected with primary visual cortex and adjoining visual areas, while having few or no inputs from the superior colliculus. A small posterior division of the pulvinar complex (Pp) appears to receive diffuse inputs from the superior colliculus while projecting to higher-order visual areas in temporal cortex. The large pulvinar of squirrels (Figure 1c) is subdivided into at least three nuclei (Figure 4b), two with superior colliculus inputs, but of different types, and one without. All three divisions differ in their connections with cortical visual areas (see Lyon *et al.*, 2003b, for review).

The pulvinar complex has been extensively studied in domestic cats, which have divisions of a lateral posterior complex as well as a pulvinar (Figure 4c). A large nucleus receives inputs from the superior colliculus while projecting to temporal visual areas. This nucleus expressed high levels of acetylcholinesterase and the neurotransmitter, substance P. Another large nucleus is interconnected with primary and secondary visual areas, and a third nucleus projects to visual areas of temporal cortex. Monkeys have even more subdivisions of the pulvinar complex, which is large and dominates the posterior thalamus (Figure 4d). The classical inferior pulvinar consists of four nuclei differing in histochemical characteristics and patterns of cortical connections. Two of these nuclei have dense inputs from the superior colliculus. The large lateral pulvinar has two divisions, differing in connections and patterns of retinotopic organization. The traditional medial pulvinar and anterior pulvinar have nonvisual connections, and are not subdivisions of the visual pulvinar.

The point of this brief and limited survey is that mammals in different lines of evolution have independently increased the differentiation and complexity of the pulvinar complex. The pulvinar of early mammals was probably a relatively undifferentiated mass of cells in the posterior thalamus that included one or two zones with superior colliculus inputs, and possibly a zone without such inputs, but with visual cortex connections. This ancestral condition is reflected in the posterior thalamus of many extant mammals with poorly developed visual systems. However, in some lines of descent, more nuclei appeared, but in different patterns and arrangements. As a result, some or most pulvinar nuclei will only be present in some taxa. However, it may be possible to homologize a few specific pulvinar nuclei across taxa. For example, a region of the pulvinar complex that receives the class of superior colliculus projections that

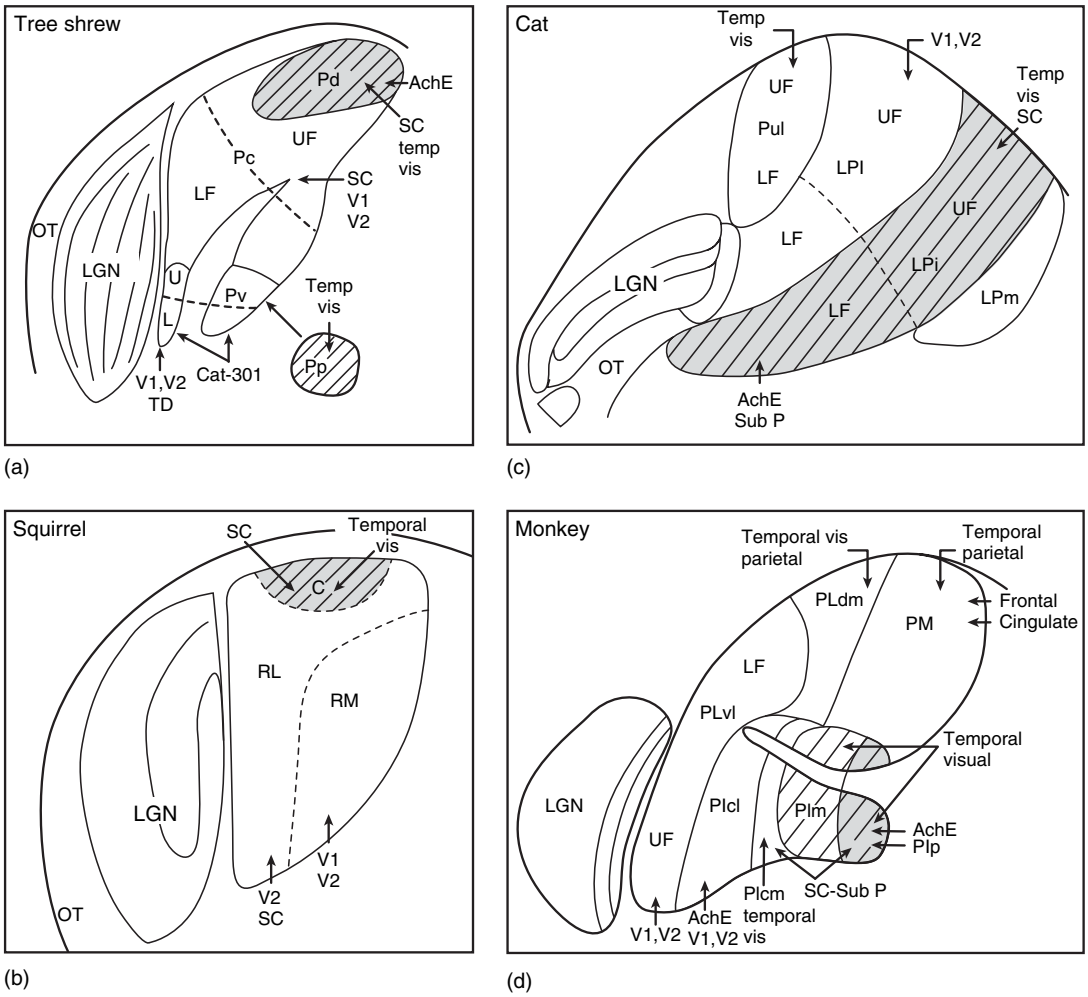

Figure 4 The organization of the pulvinar complex in four mammalian taxa. The drawings are of coronal brain sections through the caudal thalamus where the lateral border is defined by the optic tract (OT). a, In tree shrews, the four subdivisions or nuclei of the pulvinar complex include a large central pulvinar (Pc) with inputs from the superior colliculus (SC) and projections to visual areas V1 and V2, as well as a temporal dorsal (TD) visual area. Pc forms a systematic representation of the contralateral visual hemifield with the upper field (UF) represented dorsal to the lower field (LF). A dashed line approximates the zero horizontal meridian. The ventral pulvinar (Pv) also contains representations of the upper (U) and lower (L) hemifields, while expressing the antigen for the Cat-301 antibody and projecting to V1, V2, and TD. A posterior pulvinar (Pp) lies behind Pv and Pc, as indicated by an arrow, and projects to visual cortex of the temporal lobe. A dorsal pulvinar (Pd) expresses acetylcholinesterase (AchE), receives diffuse inputs from the SC, and projects to temporal visual cortex. b, In squirrels, only rostrolateral (RL), rostromedial (RM), and caudal (C) divisions of the pulvinar have been distinguished by differing connection patterns (abbreviations as in (a). c, In cats, one division of the pulvinar complex is called the pulvinar (Pul), while three others are called lateral (LPl), intermediate (LPi), or medial (LPm) nuclei of the lateral posterior complex. Note that three nuclei form separate representations of the visual hemifield, that nuclei differ in connections, and that LPi is distinguished by expressing AchE and the neurotransmitter, substance P (Sub P) that is expressed in one class of inputs from the SC but not others. d, Monkeys and other primates have a pulvinar complex that includes central lateral (PIcl), central medial (PIcm), medial (PIm), and posterior (PIp) nuclei of the inferior pulvinar, and ventrolateral (PLvl) and dorsomedial (PLdm) nuclei of the lateral pulvinar. The medial pulvinar (PM) is not completely visual and the anterior pulvinar (not shown) is somatosensory in function. Note that nuclei differ in connections and expression of AchE and Sub P. PLvl has the most precise representation of the contralateral visual hemifield, but other representations are in nuclei of the inferior pulvinar. Reproduced from Lyon, D. C., Jain, N., and Kaas, J. H. 2003b. The visual pulvinar in tree shrews. II: Projections of four nuclei to areas of visual cortex. *J. Comp. Neurol.* 467, 607–627, with permission.

express substance P may be revealed by further study to be homologous in a wide range of mammals (Hutsler and Chalupa, 1991; Stepniewska *et al.*, 2000). A broader survey of extant mammals with well-developed visual systems could usefully extend our appreciation of the variety of specializations that have emerged in pulvinar organization, and comparisons within and across taxa could provide an understanding of how specializations emerged.

3.35.4 The Somatosensory Thalamus: The Ventroposterior Complex and the Adjoining Posterior Complex

In all studied mammals, a VP nucleus can be identified by its characteristic position in the ventral thalamus, its inputs from the dorsal column and trigeminal somatosensory brainstem nuclei, and its projections to primary somatosensory cortex, S1 (area 3b) (Jones, 1985; Kaas and Pons, 1988). Commonly, investigators have divided VP into VPM and VPL. However, these represent only major divisions of VP, as VPM receives inputs from the trigeminal nuclei for the face, oral cavity, and head, while VPL receives inputs from the gracile nucleus for the lower body (hindlimb and tail) and the cuneate nucleus for the upper body (forelimb and trunk). Note, by the same logic, the trigeminal, cuneate, and gracile 'nuclei' are subnuclei. These inputs to VP via the medial lemniscus provide cutaneous receptor information about touch on the skin and hair movement. Other somatosensory afferents involving a larger range of modalities including touch, pain, and temperature course in the spinothalamic pathway (and the equivalent component of the trigeminal complex) to terminate in and around VP, sometimes including a segregated nucleus on the ventral margin of VP, the VP inferior (VPI) nucleus. Another collection of small brainstem subnuclei, including the external cuneate nucleus, relays proprioceptive information, mainly from muscle spindle receptors, to the region of VP in the thalamus. In some mammals (see below), the proprioceptive inputs are now recognized as terminating within a separate cell group on the dorsorostral margin of VP, termed in primates the VP superior (VPS) nucleus (Krubitzer and Kaas, 1992).

Adjoining VP along its dorsocaudal margin, a poorly differentiated region termed the posterior nucleus or posterior group contains neurons that respond to somatosensory, but also auditory and visual stimuli. In addition, in primates, an anterior pulvinar nucleus is recognized just dorsal to part of VP. This nucleus is interconnected with subdivisions of somatosensory cortex while receiving little or no other sources of sensory inputs (Kaas and Pons, 1988). Finally, a taste nucleus (with much broader functions), the parvocellular ventroposterior medial nucleus (VPMpc), receives gustatory, tactile, and visceral information from the brainstem, and projects to somatosensory and adjoining cortex (Kaas *et al.*, 2006b). At least some of these subdivisions of the mammalian somatosensory thalamus seem to be common to all mammals, and therefore they must have originated with or before the first mammals and diversified in various ways.

The stem reptilian amniotes that gave rise to modern reptiles, birds, and mammals likely had features of the dorsal thalamus that have been retained in all three groups. In present-day reptiles and birds, somatosensory inputs relayed from the dorsal column nuclei reach a nucleus and a perinuclear region in the dorsal thalamus. These inputs in turn project to the rostral part of the dorsal cortex of reptiles or Wulst of birds (Wild, 1997; Medina and Reiner, 2000), forming much of the evidence for the conclusion that a homologue of at least the mammalian VP nucleus was present in the common ancestor. Stem reptiles may also have had some segregation of proprioceptive and spinothalamic inputs in the thalamus, but this seems uncertain. Gustatory and related sensory inputs may have been segregated in the ventral thalamus, but the dorsal thalamus and the pallium were probably not the important processing centers for gustatory inputs (Finger, 1997; Pritchard and Norgren, 2004).

The mammalian VP nucleus can be identified by its ventral position and histological characteristics in all examined extant mammals. In some mammals, such as opossums (Pubols and Pubols, 1966; Donoghue and Ebner, 1981; Wild, 1997) and hedgehogs (Erickson *et al.*, 1967), the neurons stain somewhat darker in Nissl preparations, and they are grouped to form an identifiable nucleus, but the nucleus is only marginally different from the adjoining thalamus (see Ebbesson *et al.*, 1972 for review). The VPM subnucleus forms the largest component of the VP nucleus in most mammals. This observation suggests that, in many taxa of extant mammals, the important somatosensory information came from the whiskers of the face, the nose, and the tactile receptors of the mouth. A comparative analysis suggests that VP in early mammals was poorly differentiated, and was dominated by a large representation of the face and oral cavity within the medial division of the nucleus. As with most studied extant mammals, VP of early mammals projected not only to primary somatosensory cortex, S1, but also to a second somatosensory area, S2, and possibly to a more recently defined somatosensory area, the parietal ventral area, PV (Kaas, 2004). This basic VP has been modified in mammalian evolution in many ways to accommodate changes in somatosensory receptor distributions and functions.

One of the variables in the organization of VP is in the proportions of the nucleus that are devoted to representing different body parts. For example, rats, mice, and many other rodents devote much of VP to representing the inputs from receptors

associated with their facial vibrissae, as they use these vibrissae extensively as they explore their tactile world (Woolsey *et al.*, 1974). In a similar manner, the star-nosed mole, with its unique fleshy appendages of the nose as its major tactile organ, has much of VP devoted to the receptors of those nose appendages (Catania and Kaas, 1996). In the naked mole rat, a rodent that uses its enlarged front teeth, the incisors, to carry and manipulate food, young, and other objects, approximately one-third of VP represents the teeth (Catania and Remple, 2005). Primates (Kaas *et al.*, 1984) and raccoons (Welker and Johnson, 1965) have disproportionately large representations of their glabrous forepaws, as they use this skin surface to explore the environment. In addition, spider monkeys have a prehensile tail with a sensitive glabrous ventral surface near the tip, and this tail has a large representation in VP (Pubols, 1968). Bats have an enlarged representation of the tactile receptors of the wing that are used to guide flight (see Somatosensory Adaptations of Flying Mammals). Thus, the proportion of VP devoted to representing different body parts has been adjusted in both different and sometimes parallel ways in the various branches of mammalian evolution (Welker, 1973).

Another feature of VP that varies across species is the degree to which subnuclei are histologically obvious. In species where VP is histologically more distinct from the adjacent thalamus, cell-poor septa of fibers are typically present between highly innervated positions of the receptor sheet (skin) that are discontinuous but represented in VP next to each other. In primates, the glabrous foot, hand, and the face are separated from each other in VP by fiber bands into subnuclei for the head (VPM), hand, and foot (Kaas *et al.*, 1984). Often a subnucleus can be distinguished for the tail as well, and in some instances thin septa separating the representations of the digits in the hand representation can be detected. Fiber septa separating the representation of individual digits are even more obvious in VP of raccoons (Welker and Johnson, 1965; Wiener *et al.*, 1987a). The star-nosed mole has fiber bands that separate subnuclei in VP for each of the 11 long appendages on each side of the nose (Kaas and Catania, 2002). Rats and mice have visible subnuclei (barreloids) for each of the long sensory mystacial vibrissae on the side of the face (Akers and Killackey, 1979; Haidarliu and Ahissar, 2001). Other instances of visible subnuclei in VP have been reviewed by Welker (1973), and they appear to reflect a developmental mechanism that tends to segregate groups of neurons with different patterns of activation (Kaas, 1982; Kaas and Catania, 2002).

The connections of VP have also been altered in evolution. For example, the primitive mammalian pattern of cortical projections was to S1 and S2 (Kaas, 2004). The projections to S2 were lost in anthropoid primates, while a dense projection emerged in area 1 along the caudal border of area 3b (S1). A sparse but notable projection of VP to area 2, just caudal to area 1, developed in macaque monkeys and perhaps other catarrhine primates (Kaas, 2004). While VP independently activates S1 (area 3b) and S2 in most mammals, including prosimian primates, VP activates area 3b (S1 proper) and modulates areas 1 and 2 in catarrhine primates, reflecting the important roles of area 1 and 2 in these primates, and increased emphasis on higher-order processing in S2.

The VPI nucleus is a nucleus that has been described as only distinct in the primate brain (Jones, 1985), although it is also quite distinct in raccoons (Herron, 1983). In these mammals, VPI is composed of small, pale-staining neurons grouped just ventral to VP (Figure 5). VPI receives inputs from the spinothalamic somatosensory pathway and projects densely to the second somatosensory area, S2, as well as less densely to other somatosensory areas, including S1 (area 3b). Because similar connections exist for the cell-poor fiber septa that subdivide VP into subnuclei, and these septa have small pale-staining neurons, it seems likely that VPI is a nucleus that became largely segregated from VP in primates and raccoons, but much less so or not at all in other mammals (Krubitzer and Kaas, 1992). Thus, VPI may be an example of a new thalamic structure that arose independently via the process of differentiation and segregation in primates and raccoons from a mixed-cell population in ancestral VP. In both primates and raccoons, this birth of a new nucleus may be related to the increased use of the hand in skilled movements (Iwaniuk and Whishaw, 1999).

The VPS nucleus is another somatosensory nucleus with an uncertain history in mammalian evolution. In early studies of the primate thalamus, VPS was often included in VP. However, the recognition of VPS as a separate nucleus (Kaas *et al.*, 1984) was motivated by the evidence that it receives inputs from a brainstem relay of deep body (muscle spindle) receptors rather than cutaneous receptors. VPS forms a separate somatotopic representation of body receptors, but of deep rather than cutaneous receptors, as in VP. VPS is histologically distinct from VP in prosimians and New World monkeys, but not as distinct in Old World monkeys. VPS projects to area 3a and 2 in monkeys, rather than

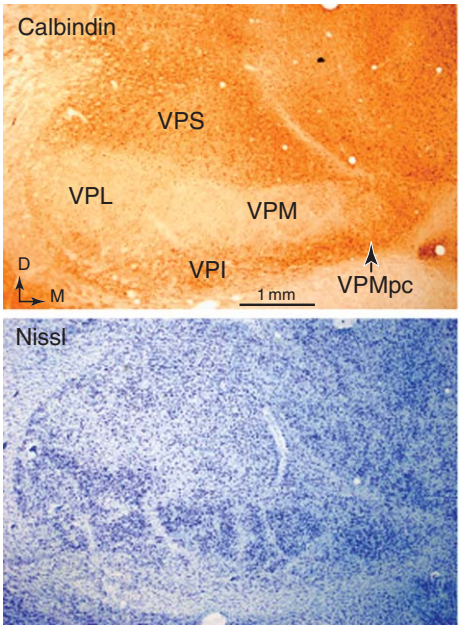

Figure 5 Above: Coronal brain section through the ventroposterior complex of a galago, a prosimian primate, which has been processed for the calcium-binding protein, calbindin. The ventroposterior, VP, nucleus, has two traditional subdivisions, the VPL and VPM, separated by a cell-poor septum. In this preparation, VP (VPL plus VPM) stands out as a light calbindin-poor region. Just ventral to VP, the ventroposterior inferior nucleus expresses a moderate amount of calbindin, while more is expressed in the taste nucleus of the thalamus, the VPMpc. Just above VP, the ventroposterior superior nucleus expresses moderate amounts of calbindin. Thus, calbindin levels usefully distinguish nuclei within the ventroposterior complex. Arrows indicate medial (M) and dorsal (D) in the photomicrograph. Below: An adjacent brain section processed for Nissl substance to reveal cell bodies. Note the cell-poor septa that divide VP into subnuclei.

area 3b and 1, as does VP (Kaas, 2004). While VPS has only been recognized in primates, the same nucleus likely exists in most mammals, and may have been present in the reptilian ancestors of mammals, given that they likely had distinct brainstem nuclei for the relay of muscle spindle information to the cerebellum and to the thalamus (Butler and Hodos, 2005). More significantly, a VPS-like nucleus or subnucleus has been identified in a range of mammalian taxa, including cats, raccoons, opossums, rats, and squirrels (Wiener *et al.*, 1987b; Gould *et al.*, 1989). The VPS-like nucleus is variously considered part of VP, part of the motor thalamus, or part of the posterior group of nuclei adjoining VP, but this kinesthetic or proprioceptive nucleus is always located on the dorsorostral border of VP, and is highly likely to be homologous across mammals, rather than independently derived.

The existence of VPS as a relay of deep receptor information raises the issue of what is included in the posterior nuclear group or complex. In rodents and cats, the part of the thalamus just dorsal to VP is commonly subdivided into a medial posterior nucleus (POM) and a lateral posterior nucleus (POL). The VPS relay nucleus in these mammals appears to occupy the ventral parts of these nuclei (Gould *et al.*, 1989), suggesting that some revision in nuclear boundaries and terminology is justified. Historically, the concept of a posterior group emerged from architectonic studies of the thalamus of sheep, cats, and rabbits by Rose (Rose and Woolsey, 1949). The complex includes parts of the thalamus dorsal and caudal to VP, and between VP and the medial geniculate complex. The region has poorly defined boundaries, and nuclear boundaries. Part of the region contains neurons that are responsive to more than one modality, often to both somatosensory and auditory, but sometimes visual stimuli, and the region has connections with nonprimary regions of sensory cortex (Jones, 1985). As nuclei of the posterior complex are difficult to identify and delimit, it seems likely that nuclei have been misidentified and misnamed across taxa. Thus, it is difficult to speculate on the evolution of the complex. As noted above, part of the complex probably includes the VPS of nonprimate mammals. Another part may well include the anterior (oral) pulvinar of primates. The anterior pulvinar is part of the somatosensory rather than the visual thalamus, and probably, it should not be grouped with the nuclei of the visual pulvinar. The anterior pulvinar receives inputs from somatosensory areas of cortex and projects back to somatosensory areas of cortex (Kaas and Pons, 1988). It has only been described in primates, and it has no known homologue in other mammals. The location of the anterior pulvinar on the mediodorsal (MD) border of VP, and the connections of the anterior pulvinar with somatosensory cortex, suggest that part of the posterior complex of other mammals is homologous with the anterior pulvinar of primates. The anterior pulvinar could also correspond to part of the lateral posterior region that is identified in most mammals, although most of the lateral posterior region corresponds to the pulvinar complex in mammals where the visual pulvinar is not recognized, or only part of the complex is recognized as the pulvinar.

3.35.5 The Auditory Thalamus: The Medial Geniculate Complex

The medial geniculate complex is another part of the mammalian thalamus that was retained from

reptilian ancestors. The reptilian homologue, nucleus medialis (also known as nucleus reuniens), has a core of more densely packed neurons, and a shell of more diffusely distributed neurons, already suggesting the existence of a nuclear complex rather than a single nucleus. The medial geniculate of reptiles resembles that of mammals in having its activating auditory input relayed from the auditory midbrain (the inferior colliculus in mammals), but differs from the mammalian medial geniculate by having a medial rather than a lateral location in the thalamus, and by projecting to the striatum and ventral pallium rather than to dorsal cortex, the homologue of neocortex. Puelles (2001) suggests that most of the auditory complex in the thalamus of reptiles migrated laterally in ancestral mammals, while keeping the auditory input from the midbrain developing a new collateral projection to the emerging neocortex, thereby forming several auditory fields. Parts of the auditory complex of the reptilian thalamus that did not migrate all the way may have led to the emergence of some of the intralaminar nuclei of mammals.

Three nuclei are widely recognized in the medial geniculate complex (Figure 6) of mammals: (1) the ventral medial geniculate (MGv); (2) the dorsal medial geniculate (MGd); and (3) the magnocellular medial geniculate (MGm), also termed the internal nucleus, as the neurons in the nucleus are not particularly large in mammals where the complex is not well differentiated (Jones, 1985). The ventral nucleus projects to one or more primary or primary-like areas

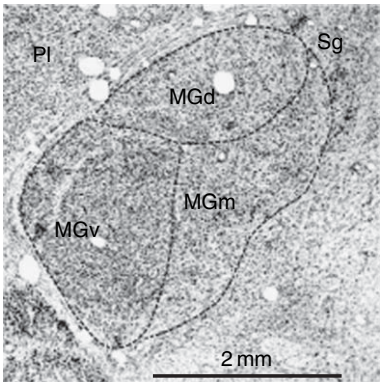

Figure 6 The medial geniculate complex of a macaque monkey. The coronal brain section has been stained for Nissl substance to reveal cell bodies. The ventral nucleus (MGv) is ventrolateral and composed of tightly packed small neurons. The dorsal nucleus (MGd) and the medial or magnocellular nucleus (MGm) have less densely packed neurons, and MGm has larger neurons. The suprageniculate nucleus (Sg) also has auditory functions, while the adjoining inferior pulvinar (Pl) is visual. Some investigators have defined further subdivisions of the medial geniculate complex in cats and monkeys (see text).

of a core region of auditory cortex in topographic patterns that preserve tonotopic organization in this cortex. As high-frequency hearing emerged with mammals, a major change from reptiles was the representation of high frequencies in MGv. The dorsal and magnocellular nuclei project more broadly to auditory cortex, to the secondary areas of the auditory belt and parabelt, with little input to the primary core (see Hackett *et al.*, 1998, for review). The divisions of the medial geniculate nucleus are distinct and well differentiated from each other and the adjoining thalamus in well-studied cats and monkeys, but much less so in hedgehogs and opossums (Winer *et al.*, 1988), supporting the contention that these nuclei became more differentiated independently in several lines of mammalian evolution. It is likely that in all mammals, the ventral nucleus is characterized by a dense distribution of the calcium-binding protein, parvalbumin, while the adjoining dorsal and magnocellular nuclei express the calcium-binding protein, calbindin (Cruikshank *et al.*, 2001). These two proteins distinguish other thalamic nuclei as well: parvalbumin distributions are high in the core nuclei that activate cortical areas, and calbindin distributions are high in the nuclei that largely modulate cortical activity.

As for other thalamic nuclei, those of the medial geniculate complex vary in differentiation and relative size in members of different taxa. As one would expect from the auditory needs of echolocating bats, the medial geniculate complex in these bats is the largest nuclear complex of the dorsal thalamus, occupying three-fifths of the rostrocaudal extent of the thalamus (Radtke-Schuller, 2004). The MGv occupies about 40% of the complex, somewhat less than 50% or more found in most mammals. The three divisions of the medial geniculate complex do vary in proportions relative to the total size of the complex in various mammals (reviewed by Radtke-Schuller, 2004). MGv occupies more of the complex in species where primary auditory cortex is prominent, while a relatively large MGd is found in species with expanded nonprimary cortical areas, as in macaque monkeys (Kaas and Hackett, 1998).

Of course, the medial geniculate complex in echolocating bats is also specialized in that the echo frequencies have a disproportionately large representation in MGv (Wenstrup, 1999). The ultrasonic communication calls of mice also appear to have an enlarged thalamic representation (Hofstetter and Ehret, 1992). Another distinctive feature of echolocating bats is that many neurons in both the MGv and MGd have complex sensitivities to sound frequencies, often having responses that are facilitated by combinations of frequencies

in the range of the biosonar signals (Wenstrup, 1999). However, the most remarkable specializations of the auditory thalamus of echolocating bats may be the existence of a direct auditory pathway from the suprageniculate nucleus to the unique frontal auditory area of cortex just rostral to motor cortex (Kobler *et al.*, 1987). While only three divisions of the medial geniculate complex have been considered here, the complex of cats and monkeys appears to have more subdivisions (Winer *et al.*, 2001; de la Mothe *et al.*, 2006), suggesting the independent evolution of new divisions.

3.35.6 The Motor Thalamus: The Ventral Lateral Complex and the Ventral Anterior Complex

The VL complex and the ventral anterior (VA) complex occupy the rostral pole of the thalamus just rostral to the somatosensory nuclei, VP, VPS, and VPI. The VL and VA nuclei are highly variable in size and differentiation, forming a small, poorly differentiated portion of the thalamus in many mammals such as hedgehogs, opossums, and even rats, while being large and architectonically subdivided into several nuclei in anthropoid primates. In addition to VL and VA, a ventromedial nucleus (VM) is often distinguished.

Much of the motor thalamus is defined by inputs from the deep nuclei of the cerebellum and from the substantia nigra and globus pallidus of the basal ganglia. The cerebellum and the basal ganglia, together with the motor cortex, are the main structures that regulate motor behavior. Thus, they provide important sources of information to the motor thalamus, which in turn projects to motor cortex. The cerebellothalamic afferents tend to project predominantly to VLp, a posterior division of the ventrolateral complex, while basal ganglia (pallidal) projections terminate more rostrally in the VLa, the anterior division of the ventrolateral complex, and in the caudal aspect of the VA complex (Ilinsky and Kultas-Ilinsky, 1984; Sakai *et al.*, 2000), although it is uncertain to what degree this segregation of inputs applies to all mammals. In tenrecs, a small insectivore from Madagascar with little thalamic differentiation, the region identified as VM receives a prominent input from the cerebellum (Künzle, 1998), but cerebellar input to VM is considerably less in rats (Herkenham, 1979).

In most mammals, VLp projects mainly to primary motor cortex, while VLa and VA project mainly to premotor cortical areas (Jones, 1985). The notable exception is in mammals that appear to have no motor cortex. Thus, in opossums and perhaps other marsupials (see Beck *et al.*, 1996, for review), there is no evidence for a primary motor area, M1, or any other motor area rostral to primary somatosensory cortex, S1. Instead, S1 is bordered rostrally by a narrow somatosensory area that resembles area 3a of cats and primates. All of the corticospinal projections in opossums originate from S1, the narrow 3a-like area, and S2 (Cabana and Martin, 1984). In other mammals, S1 and the narrow 3a-like strip of opossums have motor functions and electrical stimulation of these areas evokes motor movements. When this was noted by investigators, the S1 area was conceptualized as a sensorimotor amalgam in marsupials (Lende, 1963). Alternatively, this area in opossums can be considered to be simply S1, as it has the identifying features of S1, including sensory architecture, a characteristic somatotopic organization, bordering somatosensory areas, and input from the VP nucleus (Beck *et al.*, 1996).

However, S1 of opossums apparently receives inputs from both VP and VL (Killackey and Ebner, 1973), although these nuclei are poorly differentiated in opossums and difficult to separate. This apparent distinction between metatherian and placental mammals (where a primary motor area, M1, with inputs from VL has been consistently identified) suggests that a motor thalamus existed in early therian mammals, but separate motor cortex targets only evolved in eutherian (placental) mammals (note that projections from VL to parts of the rostral margin of parts of S1 have been reported for some eutherian mammals, but technical difficulties in obtaining relevant data make this uncertain). Arguing against the hypothesis that motor cortex emerged with placental mammals is the questionable evidence that protherian mammals (monotremes) do have a separate motor cortex (Ulinski, 1984; Krubitzer *et al.*, 1995). If so, it might be more parsimonious to argue that opossums and perhaps other marsupials have regressed from the ancestral plan, and have lost a separate primary motor area, M1. This uncertainty relates to the issue of whether reptilian ancestors of mammals had a motor thalamus. Although a region of the thalamus of birds and reptiles has been suggested as a homologue of the mammalian motor thalamus, the evidence has been described as inconclusive (Medina *et al.*, 1997; Medina and Reiner, 2000; see Field Homologies).

An unusual feature of hedgehogs is that the VL nucleus projects bilaterally to motor cortex (Dinopoulos, 1993). While crossed thalamocortical projections have been reported for midline nuclei in a range of mammals, they have not been described for VL of other mammals, including opossums

(Donoghue and Ebner, 1981) and echidnas (Ulinski, 1984). Given this apparently unique feature of the motor thalamus of hedgehogs, the most logical conclusion is that the immediate ancestors of hedgehogs developed a crossed projection that earlier ancestors did not have. However, Dinopoulos (1993) suggested instead that hedgehogs have retained a primitive trait, as their forebrain appears to be primitive in general and such connections exist in postnatal rats that are lost during development (Minciacchi and Granato, 1989). Thus, hedgehogs might have retained a primitive crossed thalamocortical projection from VL to motor cortex that is preserved in other mammals only as a transient projection early in development.

In anthropoid primates, VL and VA are large and architectonically divided into several nuclei or subnuclei (Stepniewska *et al.*, 1994). Thus, VL is commonly divided into four regions, with names varying across authors, and VA is divided into a parvocellular and a magnocellular division. This complexity likely relates to the changes that have taken place in the organization of motor cortex of primates, where dorsal and ventral premotor areas, a supplementary motor area, and a presupplementary motor area have been identified in dorsolateral frontal cortex. Such complexity has not been identified in nonprimate mammals (Kaas, 2004). Although prosimian galagos also have these additional premotor areas (Wu *et al.*, 2000), and the motor thalamus is large and subdivided, the subdivisions are architectonically much less distinct than in monkeys (Fang *et al.*, 2006).

In summary, the motor thalamus of mammals appears to be unusual in that it seems to have emerged as a subdivision or subdivisions of the thalamus before a separate motor area or motor areas existed. Alternatively, motor areas of cortex could have been lost in evolution while thalamic areas were preserved. In addition, the complexity in terms of subdivisions substantially increased at both cortical and thalamic levels in primates, but the architectonic distinctiveness of thalamic subdivisions especially increased with the advent of anthropoid primates.

3.35.7 The Anterior and Lateral Dorsal Nuclei

Three anterior nuclei and the associated lateral dorsal (LD) nucleus are well differentiated and histologically distinct in the anterior thalamus of most mammals. The anterodorsal (AD) nucleus is characterized by tightly packed, darkly stained cells in Nissl preparations, while the adjoining anteroventral (AV) nucleus is less darkly stained, but usually separated from AD by a cell-poor septum. The anteromedial (AM) nucleus resembles AV, but has a more VM position. The LD nucleus, lateral and dorsal to AD and AV, has pale-staining cells in Nissl preparations. The four nuclei are functionally related in that they project to different parts of limbic cortex on the medial wall of the cerebral hemisphere, and receive inputs from the hippocampal formation either directly from the subicular complex or indirectly via the mammillary nuclei of the hypothalamus (Price, 1995). The limbic connections of the anterior group suggest that these nuclei have roles in basic, ancient functions. Yet, this collection of nuclei has not been identified in reptiles. Butler and Hodos (2005) postulate that the elaboration of this complex involved a shift from a multisensory relay nucleus in the thalamus to a cortical–thalamic–cortical circuit resulting from an increased role of limbic cortex in learning and memory. In any case, the four nuclei vary in development across taxa in a way that suggests that their functions vary in importance. Monotremes appear to have only a single anterior nucleus (Butler and Hodos, 2005), possibly reflecting the state of early mammals, while opossums have all three anterior nuclei (Bodian, 1939). Other variations in the anterior nuclei across mammalian taxa have been reviewed by Jones (1985). In general, there seems to be an association between the architectonic representation of the nuclei and that of limbic cortex. The AM nucleus is sometimes indistinctly separated from the AV nucleus, and the AV nucleus is greatly enlarged in many primates. The AD nucleus is sometimes subdivided into regions of larger and smaller cells, and it is disproportionately large in some mammals such as echolocating bats.

3.35.8 The Mediodorsal and Intralaminar Thalamic Nuclei

A number of other nuclei of the mammalian thalamus deserve at least brief comment. The MD nucleus is a large group of cells in the AM thalamus that is often subdivided based on regional differences in cell size and packing. The nucleus or complex receives inputs from olfactory cortex, the amygdala, and entorhinal cortex while projecting to regions of frontal cortex (Price, 1995). An olfactory pathway through the thalamus to dorsal pallium appears to be widespread in reptiles and birds (Veenman *et al.*, 1997), suggesting that the reptilian ancestors of mammals had the homologue of an MD nucleus. An MD nucleus has been identified with

connections to frontal cortex in monotremes (Welker and Lende, 1980), marsupial opossums (Tobias and Ebner, 1973; Benjamin and Golden, 1985), and a wide range of placental mammals (see Benjamin and Golden, 1985, for review). One difference across mammals is that olfactory cortex projects to all of MD in opossums, but only to the medial part in placental mammals, which is further distinguished by the magnocellular division of MD in monkeys. These findings suggest that MD in early mammals functioned to relay olfactory information to frontal cortex. In some lines of evolution, especially in primates, the olfactory functions became less dominant, while MD differentiated into two or more subnuclei with olfactory and other functions.

The cell groups within the internal medullary lamina of the dorsal thalamus are called the intralaminar nuclei. The 'centre médian' is of special interest as it is a well-differentiated component of the primate thalamus, but not an obvious nucleus in most other mammals. The 'centre médian' has been suggested as one of the new components of the primate thalamus (Jones and Rubenstein, 2004). Historically, Le Gros Clark (1932) proposed that the 'centre médian' nucleus evolved from part of the intralaminar group, but the origin and primitive form of the nucleus are unclear. Veenman *et al.* (1997) propose, on the basis of immunohistochemical evidence and connection patterns, that the intralaminar, midline, and MD nuclei of the mammalian thalamus evolved from dorsal thalamic groups in reptilian ancestors that also gave rise to a dorsal thalamic zone in birds.

3.35.9 Conclusions

We have seen that there are both consistent and variable features of the mammalian dorsal thalamus. The most notable consistent feature is that a number of thalamic nuclei, such as the dorsal LGN, the medial geniculate complex, the pulvinar, and the VP nucleus, can be identified in most or all mammals studied, and likely were retained from the immediate reptilian ancestors of mammals. However, the architectonic differentiation of these nuclei was not marked, judging from the poor differentiation of thalamic nuclei in a range of extant mammals. Variable features, reflecting evolutionary changes in various lines of descent, include: increases (and, more rarely, decreases) in the absolute and relative sizes of some nuclei relative to others, changes in the proportion of nuclei devoted to some inputs over others (e.g., representation of inputs from face or hand in VP), the formation of layers and subnuclei in nuclei, alterations in the inputs and outputs of nuclei, and the emergence of new nuclei. Similar evolutionary changes have taken place in the more extensively studied neocortex (Krubitzer and Kaas, 2005).

It seems likely that few or no thalamic nuclei have been lost in mammalian evolution. Even the subterranean blind mole-rat has an identifiable, but small, dorsal LGN (Cooper *et al.*, 1993). In contrast, new nuclei appear to have emerged in many lines of mammalian evolution. A clear example is the pulvinar complex, where different nuclei have emerged in carnivore and primate lines. By using the expression of genes during development to help identify thalamic nuclei, Jones and Rubenstein (2004) listed six nuclei found in monkeys that are not found in mice, and this is certainly an underestimate. On the other hand, the patterns of gene expression suggested that some nuclei that are well differentiated in the thalamus of monkeys, such as the suprageniculate/limitans, do have a poorly differentiated homologue in the thalamus of mice. It seems likely that, as new expanses of neocortex subdivided into new areas in evolution, as is the case with much of posterior parietal cortex of anthropoid primates (Kaas *et al.*, 2006a), new thalamic nuclei with projections to the new cortical areas emerged. However, the increase in the number of cortical areas and thalamic nuclei was most likely not on a one-to-one basis, as the neocortex generally seems to have more subdivisions than does the dorsal thalamus. As an example of a new nucleus, the medial nucleus of the inferior pulvinar (IPm) of anthropoid primates projects densely to the MT visual area, but neither MT nor IPm appears to exist outside the primate order. However, in some instances, the complexity of the thalamus may exceed that of the cortex, as in opossums where the thalamic motor nucleus, VL, and the thalamic somatosensory nucleus, VP, both project to primary somatosensory cortex, S1. Also, there is no evidence of a separate primary motor area, M1, although a separate and distinct M1 with inputs from VL is characteristic of all placental mammals studied.

If new nuclei of the thalamus emerged, from where did they come? The prevailing notion is that poorly differentiated nuclei gradually differentiated to two or more nuclei in some lines of descent. This hypothesis has been stated repeatedly as an explanation for how new cortical areas emerged (Kaas, 1982; Krubitzer and Kahn, 2003; Krubitzer and Kaas, 2005). In some sense, one could argue that the new areas are not really new, and two or more nuclei in one taxon can be identified as a field homology with a single nucleus in other taxa. Alternatively, new nuclei could emerge as the result

of a change in gene expression, perhaps as a consequence of gene duplication (Allman and Kaas, 1971a, 1971b; Fukuchi-Shimogori and Grove, 2001, 2003). Finally, it is important to stress that the evolution of the thalamus is not well understood, as the great diversity of thalamic organization that must exist in mammalian radiation has been largely unexplored. For practical reasons, research efforts have focused on a few well-studied species. Clearly, there is much to learn, and many opportunities for the adventurous, comparative neuroscientist to make new discoveries of substance.

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3.36 The Dual Elaboration Hypothesis of the Evolution of the Dorsal Thalamus

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Glossary

amniote The major vertebrate taxon that comprises mammals, reptiles, and birds. Amniotes are characterized by having an amnion, which is a membrane that surrounds the embryo and contains amniotic fluid.

anamniote The major vertebrate taxon that includes fishes and amphibians. Anamniotes lack an amnion. See 'amniote'.

cladistic analysis Method in which the distribution of shared traits is used to infer phylogenetic relationships among taxa or to determine whether a particular trait was likely present in the common ancestor of a set of taxa.

collothalamus The set of dorsal thalamic nuclei that predominantly receive their inputs via a relay through the midbrain roof.

diapsid Major amniote taxon that includes modern reptiles and birds. Modern reptiles comprise lizards, snakes, turtles, the tuatara, and crocodiles. Diapsids are characterized by having two fenestrae (bony openings) bridged over by arches in the temporal region of the skull. These arches and fenestrae have become secondarily lost in modern turtles and substantially modified in modern lizards, snakes, and birds.

lemnothalamus The set of dorsal thalamic nuclei that predominantly receive their inputs directly, without relay through the midbrain roof.

sauropsid Major amniote taxon that includes modern reptiles and birds. The term 'diapsid' previously excluded turtles but can now be considered synonymous with the term 'sauropsid' for extant taxa.

synapsid Major amniote taxon that includes modern mammals. Synapsids are characterized by having one fenestra (bony opening) bridged over by an arch in the temporal region of the skull.

3.36.1 How Did the Dorsal Thalamus Evolve? The Problem and the Evidence

The dorsal thalamus consists of multiple nuclei and nuclear groups in the two major groups of amniote vertebrates (Jones, 1985; Butler and Hodos, 2005): sauropsids (reptiles and birds) and mammals. In contrast, in anamniotes (amphibians and fishes), the dorsal thalamus consists of only three nuclei: a rostrally lying nucleus anterior and two more caudally lying nuclei, the dorsal posterior and central posterior nuclei (Butler and Hodos, 2005). The latter two nuclei receive their predominant input from the midbrain roof and project predominantly to the striatum and/or to the neighboring part of the pallium, whereas the nucleus anterior receives its predominant input directly from the retina and/or from other sources and projects to the developmentally medial part of the pallium.

In sauropsids, several nuclei receive a major midbrain roof input, including nuclei rotundus, ovoidalis, and the caudal part of nucleus dorsolateralis posterior, or cDLP. (Avian nomenclature is used here for sauropsids, including the revised nomenclature of Reiner *et al.*, 2004.) Other nuclei, including several dorsomedial and dorsolateral nuclei, and the dorsal lateral geniculate nucleus (DLGN), predominantly receive more direct sensory inputs, which are not relayed from the midbrain roof. Likewise in mammals, nuclei that receive a major midbrain roof input include the posterior nuclear group (and the posterior intralaminar nuclei), the medial geniculate

body, and the lateral posterior–pulvinar complex, or LP–pulvinar. A large number of dorsal thalamic nuclei in mammals receive predominantly lemniscal, or direct, inputs, including those of the medial, midline, rostral intralaminar, anterior, and ventral groups, and the DLGN.

While multiple nuclei are present in the dorsal thalamus of all amniotes, and some nuclei, such as the DLGN (previously called OPT in birds), could be identified as homologous across sauropsids and mammals, the evolutionary origin of most nuclei had been enigmatic (Jones, 1985; see Evolution of the Visual Tectogeniculate and Pretectogeniculate Pathways in the Brain of Amniote Vertebrates). In 1942, Rose had identified five pronuclei in the developing rabbit thalamus (Figure 1) that subsequently differentiated into the adult dorsal thalamic nuclei, but their correspondence to sauropsid or anamniote dorsal thalamic nuclei was also unknown. The dorsal pronucleus differentiates into two entities, LP–pulvinar and the posterior group, whereas the medial geniculate pronucleus differentiates into the nucleus, or body, of the same name. These nuclei all receive substantial midbrain roof inputs and thus could be compared as a group to the dorsal posterior and central posterior nuclei of anamniotes and to the rotundus-ovoidalis-cDLP and related nuclei of sauropsids. The puzzle concerned the evolutionary source of all of the other dorsal thalamic groups in mammals, which are derived from the medial, central, and dorsal lateral geniculate pronuclei.

As the result of a cladistic analysis of the dorsal thalamus, Butler (1994a) recognized that the latter groups of nuclei and the DLGN were united by a number of common features of connections and position. She posited that these nuclei (and the three pronuclei that give rise to them) were all derived from a common embryological anlagen that is homologous to the nucleus anterior of anamniotes and that the similarly connected nuclei in sauropsids – the dorsomedial and dorsolateral nuclei, and the DLGN – were likewise derived. She thus identified two fundamental divisions of the dorsal thalamus across amniotes (Figure 2): the lemnothalamus, which receives most of its inputs directly rather than through midbrain roof relays, and the collothalamus, which receives its predominant inputs via the midbrain roof. Corresponding divisions of the pallium that are lemnothalamic-recipient (in mammals, the primary visual and somatosensory cortices and frontal lobe; in birds, the Wulst, or hyperpallium) and collothalamic-recipient (in mammals, including

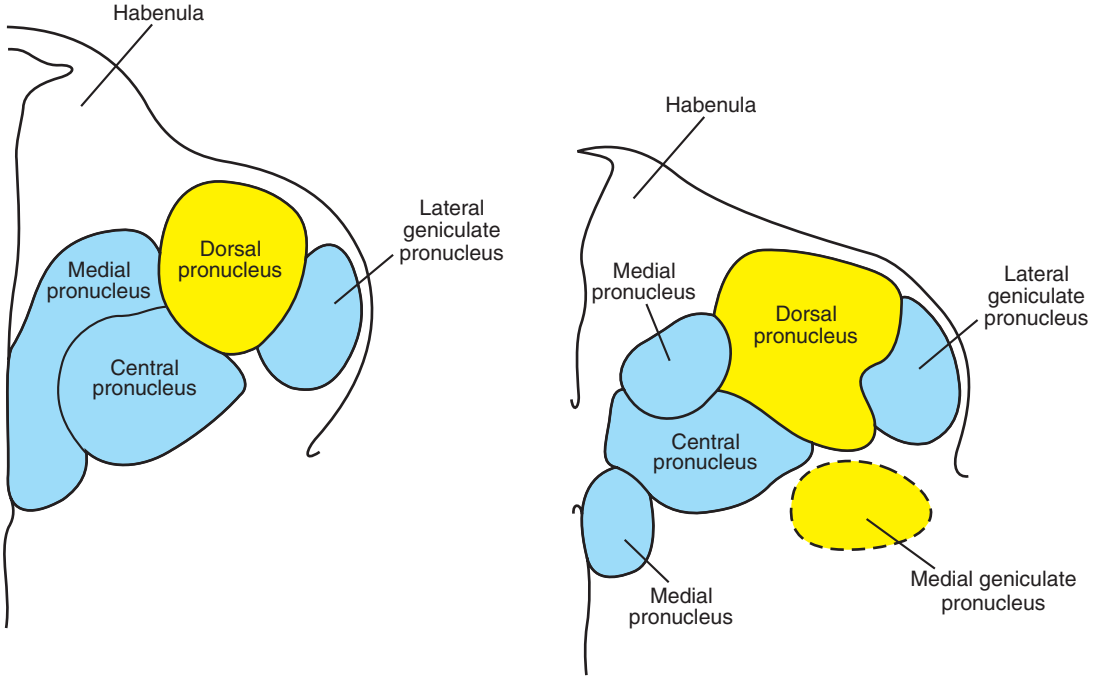

Figure 1 Drawings of pronuclei on the right side of the developing dorsal thalamus in the rabbit. The more rostral section is on the left. In the section on the right, the position of the medial geniculate pronucleus is outlined in dashes, since it actually occurs at a somewhat more caudal level than the one shown here. Blue indicates the lemnothalamus and yellow the collothalamus. Based on the findings of Rose, J. E. 1942. The ontogenic development of the rabbit's diencephalon. *J. Comp. Neurol.* 77, 61–129.

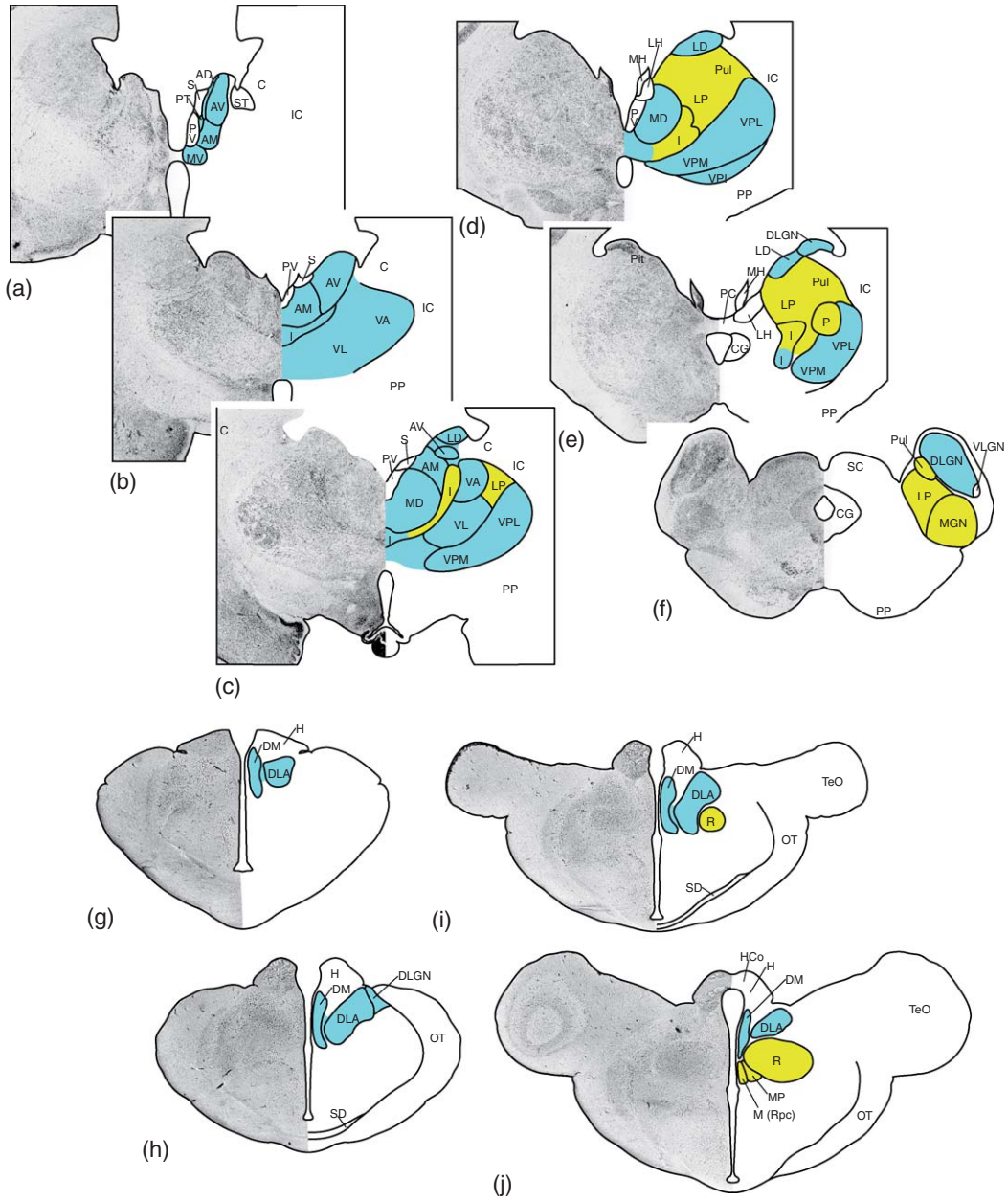

Figure 2 Series of transverse hemisections with mirror image drawings to show the distribution of lemnothalamic and collothalamic nuclei in the dorsal thalamus of an adult mammal, the raccoon *Procyon lotor* (a–f), and an adult sauropsid, the lizard *Iguana iguana* (g–j). For both species, the most rostral section is at the upper right. In mammals, the intralaminar nuclei are divided into rostral, lemnothalamic, and caudolateral, collothalamic groups, as indicated by their blue or yellow shading, respectively, with the former including the central medial, rhomboid, and central median nuclei and the latter the parafascicular, paracentral, and central lateral nuclei. Abbreviations for dorsal thalamic nuclei in a–f are: AD, anterodorsal nucleus; AM, anteromedial nucleus; AV, anteroventral nucleus; DLGN, dorsal lateral geniculate nucleus; I, intralaminar nuclear group; LD, lateral dorsal nucleus; LP, lateral posterior nucleus; MD, mediodorsal nucleus; MGN, medial geniculate body; MV, medioventral (reuniens) nucleus; P, posterior nuclear group; PT, parataenial nucleus; Pul, pulvinar; VA, ventral anterior nucleus; VL, ventral lateral complex; VPL, ventral posterolateral nucleus; VPM, ventral posteromedial nucleus; VPI, ventral posterior inferior nucleus. Abbreviations for dorsal thalamic nuclei in g–j are: DLA, nucleus dorsolateralis anterior; DLGN, dorsal lateral geniculate nucleus; DM, nucleus dorsomedialis; R, nucleus rotundus; MP, nucleus medialis posterior; M (Rpc), nucleus medialis (reuniens pars compacta). Abbreviations for additional structures labeled for orientation are: C, caudate nucleus; CG, central gray; IC, internal capsule; H, habenula; HCo, habenular commissure; LH, lateral habenula; MH, medial habenula; OT, optic tract; PC, posterior commissure; PP, pes pedunculi; PV, paraventricular nuclear group of the epithalamus; S, stria medullaris; SC, superior colliculus; SD, supraoptic decussation; ST, stria terminalis; TeO, optic tectum; VLGN, ventral lateral geniculate nucleus. Photomicrographs and guidance on nuclear boundaries for *Procyon lotor* were generously provided by Wally Welker. Identification of the DLGN in *Iguana iguana* is based on Kenigfest, N., Martínez-Marcos, A., Belekhova, M., *et al.* 1997. A lacertilian dorsal retinorecipient thalamus: A re-investigation in the Old-World lizard *Podarcis hispanica*. *Brain Behav. Evol.* 50, 313–334.

extrastriate, association somatosensory, and auditory cortices, as well as the lateral nucleus of the amygdala; in birds, the dorsal ventricular ridge – particularly its mesopallial and nidopallial components – likewise can be identified (Butler, 1994b; Bruce and Neary, 1995)).

Based on developmental, histochemical, and other criteria, divisions corresponding to Butler's lemnothalamus and collothalamus also had been recognized independently by Martínez-de-la-Torre (1985), Caballero-Bleda (1988), and Guillén (1991), working in the laboratory of Luis Puelles. Likewise, Reiner (1993) independently identified the two corresponding major divisions of the nonlimbic pallium to which the lemnothalamic and collothalamic divisions of the thalamus project (see The Evolution of the Dorsal Thalamus in Mammals).

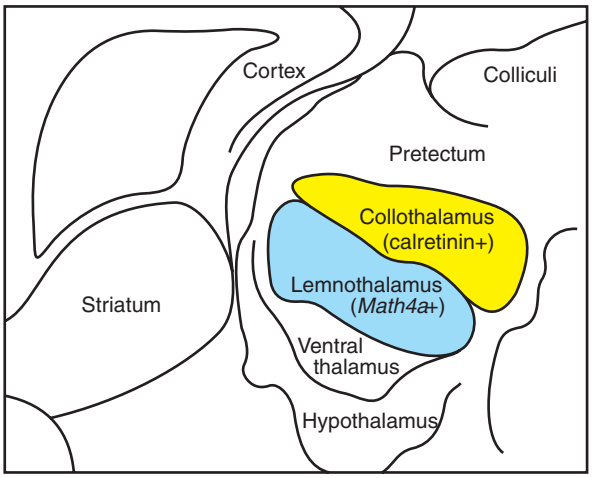

Figure 3 Drawing of part of a sagittal section through the diencephalon of the developing mouse brain, with rostral toward the left. The lemnothalamus (in blue) expresses the gene *Math4a*, while the collothalamus (in yellow) is calretinin-positive. Redrawn from photomicrograph from González, G., Puelles, L., and Medina, L. 2002. Organization of the mouse dorsal thalamus based on topology, calretinin immunostaining, and gene expression. *Brain Res. Bull.* 57, 439–442.

3.36.2 Recent Supporting Evidence for the Lemnothalamus and Collothalamus

Besides the defining issue of predominant input from either direct or midbrain-relayed sources, the basic pattern of connections of the lemnothalamic and collothalamic nuclei across amniotes (Butler, 1994a) includes: (1) projections from the lemnothalamus to dorsomedial pallial areas, some of which are bilateral, and (2) projections from the collothalamus that are solely ipsilateral and are to the striatum as well as to dorsolateral pallial areas. Additional findings of bilateral lemnothalamic projections to cortex in mammals (Dermon and Barbas, 1994; Dinopoulos, 1994; Carretta *et al.*, 1996) as well as similar previous findings by Preuss and Goldman-Rakic (1987) support this distinction. Additionally, the presence of ipsilateral striatal projections that arise from the extrageniculate visual nuclei in the cat (Harting *et al.*, 2001) is consistent with the collothalamic pattern.

Several studies in birds also have provided support for the unity of the lemnothalamus and its presence across amniotes. The afferent and efferent connections of a number of specific nuclei or nuclear regions within the lemnothalamus of birds support homology with various discrete components of the lemnothalamus in mammals, as predicted by Butler (1994a). These include somatosensory, basal ganglia- and cerebellum-related feedback, rostral intralaminar, and olfactory cell groups (Medina *et al.*, 1997; Veenman *et al.*, 1997; Wild, 1997; Montagnese *et al.*, 2003).

Recently, González *et al.* (2002) identified molecular markers that selectively label the lemnothalamus and collothalamus. In the developing mouse dorsal thalamus (Figure 3), the lemnothalamus is distinguished by expression of the gene *Math4a* and lack of calretinin immunostaining, whereas the collothalamus is negative for *Math4a* and positive for calretinin. Likewise in amphibians, acetylcholinesterase differentiates the two hypothesized divisions, with nucleus anterior (lemnothalamus) being strongly positive for it and the dorsal posterior and central posterior nuclei (collothalamus) being light to negative (Puelles *et al.*, 1996).

3.36.3 Independent Evolution of the Collothalamus and Lemnothalamus among Amniotes: Different Patterns of Evolution

Early stem amniotes gave rise to the synapsid lineage, which led to modern mammals, approximately 320 Mya, and to the diapsid lineage, which led to modern sauropsids (reptiles and birds), approximately 310 Mya (Evans, 2000). The lemnothalamus and collothalamus exhibit different relative patterns of evolution in these two lineages (Butler, 1994a, 1995). In sauropsids, the collothalamus was initially favored for elaboration, as is still evident, particularly with the relative size of nucleus rotundus in reptiles. Secondarily, in birds, the lemnothalamus also has undergone some degree of elaboration. These elaborations similarly are evident in the pallium (Butler, 1994b), with the enlargement in birds of the lemnothalamic-recipient

Wulst and the collothalamic-recipient nidopallium. Conversely, in mammals, the lemnothalamus was the first division to undergo elaboration, likely in correlation with elaboration of the medial-most part of the pallium, the hippocampal formation, and increased spatial mapping and sensory challenges. Extant monotremes have a large lemnothalamus and a large lemnopallium, particularly as evinced in the platypus with the expansive primary somatosensory cortex and echidnas with the expansive frontal lobe, combined with very small collothalamic-recipient regions (Krubitzer, 2000). Secondarily, within some orders of mammals, particularly as seen in carnivores and primates, the collothalamus elaborated markedly with expansion of the LP–pulvinar complex and corresponding extrastriate visual cortical areas (Kaas, 1995).

3.36.4 Homologies of Lemnothalamic and Collothalamic Components Across Amniotes

Homologous relationships of various lemnothalamic components and their pallial projection targets across amniotes are relatively well established and noncontroversial (Medina *et al.*, 1997; Veenman *et al.*, 1997; Wild, 1997; Medina and Reiner, 2000; Desfilis *et al.*, 2002; Montagnese *et al.*, 2003), as is that of the medial geniculate nucleus of mammals to the sauropsidian nucleus ovoidalis (Butler, 1994a; Puelles, 2001). Possible homologies of some other collothalamic components are the subject of current debate. In particular, whether LP–pulvinar, the posterior nuclear group, or both are homologous to the sauropsidian nucleus rotundus needs to be resolved. The possibility that at least part of LP–pulvinar is not a collothalamic but rather a lemnothalamic derivative has been broached (Puelles, 2001). However, cell migration patterns in the developing rat dorsal thalamus, as evinced by elongated cellular profiles between the region of the posterior nuclear group and LP (Figure 4), support the derivation of both LP and the posterior nuclear group from the unitary, collothalamic dorsal pronucleus, in agreement with Rose's (1942) observations in the rabbit. Recent molecular evidence indicating that some nuclei in the pulvinar of primates are unique to this order (Jones and Rubenstein, 2004) does not contradict the fundamental developmental relationship of LP and the posterior nuclear group.

3.36.5 Summary and Concluding Remarks

Delineation of the lemnothalamus and collothalamus allowed the evolutionary history of the dorsal thalamus to be understood in terms of differential elaboration of these two divisions within the two major amniote taxa: sauropsids and mammals. Initial elaboration of collothalamic nuclei in sauropsids was correlated with elaboration of their pallial targets within the dorsal ventricular ridge.

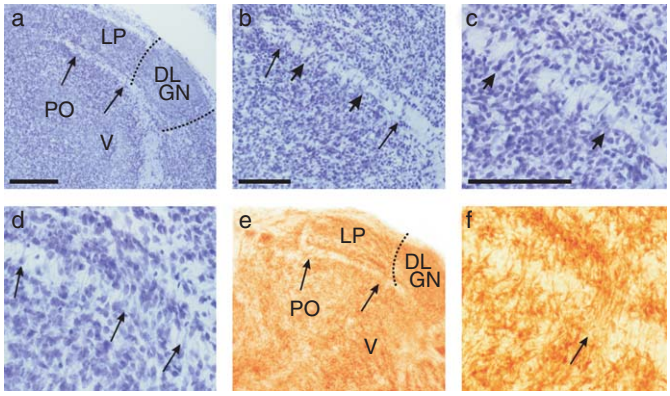

Figure 4 Photomicrographs of transverse sections through the diencephalon of the developing rat brain stained with cresyl violet (a–d) or reacted for cytochrome oxidase (e–f). The section shown in (a) is from embryonic day 21 and is enlarged in (b) and further in (c). Arrows in (a) indicate the same region between the posterior nuclear group (PO) and the lateral posterior nucleus (LP) as indicated in (b) by the longer, outside set of arrows. The shorter, arrows in (b) indicate the same region shown between the arrows in (c). Note the elongated profiles of cells, indicating migration of developing neurons from PO into the region of LP. A second example of PO to LP elongated cells, indicated by arrows, in this same region is shown in (d) from a case at postnatal day 1. A third example, also at postnatal day 1, is shown in (e), with a bridge-like cluster of elongated cells indicated by the arrow to the right also shown in (f), as indicated by the arrow, with a number of elongated cells also to the left of it. Elongated cell profiles also occur between the ventral nuclear group (V) and the DLGN, the borders of which are indicated in (a) by the dotted lines and likewise its dorsal border in (e). Scale bars: 0.25mm (a, e); 0.1mm (b); 0.1mm (c, d, f).

Initial elaboration of the lemnothalamus in mammals was correlated with elaboration of their pallial targets as well, in this case the primary visual and somatomotor areas and the frontal lobe. Subsequent elaboration of the complementary division of the dorsal thalamus occurred in each lineage. The previously cryptic but shared organization of the thalamus across mammals and sauropsids and their evolution from the relatively modest thalamic components in anamniotes can now be understood in terms of relative and sequential development of the two shared components.

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4.01 Primate Brain Evolution in Phylogenetic Context

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Glossary

anthropoids The primate group comprised of the New World monkeys (platyrrhines) and the Old World monkeys, apes, and humans (catarrhines).

Archonta The group of mammals consisting of primates and their closest relatives, currently thought to include tree shrews and flying lemurs.

catarrhines Members of the suborder Catarrhini, the primate group comprised of the Old World monkeys and the hominoids (apes and humans).

clade Any monophyletic group; that is, the set of species descended from a common ancestral species.

encephalization The disparity (usually expressed as a ratio) between the size of the brain in a particular species and the size that would be expected based on its body size.

haplorhines Members of the suborder Haplorhini, the primate group comprised of the tarsiers and anthropoids.

neomorphism An evolutionarily new feature, that is, a derived feature of a group of animals that has no homologue at the same level of organization in related mammals.

platyrrhines Members of the infraorder Platyrrhini, the New World monkeys.

prosimians Members of the primate group comprised of strepsirhines plus tarsiers; most workers think this is not a monophyletic group, so the term is not as widely used as a formal taxonomic term as it once was.

shared derived character Also known as synapomorphy. A character that evolved in the stem lineage of a group; typically, such a character is present in many descendents of the stem lineage and serves to distinguish that group from related groups.

stem lineage The segment of the evolutionary tree that connects the last common ancestor of a group of interest to the last ancestor it shares with its closest extant relatives. For example, stem primates are animals on the lineage, or on branches extending from the lineage, that connects the last ancestor of modern primates to the last ancestor primates share with other archontans (such as tree shrews).

strepsirhines Members of the suborder Strepsirhini, the branch of the primate tree consisting of the lemur group and the loris–bush baby group.

taxon (pl. taxa) Any formal grouping of related organisms, for example, any particular species, genus, family, etc.

4.01.1 Introduction

Almost anyone with some exposure to the neurosciences can tell you the story of primate brain evolution: through evolutionary time, brains became bigger and more complex, and their bearers increasingly intelligent, a process that culminated in the appearance of *Homo sapiens*, the brainiest and most intelligent animal of them all. This conception of primate brain evolution is so deeply embedded in

the foundations of neuroscience that it is easy to lose sight of the fact that the idea actually has a history, that it was the intellectual product of particular scientists at particular time points in the development of ideas about primatology and about neurobiology.

Times change. Neuroscience, as a discipline, has of course enjoyed tremendous growth over the past several decades, fueled in part by the development of a succession of new techniques that make it possible to explore brain organization and function in finer and finer detail. Less generally appreciated, perhaps, is that evolutionary biology has also undergone profound developments, with the introduction of new methods for determining how species are related to each other and for reconstructing the history of evolutionary change. The phylogenetic scale is gone, replaced by the branching tree of life. Evolutionary biologists now no longer read the history of life as the story of the Ascent of Man: humans are regarded as one of numerous specialized end-points of evolution. Unquestionably, we are justified in taking a particular interest in our own species, but we misunderstand ourselves, and other species, if we conceive of evolution as being mainly about how to get to *H. sapiens*.

As a consequence of the fundamental methodological and conceptual changes in evolutionary biology, we have a much better understanding of the evolutionary history of primates and the relationship of primates to other mammals, and textbooks on primate evolution that were current in, say, 1975, seem about as quaintly dated today as textbooks of neurobiology or physiological psychology from the same era. These new ideas and approaches to evolutionary biology have begun to make their mark on the neurosciences, as witnessed, for example, by the publication of Georg Striedter's landmark textbook on vertebrate brain evolution (Striedter, 2005) and of the volumes in the present series, but there is a long way to go, as so much of the neuroscientific enterprise takes place without any explicit reference to evolution. My main purpose in writing this article is to further the integration of primate evolutionary biology and primate neuroscience. I begin by setting the comparative context for understanding primate brain evolution with an overview of primate taxonomy, past and present ideas about primate origins and adaptations, and current thinking about how primates are related to other mammalian groups. I then review selected issues in primate brain evolution, attempting to localize specializations of primate brain organization within the context of primate evolutionary history.

4.01.2 Primate Origins and Evolution

4.01.2.1 The Primates

Primatologists currently recognize the existence of over 200 extant species of primates (Purvis, 1995; Fleagle, 1999). Traditionally, the order Primates was divided into two main groups, termed prosimians and anthropoids (the latter also known as 'simians'), or more formally, suborder Prosimii and suborder Anthropoidea. The familiar anthropoids consist of a group native to the Old World – the Old World monkeys, plus the hominoid (ape-human) group – and a group native to the New World, the New World monkeys. Formally, the Old World group is known as the Catarrhini and the New World group as the Platyrrhini. The Old World monkey group is the most successful primate group in terms of diversity, comprising no fewer than 87 extant species distributed from South Africa to Japan. These include the familiar macaque monkeys, widely used as research subjects by neuroscientists. By contrast, there are only 14 extant species in the ape-human group, and most of these are the so-called 'lesser' apes, that is, the gibbons. The prosimians consist of the lemurs, found today only on the island of Madagascar, the loris–bush baby group, with species distributed across sub-Saharan Africa and south Asia, and the tarsiers, native to the islands of the western Pacific.

Although this conventional taxonomy groups tarsiers with the lemurs and lorises, there has long been a body of opinion holding that tarsiers are actually more closely related to the anthropoids (Figure 1a). This relationship implies a different classification, in which the two major groups of primates become suborder Strepsirhini (the lemur and loris–bush baby group) and suborder Haplorhini (tarsiers plus the anthropoids). The strepsirhine–haplorhine taxonomy appears to be the preferred taxonomy among primatologists at the present time, because the majority of primatologists accept that tarsiers are the closest relatives of the anthropoids, although this matter is not entirely settled (Ross and Kay, 2004b).

Certain useful generalizations about the biology and behavior of the major primate groups can be stated (Martin, 1990; Fleagle, 1999). Compared to anthropoids, the strepsirhines tend to have small bodies and relatively small brains (when brain size is adjusted for body size). Many of the strepsirhines are nocturnally active. The smaller, nocturnal strepsirhines – such as bush babies (galagos), lorises, and mouse lemurs – tend to be solitary and subsist on a diet of insects, small vertebrates, fruit, and flowers. Some of the larger lemurs on Madagascar, however,

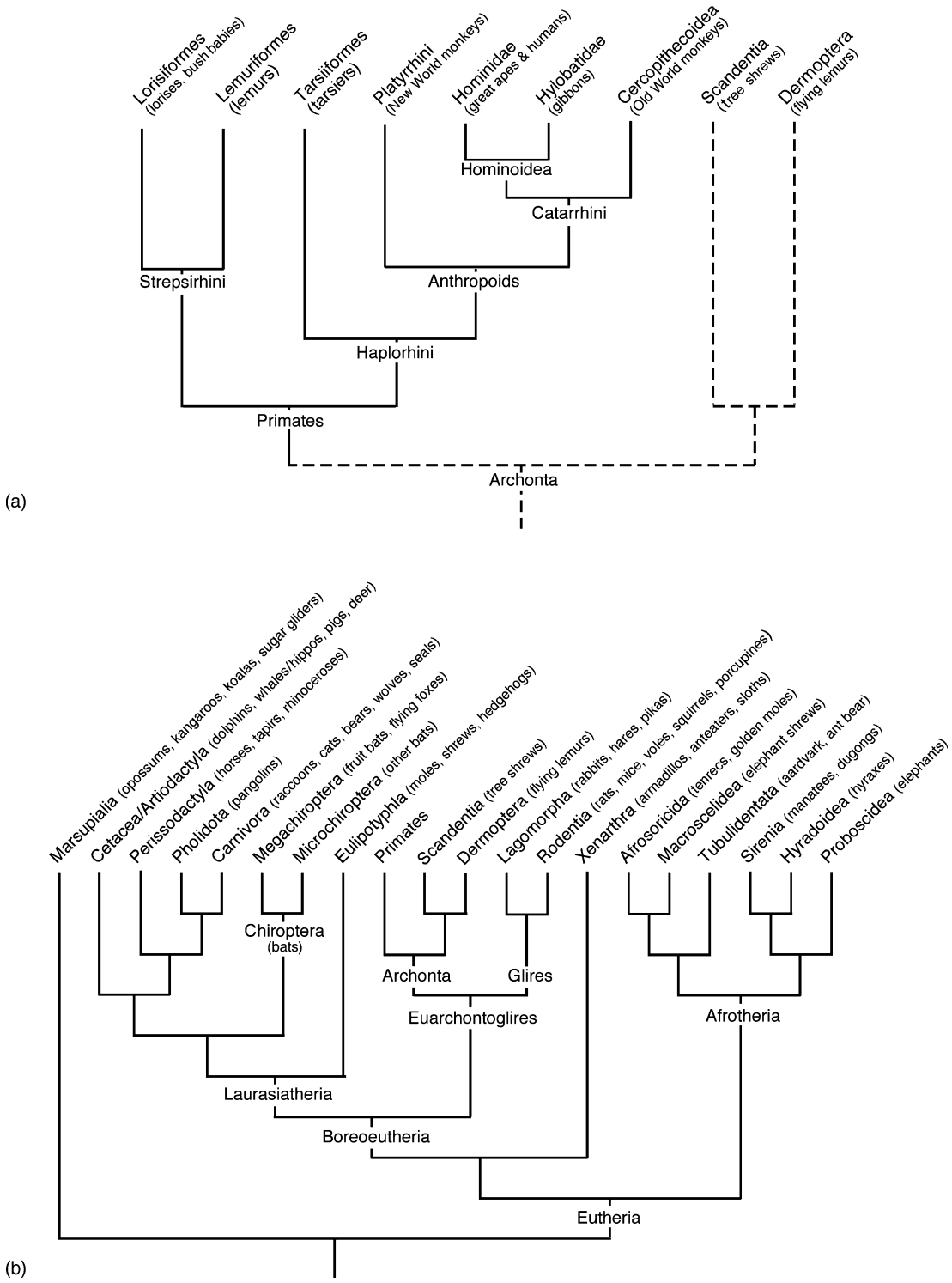

Figure 1 The evolutionary relationships of primates and mammals. a, The phyletic relationships of the major primate groups. Currently, tree shrews (order Scandentia) and flying lemurs (order Dermoptera) are the animals thought to be most closely related to primates. The Archonta is the higher-order group that includes Primates, Scandentia, and Dermoptera. b, A modern interpretation of the relationships of therian mammals, based on the comparative DNA studies of Murphy *et al.* (2001b). Murphy and colleagues recognized the close relationship between Primates, Scandentia, and Dermoptera (which they designated as 'Euarchonta' rather than Archonta). Note that the bats (order Chiroptera), in contrast to some other interpretations, are regarded here as monophyletic, but distantly related to primates.

are diurnal, live in social groups, and have a diet consisting primarily of plant material. By contrast to strepsirhines, the anthropoids are diurnally active (with the sole exception of the New World owl monkey, *Aotus*), and most live in sizable social groups, subsisting on fruit, flowers, gum, and leaves, and in some cases also insects and vertebrates. The tarsiers, although probably closely related to the anthropoids, resemble the smaller strepsirhines in being very small bodied, nocturnal, and solitary, and subsisting on a diet of invertebrates and small vertebrates. Tarsiers are readily identified by their enormous eyes.

4.01.2.2 Primate Evolution

4.01.2.2.1 Early views Not surprisingly, the history of ideas about primate and human evolution carries the very significant imprint of Charles Darwin (see especially Darwin, 1859, 1871). Darwin, of course, is a hero to modern evolutionary biologists, who tend to attribute to him a very modern view of evolution. In order to understand how Darwin influenced thinking about primate evolution, however, it is necessary to place him in proper historical context. Although Darwin is sometimes seen as a champion of the modern idea that phylogeny is like a branching tree rather than like an ascending scale – and there is indeed a prescient remark in one of his notebooks that suggests the primacy of the branching view of evolution – any fair reading of Darwin's large corpus of published writings makes it clear that he considered natural selection to be a process leading to progressive improvement, and that humans are, among animals, the most improved forms (Richards, 1992; Preuss, 1993, 1995b). Darwin, like most of the first generation of evolutionists, simply did not perceive a conflict between the branching-tree conception of evolution and the idea that evolution is a scale. As a result, early drawings of the tree of life generally have very stout vertical trunks, with humans at the top and little twigs branching off at lower levels of the trunk representing less-evolved (although nonetheless modern) forms. The archetype of this genre is the famous tree published by Haeckel (1874) and widely reproduced (see, e.g., figure 1 of Preuss, 2004b). Darwin, it should be said, was an unabashed fan of Haeckel (Richards, 1992).

For Darwin, then, an important part of the evolutionary story was about how to account for the ascendancy of humans among animals. In Darwin's view, the key to human success is not the human body, weak and feeble as it is, but rather the brain and the intellectual abilities it confers. It is important also to note, however, that Darwin – perhaps being concerned about drawing such a sharp line between humans and other animals as to create doubt about the theory of evolution – emphasized that the differences between the intellectual abilities of humans and other animals are matters of degree rather than kind. This is Darwin's 'Principle of Continuity', which has cast a very long shadow on the history of comparative psychology and neuroscience (Povinelli, 1993; Preuss, 1993, 1995b)

Theories of primate and human evolution developed during the early part of the twentieth century took their lead from Darwin, focusing on brain size and the conditions under which brain-size increases would be selected for (Cartmill, 1982). The British anatomists G. Elliot Smith and F. Wood Jones championed the idea that life in the trees selected for greater intelligence and enhanced vision (including the stereoscopic vision supposedly necessary for arboreal living). W. E. Le Gros Clark popularized these views in a series of books, including his widely read treatise, *The Antecedents of Man* (Le Gros Clark, 1959). Not only did Le Gros Clark promote the arboreal theory, he argued that the key to the success of primates was that they remained fundamentally unspecialized anatomically, the retention of a generalized, behaviorally adaptable skeleton serving as a better vehicle for the big brains of primates than the behaviorally limiting, specialized body forms evolved by terrestrial mammals. Thus, rather than being defined by a set of morphological specializations, primates were to be defined by a set of adaptive trends set in motion by an early commitment to arboreality: progressive enhancement of vision, reduction of olfaction, enlargement of the brain, and increasing intelligence. With respect to the brain, Le Gros Clark argued that as it became enlarged, the differences between regions became more sharply defined; however, the increased differentiation reflected the refinement of structural characteristics already present in more primitive forms rather the addition of new structural elements. This stance distinguishes Le Gros Clark from some other early neuroanatomists, notably Brodmann (1909), who believed that the greater histological differentiation of anthropoid and human cortex compared to other mammals represented the addition of new organs of mental function (Preuss, 1993).

Le Gros Clark also advanced a particular view of primate relationships and evolutionary history that proved extremely influential. He viewed primates as progressing through a series of ascending steps or grades reflecting the extent to which animals had

progressed along the adaptive trajectory initiated by arboreality, each grade being represented by some currently living forms. Arising from an ancestral insectivoran stock, the most primitive grade is represented by tree shrews, with lemurs, tarsiers, monkeys, apes, and humans representing successively higher evolutionary grades. Le Gros Clark did not think that humans are actually derived from modern lemurs, monkeys, or apes, of course, but rather that the modern forms have departed little from the ancient forms from which humans are descended. His inclusion of tree shrews among primates was controversial, although it enjoyed the support of the prominent paleontologist G. G. Simpson, and modern workers reject this view for reasons to be explained below.

The work of Le Gros Clark had a large impact on neuroscience. In part, this was because Le Gros Clark was himself an important neuroanatomist and had much to say about brain evolution. Perhaps more significant, however, was his treatment of the different groups of living primates as representatives of the stages of primate and human evolution. This led neuroscientists to bring insectivores (such as hedgehogs), tree shrews, and bush babies into the laboratory for study, particularly by Irving Diamond (Diamond and Hall, 1969; see also Hodos and Campbell, 1969). Diamond trained many students and had a strong hand in shaping modern comparative neuroscience. Le Gros Clark's impact is also apparent in the work of Heinz Stephan and his associates (Stephan and Andy, 1969) on evolutionary changes in the sizes of brain structures in primate evolution, who used hedgehogs and other supposedly 'basal' insectivores as stand-ins for the initial stage of primate evolution, tree shrews and other supposedly 'advanced' insectivores as stand-ins for the next higher stage, the living prosimians as representatives of the next stage, and so forth. Although the evolutionary concepts that inspired the work of Diamond, Stephan, and their colleagues are now considered to be flawed, the data generated remain invaluable.

4.01.2.2.2 Modern ideas about primate origins The 1960s and 1970s witnessed the emergence of a new movement in evolutionary biology, initiated by the writings of Willi Hennig (especially Hennig, 1966), and usually referred to as 'cladism'. Cladism comprises a set of methods for reconstructing the phyletic relationships of species based on comparative studies of their characteristics, and a set of rules for classifying organisms based on these relationships. (Not all who subscribe to cladistic ideas about reconstructing phylogeny adhere to these rules of classification.) The basic goal of a cladistic analysis is to identify groups of species that constitute the complete set of species descended from a common ancestral species; these groups are described as being 'monophyletic' and are called 'clades'. A clade is, simply, any complete branch of the evolutionary tree, complete with subsidiary branches. Cladistic analysis leads to the enumeration of sets of features of the last common ancestor (LCA) of a monophyletic group that distinguish that ancestor (and its descendants) from other taxa. These features are referred to as 'shared derived characters' or 'synapomorphies'. These are the features that define the group. Of course, the species making up the group will have many other features that arose much earlier in evolution and that are shared with a wide array of other species, but these features are not helpful for determining the relationships of the group with its close relatives. So, for example, the fact that primates and carnivores both have hair says nothing about whether or not carnivores are close relatives of primates, because hair is an ancestral feature of mammals and is present in most living mammalian forms. Cladism, then, inspires efforts to identify the set of shared, derived characters that define monophyletic groups (clades) and requires workers to grapple with the issue of whether characters shared by different groups of animals are shared by virtue of common descent, and thus should be considered synapomorphies, or as the result of independent, convergent evolution, and thus of no value in determining relationships (although convergence can be very helpful in understanding how organisms adapt to particular environmental circumstances).

With the rise of cladism, Le Gros Clark's approach to primate evolution became passé, his focus on grades giving way to an emphasis on identifying sets of characters that could serve as defining features of the primate clade and its subclades. This approach was explicitly adopted by Robert Martin and is now an accepted part of evolutionary primatology (see especially Martin, 1990, and the contributions in Ravosa and Dagosto, 2006). A survey of generally accepted primate synapomorphies would include: a divergent (grasping) big toe (hallux) and possibly also a grasping thumb; the presence of flat nails, rather than claws, on at least some digits; the presence of a complete ring of bone around the eye (i.e., a 'postorbital bar'); large, forward-facing eyes; brain enlargement; reduction of the olfactory apparatus; and enlargement of the visual apparatus.

One consequence of defining primates in this way was to remove tree shrews from the order

(see Martin, 1990, and the contributions in Luckett, 1980). Not only do tree shrews lack many of the defining anatomical characteristics of primates, but some of the ways in which tree shrews resemble primates, for example, the presence of an enlarged visual apparatus and of a complete orbital ring, came to be judged as convergences not relevant to common ancestry. Comparative studies of the nervous system have revealed many other ways in which tree shrews differ from undisputed primates (see especially the reviews of Campbell, 1980, and Kaas and Preuss, 1993).

Once one is focused on the character states that define a group, it is natural to try to reconstruct the anatomy and behavior of the LCA of the group and to ask why the defining features of the group evolved. The modern project of framing adaptive explanations of primate origins begins with the work of Cartmill (1972, 1974b) and Martin (1973) and continues today (for major reviews, see Martin, 1990; Cartmill, 1992; and the contributions in Ravosa and Dagosto, 2006). This led to a major re-evaluation of the arboreal hypothesis. By the 1970s, it was appreciated that the earliest primates were anatomically similar to some of the smaller-bodied living prosimians, such as the smaller bush babies (genus *Galago*), mouse and dwarf lemurs (*Microcebus*, *Cheirogaleus*), and tarsiers (*Tarsius*). Examination of their behavior suggests why primates have opposable first digits, and digits tipped with nails, rather than claws: animals such as *Galago* and *Microcebus* make their living in a particular kind of arboreal environment, specifically, in the fine, terminal branches of trees and shrubs (Charles-Dominique, 1977). Claws are of less utility for grasping fine branches than are opposable thumbs and big toes, and nails would provide support for the broad terminal segments for digital grasping. This, in brief, is the 'fine-branch niche' hypothesis (Martin, 1990).

Classically, the convergent, front-facing orbits of primates were explained as adaptations for binocular overlap and stereopsis, presumably required for safe arboreal locomotion. As Cartmill noted, however, there are many arboreal mammals (e.g., squirrels), that manage quite well in an arboreal environment despite having eyes on the sides of the head. Cartmill pointed out that living vertebrates such as raptors and cats that have convergent eyes tend to be predators. Recognizing that many of extant, small-bodied prosimians make their living at least in part by capturing insects and small vertebrates, often grabbing them with their hands, Cartmill proposed that the large, convergent eyes of primates are adaptations for predation, with stereoscopic vision enabling an accurate hand grab. Allman (1977) and Pettigrew (1978) helped flesh out this theory by suggesting that front-facing eyes might have less to do with stereoscopic vision than with establishing a direct light path between objects in the environment and the central retina, resulting in a sharper image. This advantage would be accentuated in a nocturnal environment.

Combining the arguments discussed above, we have a picture of the primate LCA as a small-bodied, nocturnal predator foraging in a fine-branched niche. This constitutes a sort of working hypothesis that frames modern research on primate origins. One matter of controversy is the relative importance of predation in shaping primate anatomy and behavior. Sussman, for example, has emphasized the importance of foraging for fruit and/or flowers – both available in terminal branches – in selecting for the grasping extremities of primates (Sussman and Raven, 1978; Sussman, 1991). Recent paleontological evidence suggests that the defining characteristics of primates did not emerge as a unitary adaptive package, but rather that grasping extremities evolved prior to orbital convergence (Bloch and Boyer, 2002).

4.01.2.2.3 Primates among mammals: Grandorder Archonta In order to understand primate specializations, it is necessary to reconstruct the ancestral condition from which the specializations evolved. In phylogenetics, one reconstructs ancestral organization by 'out group' analysis, that is, by studying the animals most closely related to the in-group. To understand primate specializations, then, we need to know who primates are related to. Recall that in the work of Le Gros Clark and G. G. Simpson, primates were regarded as emerging from an insectivoran stock. Indeed, for much of the twentieth century, insectivores such as hedgehogs and shrews were regarded as the wellspring from which all eutherian orders emerged, with the result that mammalian evolution was depicted as more like a bush than a tree. Not everyone took this view, however: early in the twentieth century, W. K. Gregory proposed that primates did not simply emerge from a generalized insectivore form, but instead were part of a small set of related mammalian taxa consisting of elephant shrews, tree shrews, bats, and the so-called 'flying lemurs', who are not lemurs at all but rather small animals adapted for gliding. He called this collection of primates and related mammals the grandorder Archonta (Gregory, 1910).

As cladism began to exert its influence, Gregory's ideas were resurrected by a number of workers, notably McKenna (1975). McKenna modified

Gregory's concept of a grandorder Archonta to consist of primates (order Primates), bats (order Chiroptera), flying lemurs (order Dermoptera), and tree shrews (order Scandentia). Subsequent research marshaled comparative data on many aspects of the biological organization of primates and their putative relatives, including skeletal anatomy, soft-tissue anatomy, and molecular biology, with the result that the Archonta concept is now widely endorsed (MacPhee, 1993; Ravosa and Dagosto, 2006). Despite this, there has been considerable wrangling over the pattern of interrelationships among the putative archontans as well as disagreements about which taxa should be included in Archonta. Some argued that bats were not a natural, monophyletic group and that only one group of bats, the megachiropterans, are closely related to primates, and are, indeed, the sister group of primates (Pettigrew, 1986; Pettigrew *et al.*, 1989). This is the 'flying primate hypothesis', which has spurred much debate (MacPhee, 1993).

Recently, comparative genomic studies were undertaken by O'Brien and colleagues with the goal of settling the question of how the different mammalian orders are interrelated (Murphy *et al.*, 2001a, 2001b). They examined 18 homologous DNA sequences from a wide array of mammals, drawn from genomic databases, and reconstructed the mammalian branching order using maximum-likelihood and Bayesian techniques (Figure 1b). It is perhaps too soon to conclude that these studies have settled all the really significant questions about mammalian relationships, but they have important implications for students of primate evolution. In particular, they support a modified version of the Archonta hypothesis. Interestingly, although their results support the monophyly of bats, they indicate that bats are distantly related to primates, and cannot be considered archontans, which in their system consist of primates, tree shrews, and flying lemurs. In addition, their results indicate that the rodents and lagomorphs form a monophyletic group (superorder Glires), which may be the sister group of the Archonta. Collectively, the clade consisting of Archonta (now also known as Euarchonta) plus Glires is referred to as Euarchontoglires.

4.01.2.2.4 Haplorhine/anthropoid origins and hominin origins In principle, any subsidiary branch of the evolutionary tree of primates can be subjected to the sort of analysis that has been applied to the tree as a whole, that is, to identify the shared, derived characters that define the group and to develop and test adaptive accounts to explain the evolution of those characters. Much effort has been devoted to exploring the origins of the haplorhine anthropoid primates (for reviews, see The Role of Vision in the Origin and Evolution of Primates and the contributions in Ross and Kay, 2004a). Among the derived features of haplorhines and anthropoids are several specializations of the eye: tarsiers and anthropoids possess a true avascular fovea and they lack the reflecting tapetum lucidum (presumably an adaptation for nocturnal vision) found in strepsirhines. In anthropoids, the eye is isolated in a bony cup that may help to stabilize the image on the retina and the concentration of cones in the central retina is much higher than in strepsirhines. These changes, plus the fact that all living anthropoids save *Aotus* are diurnal, suggest an early shift to diurnality in stem anthropoids and concomitant specializations for high-acuity vision under daylight conditions. The olfactory apparatus is reduced in haplorhines compared to strepsirhines. Anthropoids also tend to be larger bodied than strepsirhines and live in larger social groups, consisting of multiple adults. Early anthropoids known from the fossil record, however, were very small, suggesting that increased body size evolved after the emergence of stem anthropoids. The larger size of anthropoids implies dietary changes, as larger-bodied primates tend to meet their protein needs by consuming leaves more than insects. The masticatory apparatus of anthropoids is more robust than that of strepsirhines, which makes it possible for them to include harder food items (such as unripe fruit) in their diet.

An evolutionary scenario that has been proposed to account for these features of anthropoids holds that the earliest (stem) haplorhines were small, diurnal predators, which selected for high visual acuity, with later stem anthropoids evolving increased body size and increased amounts of plant material in the diet (Ross and Kay, 2004b). Increases in social group size may have evolved early: some early fossil species have sexually dimorphic canines, which suggests the existence of male–male intragroup competition. In this scenario, tarsiers are seen as an early offshoot of the lineage leading to anthropoids, in part because their visual systems share certain features of anthropoids, such as the presence of a fovea and the absence of a tapetum lucidum. The living tarsiers are also obligate small-animal predators. Although living tarsiers are nocturnal, they are thought to derive from diurnal ancestors. The scenario holds that although extant tarsier species are nocturnal, they evolved from a diurnal ancestor, resulting in loss of the tapetum lucidum, and have adapted to nocturnality by evolving enormous eyes, much as owl monkeys did.

Among anthropoids, the clade that has received the most attention with regard to its origins and adaptations is of course that consisting of humans and their close relatives. Humans (*Homo sapiens*) are a recent offshoot of the African apes, the branching date being 5–10 Mya (Fleagle, 1999). Our closest relatives are a clade consisting of the common chimpanzee (*Pan troglodytes*) and the bonobo (*Pan paniscus*), followed by gorillas (*Gorilla gorilla*). Traditionally, species connected to the branch leading to humans after its split from the African apes were classified as members of the family Hominidae, or colloquially, as 'hominids', and the domain of human origins was referred to under the rubric of 'hominid evolution'. The recent recognition of the propinquity of humans to the apes has led to the expansion of the category Hominidae to include the great apes and humans, and the expansion of the subfamily Homininae to African apes and humans. As a result, the branch leading uniquely to modern humans is usually classified today at the tribe level (a tribe being a sub-subfamily). Humans, therefore, belong to the tribe Hominini, and the study of human origins is referred to as 'hominin evolution' (see The Hominin Fossil Record and the Emergence of the Modern Human Central Nervous System).

We are fortunate to have an extensive physical record of human origins; this permits us to document evolutionary increases in hominin brain size from the study of fossils (see The Hominin Fossil Record and the Emergence of the Modern Human Central Nervous System) and behavioral changes from the archeological record. Hominins underwent a fantastic increase in brain size – modern human brains are approximately three times larger in volume than those of chimpanzees – and the fossil record indicates that most of the increase in brain volume took place during the last 2 million years. By contrast, bipedalism, that peculiar way humans have of getting around in the world, appeared much earlier in hominin evolution.

After many decades of focusing on the similarities between humans and apes, students of cognition have recently begun to appreciate the many respects in which humans are specialized relative to our ape cousins. Humans, of course, have a unique and highly developed system of symbolic representation and communication – language (see The Evolution of Language Systems in the Human Brain). The evolution of language may well be related to the extreme degree of hemispheric asymmetry exhibited by humans (see Nuclear Schizophrenic Symptoms as the Key to the Evolution of the Human Brain), although language is by no means the only function that is strongly lateralized in humans (see The Evolution of Hemispheric Specializations of the Human Brain). Recent research points to the existence of a variety of additional higher-order cognitive systems that mediate our understanding of the physical interactions of objects and the our inferences about the causes and mechanisms of the behavior of other organisms, and of ourselves (see Neurological Specializations for Manual Gesture and Tool Use in Humans, The Evolution of Human Emotion, Human Cognitive Specializations). The human propensity to concoct narrative accounts of events, and to maintain them even in face of contrary evidence (see The Interpreter in Human Psychology), would seem to reflect the interaction of the language system with systems for representing causation. Human cognitive specializations can in many instances be viewed as components of a broader human adaptation for culture, permitting individual humans to acquire the know-how, ideas, and values of their community, and making it possible for communities to adapt to an extraordinary variety of environmental conditions (Richerson and Boyd, 2005).

4.01.3 Topics and Issues in Primate Brain Evolution

4.01.3.1 Encephalization and Gross Morphology

We think of primates as being highly encephalized creatures. This is true of modern anthropoid primates, which are about twice as encephalized as 'average' modern mammals (Jerison, 1973). Modern strepsirhines, however, are not notably encephalized compared to other extant mammalian groups. Nevertheless, there is reason to think that brain size expansion was an early feature of primate evolution. As with other groups of mammals, notably carnivores, ungulates, and cetaceans, the order Primates underwent expansion of the brain relative to body size (encephalization) throughout its history (Jerison, 1973). At any given time period in the Cenozoic, however, primates appear to have been more encephalized than most of their contemporaries (Jerison, 1973). This was the case even early in the Cenozoic, prior to the diversification of the anthropoids. Moreover, Sacher (1982) argued that primates (including strepsirhines) commit a disproportionately large fraction of metabolic resources to brain growth in early development, so that at birth, primate brains are about 12% of total body size, compared to about 6% for most other mammalian orders.

Just as relative brain size increased independently in different mammalian orders, it is likely that there was some degree of independent encephalization among primate groups. Early primates from the Eocene (~55–34 Mya) that resemble modern strepsirhines in many features of anatomy were substantially less encephalized than modern strepsirhines. Early anthropoids may also have been less encephalized than modern anthropoids. For example, it has been argued that *Aegyptopithecus*, dated to about 33 Mya and generally accepted to be a catarrhine, had a brain about half the size of modern catarrhines of similar body size (Jerison, 1979). This suggests that although relative brain sizes are similar in New World and Old World anthropoids, some brain-size enlargement occurred independently in the two groups.

Primate brains are not simply uniformly enlarged versions of some common mammalian brain – particular regions, such as the neocortex, underwent disproportionate enlargement. The quantitative neuroanatomical studies of Stephan and colleagues, alluded to above, have documented differences in the sizes of brain components across a substantial range of primate and insectivoran species (Stephan and Andy, 1969; Stephan, 1972; Stephan *et al*., 1988), and their data set has been widely used to generate and test hypotheses about primate evolution. Unfortunately, however, most of the brain structures they measured are too large (e.g., neocortex, cerebellum) to correspond to meaningful functional units of the brains. Moreover, structures can undergo substantial modifications of internal organization without undergoing substantial size change. For example, the absolute size of the primary visual area is similar in humans and chimpanzees (Frahm *et al*., 1984), but the internal organization of the area is quite different in certain respects (Preuss and Coleman, 2002). Nevertheless, it is possible to say something about the relationship between the enlargement of particular brain regions in primate evolution and the evolution of the external form of primate brains.

The external morphology of primate brains reflects, to a considerable degree, the degree and pattern of enlargement of the visual cortex, which involved the expansion of the primary visual area, area V1 (an area common to most, if not all, other mammals), as well as the addition of new areas (see especially, Allman, 1977, 1982, 1999; Kass, 1977, 1982, 1987). In most strepsirhines and haplorhines, the cortex devoted largely or exclusively to the visual modality encompasses approximately half the cortical mantle (Allman, 1977, 1982). The expansion of area V1 was probably accompanied by the evolution of a distinctive sulcal configuration, the triradiate calcarine fissure, which consists of a retrocalcarine sulcus (the familiar 'calcarine fissure' of the neuroscientific literature) and ascending and descending branches expending from the anterior end of the retrocalcarine (Martin, 1990). This triradiate configuration is found in most living primates (Martin, 1990) and was probably present in early primates.

Expansion of the visual cortex is also reflected dramatically in the unusual morphologies of primate temporal lobes. In most extant strepsirhines and anthropoids, the temporal lobe forms a distinct ventral island of cortex, demarcated from the frontal and parietal lobes by a deep lateral (Sylvian) fissure. Much of the temporal lobe consists of visual cortex (the inferotemporal cortex) and multimodal cortex with major visual inputs (the superior temporal sulcal (STS) cortex). The inferotemporal and multimodal STS regions appear to be neomorphic in primates (Preuss and Kaas, 1999; Preuss, 2006). The ventral expansion of visual cortex influenced the morphology of adjacent regions. In most mammals, the hippocampus and associated tissues form an arc around the posterior end of the corpus callosum, whereas in strepsirhine and haplorhine primates, the hippocampus has a more inferior location, as though the temporal lobe in which it resides were rotated around the posterior pole of the callosum. The orientation of auditory cortex, located superiorly in the temporal lobe, appears to reflect the torque of the temporal lobe; in most mammals, the map of auditory frequencies in the primary auditory area (A1) is arranged with high frequencies anteriorly and low frequencies posteriorly, whereas in primates, high frequencies are represented in the superior and posterior part of A1 and low frequencies inferoanteriorly (Stiebler *et al*., 1997).

Although the visual cortex evidently underwent early expansion, it has been argued that the frontal lobe initially remained quite small, undergoing major expansion only later in primate evolution (Radinsky, 1970; Jerison, 1973; Gurche, 1982). Judgments of frontal lobe size depend, however, on how you determine the border between frontal lobe and its neighbors on the lateral surface, the parietal and temporal lobes, and this is not as simple a matter as one might suppose. In most anthropoid primates, there is a deep central sulcus that separates the frontal and parietal lobes. Tarsiers and most strepsirhines lack a central sulcus, however, and it is typically not apparent in the endocasts of early primates (Radinsky, 1970; Gurche, 1982), so the frontal–parietal boundary cannot be determined with any precision in the endocasts. We should therefore be cautious in drawing conclusions about

the size of the frontal lobe relative to other cortical regions in early primates.

Identifying the frontal–temporal boundary would seem to a more straightforward affair: in most modern strepsirhine and anthropoid primates, a deep Sylvian fissure divides the temporal lobe from the frontal and temporal lobes. Some of the endocasts of early primates, such as the specimen of the lemur-like *Adapis* illustrated by Radinsky (1970) and by Gurche (1982), also preserve an impression that is plausibly interpreted as a Sylvian fissure. Thus, it is tempting to conclude that the presence of a Sylvian fissure is an ancestral feature of primate organization.

Nevertheless, Sylvian fissure is evidently not, a feature of all primates, living and extinct. Tarsiers, for example, lack a Sylvian fissure (Le Gros Clark, 1959; Collins *et al*., 2005), so that the insular cortex, which is buried in the depths of the Sylvian fissure in most primates, is exposed on the surface of the tarsier brain (Woolard, 1925). The exposed insular cortex and adjacent portions of frontoparietal cortex appear to form a nearly vertical ridge where they join the remainder of frontal cortex. Le Gros Clark (1959) suggested that the peculiar morphology of tarsier brains results from the enormous enlargement of the orbits in these animals, effectively indenting the ventral portion of the frontal lobe and anterior portion of the temporal lobe. The morphology of tarsiers, however, may not be unique among primates: illustrations of endocasts of extinct primates from the Eocene show a diversity of sulcal patterns (Radinsky, 1970; Jerison, 1973; Gurche, 1982), some with typical Sylvian fissures and some that could have morphologies more like those of extant tarsiers. Moreover, the extant lemur *Daubentonia* (the aye-aye) also appears to lack a typical Sylvian fissure (Kaufman *et al*., 2005; Le Gros Clark, 1959), and the sulci that are present seem rather shallow for a brain of its size (Kaufman *et al*., 2005). Thus, we should probably be cautious in ascribing to the LCA of primates a Sylvian fissure morphology like that found in most extant primates.

A number of additional sulci have been described in primate brains. Primates, like other mammals, possess a rhinal fissure, which separates the hippocampal and entorhinal cortex from neocortex. Most primates possess a cingulate sulcus on the medial wall superior to the corpus callosum, although in smaller primates this may be just a shallow groove. Many extant strepsirhines possess on the lateral surface of the hemisphere an elongated, anteroposteriorly situated groove, termed the coronolateral sulcus (Radinsky, 1975), which in smaller-brained forms may be broken up into two or three segments, that have been termed from posterior to anterior, the intraparietal sulcus (IPS), sulcus e, and sulcus rectus (Connolly, 1950; Haines *et al*., 1974). The sulcus rectus, a term which has also been applied to the principal sulcus (PS) of Old World monkeys (Falk, 1978, 1982), has also been referred to as sulcus frontalis in lemurs (Brodmann, 1905, 1909). The use of the terms 'intraparietal sulcus' and 'sulcus rectus' (or 'principal sulcus') suggests that the cortical areas within which these grooves are found in strepsirhines are homologous to the areas within which the corresponding grooves are found in anthropoids, and specifically in Old World monkeys. Caution is in order, once again: Preuss and Goldman-Rakic (1991c), in their studies of frontal lobe organization in *Otolemur* (formally *Galago*) *crassicaduatus*, argued that the sulcus rectus marks the border between premotor and granular prefrontal cortex, and thus differs from the PS of macaques, which lies entirely within prefrontal cortex. Similarly, they noted that whereas the IPS of macaques separates area 7 and its subdivisions from area 5, the IPS of *Otolemur* lay entirely within the territory of area 7 (Preuss and Goldman-Rakic, 1991a). Thus, inferences about the areal organization of the cortex of different primates based on the configurations of sulci are problematic, especially when comparing relatively distantly related taxa such as *Macaca* and *Otolemur*.

4.01.3.2 Chemical Senses

Whereas primates are characterized by a well-developed visual system, evolution of the chemical senses mainly involved reductions or degradative changes (Figure 2). Evolutionary reductions are especially notable in the main and accessory olfactory systems, changes that were, however, probably concentrated in haplorhine or anthropoid phylogeny. By contrast to haplorhines, strepsirhines have comparatively well-developed olfactory systems, and it is likely that they retain many features shared with other mammalian groups. Unfortunately, we have very little modern information about the olfactory systems of the taxa most closely related to primates.

Evolutionary changes in the olfactory systems in primates were accompanied by changes in the anatomy of the primate face. Most mammals possess two distinct olfactory systems: a main system, with receptors in the epithelium of the nose, and an accessory system, with receptors located in a specialized zone of epithelium buried within the upper jaw known as the vomeronasal organ (VNO; also known as Jacobson's organ) (Martin, 1990). The

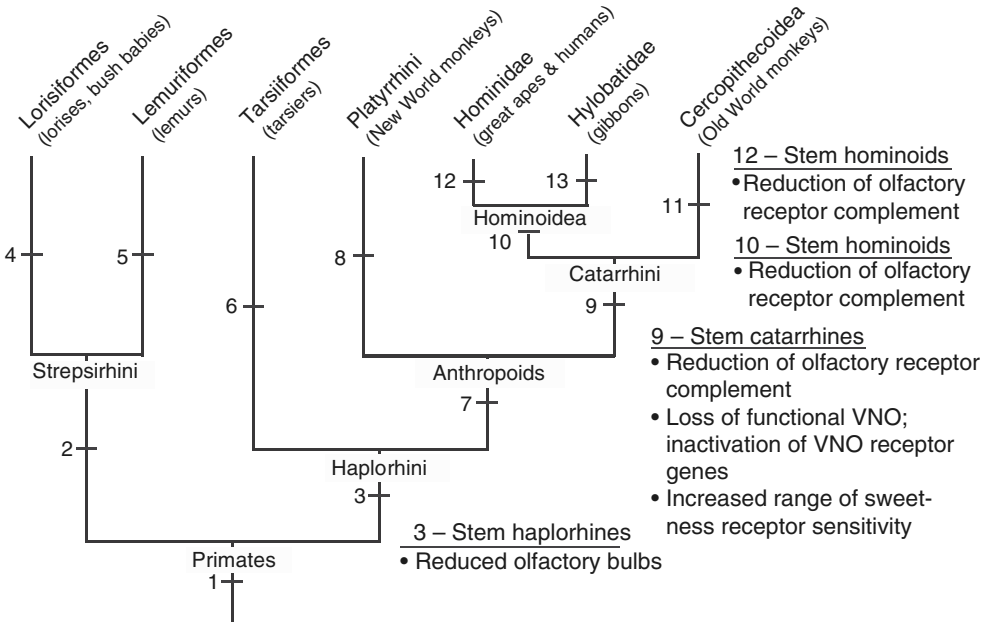

Figure 2 Evolutionary changes in the portions of the central nervous system devoted to the chemical senses. In this and following figures, characters interpreted as derived are mapped onto specific segments of the primate tree; the segments are numbered to facilitate comparisons across figures.

accessory olfactory system is usually thought to be involved principally in the detection of pheromones. In most mammals, there is a physical connection between the two olfactory systems. Most mammals have a naked, glandular external nasal membrane – a wet nose, in other words. This membrane extends down to the mouth where it is anchored to the soft tissue of the upper jaw. Some mammals (including strepsirhine primates) have a well-developed median cleft extending from the external nose to the space between the upper incisors. Ducts in the anterior palate allow passage of liquid from external nasal membrane to Jacobson's organ. Ducts also interconnect Jacobson's organ with the nasal cavity, superiorly.

Although the familiar haplorhine/anthropoid primates lack an exposed rhinarium of the type just described, the living strepsirhine primates exhibit this ancestral mammalian condition. The retention of ancestral nasal morphology in strepsirhines is accompanied by a generally well-developed central olfactory systems: compared to haplorhines, the main olfactory bulbs are quite large (Stephan *et al.*, 1988). The VNO and accessory olfactory bulbs are present, consistent with the apparent importance of pheromonal communication in extant strepsirhine primates (Charles-Dominique, 1977).

The VNO, however, underwent dramatic changes in anthropoid evolution. New World monkeys evidently possess functional VNOs, but they appear to be greatly reduced in Old World anthropoids, to the point of being vestigial (hominoids) or absent (in Old World monkeys) (see The Vomeronasal Organ and Its Evolutionary Loss in Catarrhine Primates). This doesn't necessarily imply that pheromonal communication is absent in Old World anthropoids, as it is possible that pheromones can be detected by receptors of the main olfactory system (Wysocki and Preti, 2004).

Information from olfactory receptors in the nasal epithelium reaches the brain through projections to the olfactory bulbs, which project in turn to the thalamus, amygdala, and ultimately to portions of the orbital and insular cortex. Taste inputs are relayed through the brainstem and thalamus, from which they also reach orbito-insular cortex. We know relatively little about evolutionary changes in the central pathways or structures representing the chemical senses in primates. One change that is well documented, however, is the evolutionary reduction in the size of the olfactory bulb in haplorhines: by comparison to haplorhines, the olfactory bulbs of strepsirhines are enormous (Stephan and Andy, 1969; Stephan, 1972; Stephan *et al.*, 1988). The olfactory systems of strepsirhines are clearly more conservative than those of haplorhines.

The sequencing of the genomes of a variety of mammalian species has spurred comparative studies of olfactory receptor (OR) and VNO receptor genes. These make ideal subjects for comparative

genomics, because mammals possess more than 1000 different OR genes, comprising multiple gene families, and on the order of about 250 VNO receptor genes. These investigations indicate that changes in olfactory and VNO receptor genes parallel the morphological changes described above. The morphological reduction of the olfactory system was accompanied by the accumulation of mutations that transformed functional OR genes into pseudogenes (see The Loss of Olfactory Receptor Genes in Human Evolution). The fraction of OR genes that is nonfunctional is higher in Old World monkeys than New World monkeys, higher still in hominoids, and highest in humans, among the hominoids that have been examined (Rouquier *et al*., 2000; Gilad *et al*., 2004). In humans, more than 50% of the OR genes have mutations that should render them nonfunctional (Gilad *et al*., 2005). This suggests a relaxation of selection pressures on OR genes in catarrhine primates, at least. Despite this, there is also evidence for positive selection in certain subsets of OR genes, even in humans (Gilad *et al*., 2005). Similar to the situation in the olfactory system, the morphological diminution or loss of the VNO in catarrhines was accompanied by the fixation of mutations that render inactive the *TRPC2* and *V1R* genes, which code for proteins essential for transducing signals from VNO receptors (see The Vomeronasal Organ and Its Evolutionary Loss in Catarrhine Primates; see also Liman and Innan, 2003; Zhang and Webb, 2003).

We know very little about the evolution of taste in primates, although comparative molecular and genetic studies are providing some new insights. In humans, five basic tastes are recognized: sweet, sour, bitter, salty, and umami (a savory flavor) (see The Evolution of the Sweetness Receptor in Primates). The number of taste-receptor genes is evidently much smaller than the number of OR genes. Three genes in the *TAS1R* family are believed to be involved in the perception of sweet and umami, with the protein products of the different gene family members combining to form heterodimers with different sensitivities. Bitter perception is mediated by a set of approximately 25 genes in the *TAS2R* family. The basis of salty and sour perception is poorly understood (Drayna, 2005).

Glaser has investigated the evolution of sweet perception in primates and found that humans and other catarrhines perceive more chemical compounds as being sweet than do other primates. The basis for this increased range of sensitivity reflects modifications of the structure of *TAS1R* receptors rather than the addition of new receptors (see The Evolution of the Sweetness Receptor in Primates).

4.01.3.3 The Visual System

4.01.3.3.1 Eye and retina Ancestral primates are thought to have been nocturnal animals, and the eyes of modern strepsirhine primates, most of which are nocturnal, retain the hallmarks of this heritage. For example, strepsirhines, in contrast to haplorhines, lack a retinal fovea and most strepsirhines have a reflective membrane, the tapetum lucidum, that is found in many nocturnal mammals and presumably enhances vision under low-light conditions (Figure 3). The tapetum is absent in some modern diurnal lemurs (Martin, 1990). The complement of photoreceptor cells in strepsirhine primates is also typical of nocturnal mammals: in addition to rods, which respond to light over a broad spectrum of wavelengths, strepsirhines have two varieties of cones, which respond to short-wavelength light (S cones) and medium- to long-wavelength light (M/L cones). Most nonprimate mammals that have been examined also have two-cone systems (Jacobs, 1993). Interestingly, mutations of the gene coding for the S-cone visual pigment have rendered this gene nonfunctional in the lorisiform (loris–bush baby) group of strepsirhines, which are nocturnal, and also in the nocturnal New World owl monkey, *Aotus* (Jacobs *et al*., 1996a; Kawamura and Kubotera, 2004). Although lacking a fovea, strepsirhines do possess a central specialization with a high density of photoreceptors, so that visual acuity is greatest in the central part of the visual field. The central specialization is populated by small rods and cones. In contrast to anthropoids, in which rods and cones are distinguishable by the morphology of their outer segments, strepsirhine rods and cones are similar in appearance and the existence of cones in strepsirhines was not definitively demonstrated until antibodies for cone-specific opsins were developed (Wikler and Rakic, 1990).

The eyes of tarsiers and of anthropoids differ markedly from those of strepsirhine primates. As both taxa lack a tapetum lucidum, this was probably lost prior to the tarsier–anthropoid divergence. A fovea is present in *Tarsius* and in anthropoids, and presumably evolved in stem haplorhines (Ross, 2004) although the density of retinal ganglion cells (the cells that transmit visual information to the brain) in the tarsier fovea is more typical of a nocturnal strepsirhine than a diurnal anthropoid (Collins *et al*., 2005). Tarsier foveae contain both rods and cones (Hendrickson *et al*., 2000), whereas the central retina (containing the fovea) of diurnal anthropoids is populated by small cones to the virtual exclusion of rods.

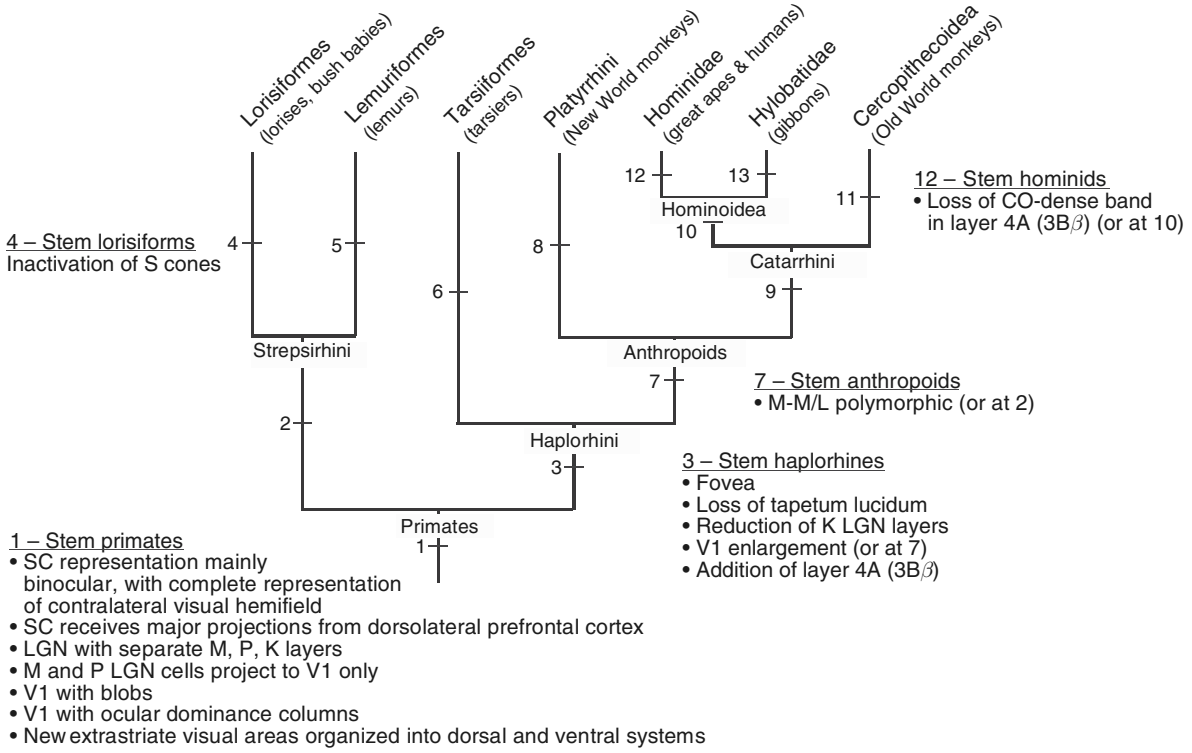

Figure 3 Interpretation of the shared derived features of the visual system in primates and major primate subgroups. For more details of the visual system specializations of hominoids, see Preuss and Coleman (2002) and Preuss (2004a).

The ancestral cone complement of haplorhines was most likely a two-cone system (Heesy and Ross, 2001), although it may have been a polymorphic system with some individuals having two cones and other individuals having three (Tan and Li, 1999). Most New World monkeys have only a single M/L gene, located on the X chromosome, but there are different alleles for this gene, each allele producing a protein with a different spectral sensitivity. Thus, most New World monkey populations are polymorphic for cone opsins, and because the M/L alleles are located on the X chromosome, many females have two different M/L alleles. These individuals are psychophysically trichromatic (see The Comparative Biology of Photopigments and Color Vision in Primates). Catarrhine primates have a three-cone system, the ancestral M/L opsin gene having duplicated to yield different M and L genes, both located on the X chromosome. (A similar event occurred independently in the New World howler monkeys, *Alouatta* spp.; Jacobs *et al.*, 1996b). Extant tarsier species have two-cone systems, but there is sufficient variation in the M/L genes of different tarsier species to prompt the suggestion that the ancestral condition for tarsiers (and thus for haplorhines, given the situation in New World monkeys), was polymorphic (Tan and Li, 1999).

The recent appreciation that many nocturnal primates have functional cones has had a substantial impact on thinking about color vision and its role in primate evolution. For one, it is now quite common to see primates referred to as 'trichromats', 'dichromats', or 'monochromats', based on their complement of cone pigments. For another, the likely existence of a two-cone system in ancestral primates is regarded by some as being incompatible with the claim that the primate LCA was nocturnal (Tan *et al.*, 2005). The latter conclusion would seem to assume that the function of cones is to permit color discrimination of the sort anthropoid primates are capable of. Anthropoid color discrimination certainly requires conditions of high illumination, so that color discrimination is essentially lost under nocturnal conditions. The relationship between cone types and color perception is by no means direct, however. So, for example, the ability to discriminate green and red in catarrhine primates is not simply a consequence of the fact that catarrhines have dedicated green and red photoreceptors. Although it is tempting to think of the M and L receptors as green and red receptors, in fact, the sensitivity peaks of the M and L pigments are about 530 and 560 nm, respectively, which correspond to the green and yellow-green parts of the visible

spectrum. The discrimination of red and green depends on the opponent (subtractive) interactions in the retina between M and L cones. These kinds of interactions evidently require high cone densities, such as those which occur in the anthropoid fovea (Dacey, 2000; Callaway, 2005). Yet anthropoids have cones in the retinal periphery, where they occur at much lower density than in the fovea, and many other mammals, both nocturnal and diurnal, have cones present at low density across the retina. The comparative psychophysical data suggest that nocturnal species have very poor color discriminative abilities, even those that have functioning S and M/L cones (see The Comparative Biology of Photopigments and Color Vision in Primates; Jacobs and Deegan, 2003). Indeed, even diurnal lemurs have very poor color discrimination (Blakeslee and Jacobs, 1985). It seems likely that the cones of most mammals are mainly doing something other than generating color-opponent signals such as those that form the basis for the fine color discrimination of anthropoids, for example, enhancing detection sensitivity in certain parts of the spectrum (e.g., Martin, 1990, p. 303; Winter *et al.*, 2003). It would be useful to have more experimental behavioral data about the vision of strepsirhine primates and nocturnal mammals; genetic, physiological, and anatomical studies of the visual systems of nonhuman species have advanced in recent years, but psychophysical and behavioral studies have not kept pace. We should be very cautious about making inferences about the color-vision capabilities of animals based on the presence or absence of cones expressing particular photopigments. To do so is, in effect, to adopt the anthropoid fovea – as specialized a bit of neural machinery as any we know of – as a general model of the mammalian retina.

4.01.3.3.2 Lateral geniculate nucleus and superior colliculus The retina sends visual information to numerous brainstem structures, of which we will consider only two here, the lateral geniculate nucleus (LGN) and the superior colliculus (SC), both of which underwent important changes in primate origins and evolution. The output from the retina is conveyed by the axons of retinal ganglion cells (RGCs). Currently, three main classes of RGCs are recognized, which in primates are usually termed M, P, and K cells, because they project to the magnocellular, parvocellular, and koniocellular layers of the LGN, respectively. These are probably homologous to the Y-, X-, and W-type cells described in other mammals (see The Evolution of Parallel Visual Pathways in the Brains of Primates). The different types of RGCs convey different types of visual information. At least some of the P-type cells carry color-opponent information, but it is likely that some P-type cells are not color selective and there is evidence that some K-type cells have a special relationship to S cones, so these may be involved in color processing as well (Callaway, 2005).

The LGN is important as a relay for visual information to the cerebral cortex. The structure typically appears to be composed of separate cell laminae, and in primates the lamination is very conspicuous. Moreover, primate LGNs are laminated in distinctive ways (see especially Kaas *et al.*, 1978; Kaas and Preuss, 1993; see The Evolution of Parallel Visual Pathways in the Brains of Primates and citations therein). In most mammals that have been studied in detail, M- and P-like cells are mixed in at least one layer of the LGN. In primates, however, M and P cells are strictly segregated, and there is a pair of M and P layers, one for each eye, yielding a fundamental four-layered pattern. In catarrhines, including humans, the P layers subdivide and interleave, yielding the 'six-layered' pattern described in neuroanatomy textbooks. Strepsirhine primates also have a pair of distinct K layers, sandwiched between the two P layers. In anthropoids, the K cells do not form distinct layers, but rather mainly occupy territories between the M and P layers. The nocturnal owl monkeys are unusual among anthropoids in having a well-developed single K zone located between the outermost M layer and the innermost P layer. Interestingly, tarsiers are reported to resemble anthropoids more than strepsirhines in their pattern of LGN lamination, and like owl monkeys, have a relatively well-developed K zone between the M and P layers (Collins *et al.*, 2005).

The SC occupies the most superior part of the midbrain. It is homologous to the structure referred to as the 'optic tectum' in most vertebrates, a name that reflects the fact that this structure plays an important role in visually guided behavior. The SC is a laminated structure; the upper layers receive visual inputs while the deeper layers receive auditory and somatosensory inputs (Huerta and Harting, 1984). Outputs from the SC descend to motor nuclei in the brainstem, especially those involved in eye movements, while ascending projections target the K layers of the LGN and the inferior pulvinar nucleus of the thalamus. Large regions of the neocortex project to the SC, although primates are distinctive among mammals in having a massive projection to the SC arising from the dorsolateral prefrontal cortex (Preuss, 2006).

Primates are also distinctive in the pattern of retinal projections to the SC. In most mammals, the SC receives its visual inputs mainly from the retina of the contralateral eye, with a small and variable contribution from the ipsilateral retina. In primates, by contrast, the SC receives strong inputs from both retinas, but only from the portion of each retina that represents the contralateral visual field. Thus, each SC in primates contains a 'map' of the contralateral half of visual space, whereas nonprimate SCs represent both contralateral and ipsilateral visual space, since the contralateral retina represents both parts of visual space (Lane *et al.*, 1973). This primate–nonprimate difference is well established (reviewed by Kaas and Preuss, 1993; Preuss, 2006). Pettigrew and colleagues have argued that megachiropteran bats have a primate-like SC, and cite this and other evidence that megachiropteran bats should be considered the sister group of primates (Pettigrew, 1986; Pettigrew *et al.*, 1989). The primate-like character of megachiropteran bats has been disputed (Thiele *et al.*, 1991), and defended (Rosa *et al.*, 1996), but in any event, there is now considerable evidence from comparative genomic studies indicating that megachiropteran bats are not closely related to primates (Murphy *et al.*, 2001b). To the extent that megachiropterans resemble primates, therefore, those similarities are probably the result of evolutionary convergence. Preuss (2006) suggests that the adaptive significance of the primate specialization of SC organization lies in the fact that individual SC neurons receive inputs from both eyes, and speculates that the primate SC uses binocular disparity to make precise adjustments of eye movements and visually guided reaching and grasping movements.

4.01.3.3.3 Primary visual area The principal target of projections from the visual system to the cortex is area V1, also known as area 17 (after Brodmann, 1909) and as the striate area, so-called because of the horizontal stripe of myelin characteristic of this area in primates. Area V1 is present in all mammalian groups that have been studied and is undoubtedly one of the 'heritage' areas that was present in the early mammals (Kaas, 1995). In primates, as in other mammals, visual information reaches area V1 via the LGN and from the pulvinar nucleus of the thalamus (in primates, from the inferior part of the pulvinar, specifically). Notwithstanding these commonalities, primates possess specializations of V1 that distinguish them from other mammals, and there are also prominent differences in V1 organization within the primate order (see The Evolution of Parallel Visual Pathways in the Brains of Primates; Kaas, 1993; Preuss, 2004a, 2006).

One distinctive feature of primate V1 is its pattern of inputs from the LGN (reviewed by Preuss, 2006). Virtually the entire cortical projection of the LGN reaches area V1 exclusively; only the K layers of the LGN project to other, 'extrastriate' cortical visual areas, and those projections are rather weak. In the nonprimates that have been examined, there are major LGN projections to extrastriate areas. The responsiveness of extrastriate cortex to visual stimulation depends more strongly on V1 in primates than in other mammals.

Another distinctive feature of primate V1 is its appearance when stained for cytochrome oxidase (CO), a metabolic enzyme. CO staining reveals a regular, repeating series of dark patches in the upper layers of area V1; these patches are technically known as 'blobs'. Blobs have been described in all strepsirhine and haplorhine primates that have been examined with appropriate histological material (Preuss and Kaas, 1996), including, most recently, tarsiers (Collins *et al.*, 2005). Tree shrews lack blobs, as do representatives of many other mammalian orders that have been examined for blobs (reviewed by Preuss, 2006). Some carnivores exhibit blob-like staining, but because carnivores are not closely related to primates, this is almost certainly a case of convergence (Preuss and Kaas, 1996; Preuss, 2000a). Although blobs are a common feature of primate organization, their connections and functions are matters of debate. Published descriptions of connectivity indicate that blobs receive direct projections from the LGN, including the K layers, but details of these connections differ between studies and there could be genuine species differences (see The Evolution of Parallel Visual Pathways in the Brains of Primates).

The strongest projections to V1 from the LGN in primates, as in most other mammals examined, terminate in the middle levels of cortex, specifically within a stratum of very densely packed small cells (granule cells) designated as cortical layer 4. Projections from the P layers target the deep part of layer 4, whereas projections from the M layers target its more superficial part. Interlaminar connections relay connections from layer 4 to more superficial and to deeper layers of cortex. This pattern of organization is common to the strepsirhine and anthropoid primates that have been examined. Anthropoid primates, however, have elaborated the organization of the upper layers of cortex. In particular, anthropoids possess an additional band of densely packed small cells above layer 4 that is separated from it by a narrow band of larger, more sparsely arranged cells. There are different ways of naming these layers: most workers follow

(Brodmann, 1909) in referring to the upper band of small cells as layer 4A, the sparse band of larger cells as layer 4B, and the deeper, thick band of small cells as layer 4C. Others believe that the bands called 4B and 4A by Brodmann should be considered as subdivisions of layer 3; in this terminology, Brodmann's layer 4A corresponds to layer 3Bβ (see The Evolution of Parallel Visual Pathways in the Brains of Primates). In most New World and Old World monkeys that have been examined, layer 4A/3Bβ receives a direct input from the parvocellular layers of the LGN, and there is a band of dense CO staining coincident with this input. Among New World and Old World monkeys, only *Aotus* is known to lack a direct LGN projection to layer 4A/3Bβ, and it lacks the corresponding CO-dense band, also (Horton, 1984). Although we do not have information about the connectivity of tarsier V1, histologically, the lamination of this area resembles that of anthropoids more than strepsirhines (Collins *et al.*, 2005). We also have virtually no information about the connectivity of area V1 in apes or humans, but its histology differs from that of monkeys: a band of small cells corresponding to layer 4A/3Bβ is present, but it lacks a CO-dense band (Preuss *et al.*, 1999), which suggests that its connectivity differs from that of monkeys. Additionally, layer 4A/3Bβ of humans exhibits histological features that differ markedly from those of apes (Preuss *et al.*, 1999; Preuss and Coleman, 2002). Since area V1 is a major source of visual information for extrastriate visual areas, these species differences in the processing of visual information in V1 could ramify through the cortical visual system.

In primates, the projections of the LGN to layer 4 of area V1 are segregated by eye, forming a set of alternating, elongated ocular dominance columns (Hubel and Wiesel, 1969). Although the degree of ocular segregation in adult individuals varies across species, some degree of ocular segregation has been found in every primate species examined, while ocular dominance is absent in the nonprimate species that have been examined with the exception, again, of carnivores (Horton and Hocking, 1996).

4.01.3.3.4 Extrastriate visual cortex Primates possess a large region of extrastriate visual cortex comprised of multiple, retinotopically organized visual areas (see, for example, Visual Cortex: Evolution of Maps and Mapping and the reviews of Kaas, 1995; Tootell *et al.*, 1996; Rosa, 1999; Orban *et al.*, 2004; Sereno and Tootell, 2005). These have been extensively studied in the New World and Old World monkeys, and to a lesser extent in strepsirhine primates, using microelectrode mapping techniques, tract-tracing experiments, and histochemical (architectonic) methods. Histochemical and functional imaging techniques have recently been applied to the study of extrastriate cortex in humans. From these studies, it is clear that there is a large number of extrastriate visual areas in anthropoids – at least 15 and perhaps many more. The diversity of opinion regarding the numbers of areas reflects in part the application of different criteria for assigning areas to the visual realm – does an area need to be exclusively visual to qualify, or need it only have a prominent visual input? – as well as differing interpretations of experimental data. Within the primate extrastriate cortex, investigators have determined that the extrastriate areas and their interconnections form two broad information processing pathways: a dorsal stream, which begins in V1, traverses the middle temporal (MT) visual area, and ends in the posterior parietal cortex, and a ventral stream, which begins in V1, traverses area V4 (also called the dorsolateral visual area, DL, in New World monkeys), and ends in the inferior temporal (IT) region. These pathways are functionally specialized: the ventral and dorsal pathways have been characterized as the 'what is it' and 'where is it' systems, respectively (Ungerleider and Mishkin, 1982). While recent work supports the idea that the ventral pathway is specialized for the visual identification of objects, it has been argued that the functional specialization of the dorsal pathway pertains to 'vision for action', that is, for organizing eye and hand movements to objects in nearby space (Goodale and Milner, 1992).

Present evidence does not provide a definite indication of differences in the complement of extrastriate areas between Old World and New World monkeys. Moreover, the evidence from humans suggests a pattern of extrastriate organization similar in many important respects to that of Old World and New World monkeys (Orban *et al.*, 2004; Sereno and Tootell, 2005). One might reasonably suppose, however, that anthropoid primates have more visual areas than strepsirhines. For one thing, the evolution of haplorhines and anthropoids was accompanied by the appearance of a fovea and modifications of retinal receptor distribution and circuitry supporting color vision. For another, the visual region makes up a large fraction of the cortical mantle in strepsirhines and haplorhines, but strepsirhines are about half as encephalized as anthropoids. Nevertheless, at present there is no clear evidence that strepsirhines have fewer visual areas than do New World or Old World monkeys. This may reflect the fact that much less effort has been devoted to the study of visual cortex in

strepsirhines than in anthropoids. This said, however, the studies that have been carried out in strepsirhines indicate that they possess many areas in common with anthropoids (e.g., Rosa *et al.*, 1997; Lyon and Kaas, 2002), and there is evidence from histological and connectional studies that the strepsirhine extrastriate region is divisible into dorsal and ventral streams, as in anthropoids (Preuss, 2006). (It would be very valuable to compare the organization of inferotemporal cortex in strepsirhines and in anthropoids, since this region would likely have been affected by the evolution of specializations of foveal vision that characterize anthropoids.) Moreover, cortical enlargement in anthropoids probably reflects in part the increased size of individual areas: area V1, in particular, is much larger in anthropoids than in strepsirhines of similar body size (Frahm *et al.*, 1984).

The organization of primate extrastriate cortex differs markedly from that of nonprimate mammals. Most of the nonprimates that have been studied – including even tree shrews of the genus *Tupaia*, which, as Le Gros Clark emphasized, are diurnal animals with well-developed visual systems – appear to have a small complement of visual areas, with homologues of the first and second visual areas (V1, V2), and perhaps four or five additional areas (Lyon *et al.*, 1998; Rosa, 1999). Certain groups of mammals, notably carnivores, independently evolved large complements of cortical visual areas. It follows, then, that many of the extrastriate areas of primates evolved after the separation of the primate lineage and therefore have no homologues in other mammals. Moreover, the visual areas of nonprimate mammals (even carnivores) do not appear to be comprised of functionally distinct dorsal and ventral streams, as they are in primates (Preuss, 2006).

Although the living primates share many common features of visual cortical organization, there are also differences. The best documented differences involve the intrinsic organization of particular visual areas. As noted above, the layering of area V1 varies markedly across primate taxa. V2 exhibits differences as well: when stained for CO, most anthropoids exhibit well-demarcated thick and thin stripes that extend from the caudal border to the rostral border of V2, while strepsirhines show much less distinct banding (Preuss *et al.*, 1993). The functional consequences of these variations in area V2 organization have not been investigated. Humans also are reported to have indistinct V2 bands (Tootell and Taylor, 1995). There are, in addition, differences in the functional properties of homologous areas between anthropoid primates. For example, area V3 of macaques is more motion sensitive than its homologue in humans, whereas area V3A of humans is more sensitive to motion and contrast than area V3A of macaques (see the reviews in Preuss, 2004a; Visual Cortex: Evolution of Maps and Mapping).

4.01.3.4 Somatosensory and Motor Systems

It is useful to consider the central somatosensory and somatic motor systems together (Figure 4), as they are intimately related structurally and functionally (see the reviews of Kaas and Pons, 1988; Kaas, 2004; see The Evolution of Sensory and Motor Systems in Primates). For example, the fine control of grasping forces is mediated by transcortical processing loops: sensory signals originating in the hands and feet are relayed through the spinal cord to the brainstem, thalamus, and somatosensory cortex, from there to motor cortex, and from motor cortex through the corticospinal tract (CST) to the motor neurons controlling digit flexion and extension (e.g., Evarts and Fromm, 1981). Similarly, the 'haptic' sense involves the assessment of somatosensory feedback about the shape, texture, compressibility, and other physical characteristics of objects resulting from grasping and manipulating them (e.g., Lederman and Klatzky, 2004).

4.01.3.4.1 Peripheral mechanisms Primates are characterized by specializations of the extremities related to grasping, including opposable first digits. Grasping abilities vary widely among primates, however (Bishop, 1964; Napier, 1993). Strepsirhines and most New World monkeys have little, if any, independent digit control. Catarrhine primates, as well as the New World *Cebus* monkeys, have a much greater degree of independent digit control. In apes and humans, the grasping ability of the thumb is greatly increased: the pad of the thumb can be opposed to the pads of other digits, yielding a precision grip. In humans, this capacity is extremely well developed and is accompanied by modifications of the hand bones and muscles (Susman, 1994). Primate grasping is facilitated by the presence on the ventral surfaces of the hands and feet of large regions of hairless (glabrous) skin with complex patterns of epidermal ridges. This 'dermatoglyph' skin, which is thought to reduce slippage, is also present in tree shrews and in arboreal marsupials (Cartmill, 1974a; Martin, 1990). Some New World monkeys have increased their grasping abilities by evolving prehensile tails, the pads of which also possess dermatoglyph skin. Primates also appear to differ from most other mammals in their fundamental pattern of gait: most primates have a

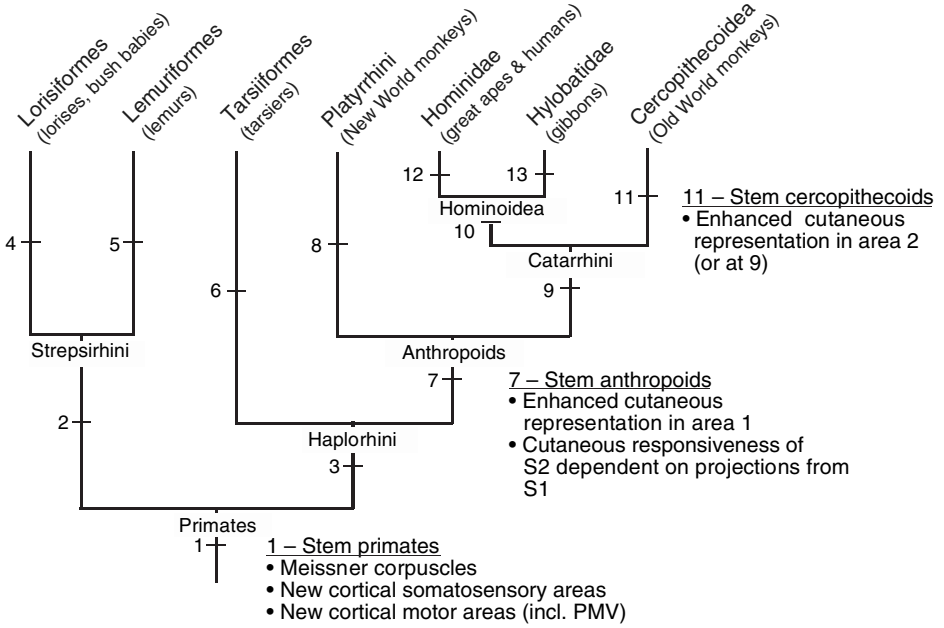

Figure 4 Interpretation of the shared derived features of the somatic sensory and motor systems in primates and major primate subgroups.

'diagonal-sequence' walk, in which a hind footfall is followed by the fall of the opposite forefoot, whereas most other mammals have a 'lateral-sequence' walk, in which a hind footfall is followed by the fall of the forefoot on the same side (Cartmill *et al.*, 2006; Lemelin and Schmitt, 2006).

We know rather little about the neurological bases of distinctively primate grasping and gait patterns at the level of spinal mechanisms. There is, however, evidence for a primate specialization on the sensory side. The sense of touch is mediated by several types of receptors, of which the Meissner corpuscles deserve special note. Meissner corpuscles consist of a nerve ending ensheathed in a capsule of non-neuronal cells, and linked to surrounding tissue in such a way as to make them exquisitely sensitive to deformation of the skin (Martin, 1990; Hoffmann *et al.*, 2004). They are concentrated in the epidermal ridges, especially of digits 1–3, which have been proposed to constitute a 'tactile fovea' (Hoffmann *et al.*, 2004). The placement of Meissner corpuscles suggests that they play an important role in the sensory control of grasping (e.g., by detecting slippage and initiating compensatory changes in grip force). Additionally, Meissner corpuscles could play an important role in the use of the hand as a haptic organ: some primates, especially catarrhines, appear to use active touch to investigate the mechanical properties of food items and other objects. Hoffmann *et al.* (2004) suggest specifically that Meissner corpuscles provide information for assessing the texture (and hence ripeness) of fruit. Meissner corpuscles are not, strictly speaking, unique to primates: morphologically similar organs are also present in the dermatoglyph skin of marsupials and on the finger-like tips of elephant trunks (Hoffmann *et al.*, 2004). They are, however, absent in tree shrews and most other mammals that have been examined (Martin, 1990; Hoffmann *et al.*, 2004). It seems likely that Meissner corpuscles evolved independently in several mammalian groups as the elaboration of a simpler type of mechanoreceptor.

4.01.3.4.2 Cortical somatosensory systems Mapping of the cortical somatosensory and motor fields has a long history, going back to the beginnings of experimental electrophysiology in the last half of the nineteenth century. It was clear from an early date that these fields are organized in an orderly, somatotopic fashion, with the tail and hindlimb represented medially in the cortex, near or within the interhemispheric fissure, and the forelimb and face represented laterally. In anthropoid primates, the principal somatic fields are located along the central sulcus, the somatosensory region posteriorly and the motor region anteriorly. In non-primates, which lack the large territory of dorsolateral prefrontal cortex present in primates, the somatic regions are located on the lateral surface near the frontal pole. Early comparative mapping

research culminated in the work of Woolsey (1958). Woolsey concluded that there were two somatosensory areas, the large primary area, SI, located along the central sulcus, and a smaller area, SII, located posterior and inferior to SI along the dorsal bank of the lateral sulcus. He also acknowledged two motor areas, the primary area, MI, located immediately anterior to SI, and a secondary area, MII (also called the supplementary motor area, SMA), located anterior and medial to MI along the mesial surface of the frontal lobe. Woolsey believed these four areas were present in most if not all eutherian mammals. The well-known neurosurgeon Wilder Penfield promulgated a similar understanding of human motor and somatosensory cortex (Penfield and Roberts, 1959). The elegance of Woolsey's schematic figures, with their artful drawings of somatosensory and motor homunculi, ensured that they would remain staples of neurobiology textbooks for many decades.

In the 1970s, the large surface electrodes that had been used for cortical mapping studies were replaced with fine-diameter electrodes that were inserted into the cortex to record from or stimulate neurons. The use of intracortical microelectrodes made it possible to map cortical areas in much greater detail, and with their application came the appreciation that Woolsey's interpretations of the somatosensory and motor regions were simplistic (Merzenich *et al*., 1978; Kaas *et al*., 1979). For example, the territory encompassed by Woolsey's 'SI' in anthropoid primates turned out to contain four separate representations of the body, two areas that receive inputs mainly from cutaneous receptors (including the Meissner corpuscles) and that correspond to cytoarchitectonic areas 3b and 1, and two areas that receive inputs mainly from muscle and joint receptors, corresponding to areas 3a and 2. Of these areas, the somatotopy of area 3b most closely resembled Woolsey's SI, so it was dubbed 'SI proper' (see the review of Kaas, 1983).

In contrast to SI, modern research has largely substantiated Woolsey's concept of an SII, an area of principally cutaneous representation located posteriorly and laterally to SI along the dorsal bank of the lateral sulcus (see the reviews of Kaas, 2004; see The Evolution of Sensory and Motor Systems in Primates). This research, however, has identified additional somatosensory representations in the lateral sulcus anterior and posterior to SII, as well as in the posterior part of the insular cortex, ventral to SII. These areas include the so-called parietal ventral (PV) and parietal rostral (PR) areas. There are also additional territories of somatosensory representation posterior to area 2, in the region termed area 5.

Comparative studies of strepsirhines (mainly *Galago* and *Otolemur*) and a variety of New World and Old World monkeys suggest that the areas discussed above are common to all major primate groups and were likely present in stem primates. It is also likely that at least some of these areas are neomorphic in primates, as most nonprimate mammals that have been studied, including tree shrews, have only a single main cutaneous representation in the region of SI, presumably homologous to area 3b/SI of primates (Kaas, 1983), and only a small region of somatosensory representation in the parietal cortex posterior to SI, which in primates contains areas 1, 2, and 5 (see The Evolution of Sensory and Motor Systems in Primates; Kaas, 1983; Beck *et al*., 1996).

There are some important variations in cortical somatosensory organization between primate groups. For one, New World monkeys that have prehensile tails, such as capuchins (*Cebus*) and spider monkeys (*Ateles*), possess prominent representations of the tail pads in SI (Pubols and Pubols, 1971; Felleman *et al*., 1983). In addition, cutaneous representation appears to be more extensive in anthropoids, and particularly in catarrhines, than in strepsirhines. Most strepsirhines that have been studied lack the second strip of cutaneous representation in area 1 caudal to area 3b/1 that is present in most anthropoids, although the slow loris, *Nycticebus coucang*, is reported to have a second representation of the glabrous skin of the hand (Carlson and Fitzpatrick, 1982). Although present in most New World and Old World monkeys, cutaneous representation in area 1 is reduced or lacking in tamarins (Carlson *et al*., 1986) and marmosets (Krubitzer and Kaas, 1990), both New World monkeys of the family Callithricidae that re-evolved claws in place of nails. Also, whereas areas 2 and 5 in strepsirhines and platyrrhines show little responsiveness to cutaneous stimulation, Old World macaque monkeys have a zone of cutaneous responsiveness extending across these areas (Pons *et al*., 1985). Finally, there is evidence for changes in the connectivity of the forebrain somatosensory network in primates. In galagos, there are parallel thalamic projections of cutaneous information to areas SI and SII, so that lesions of SI do not greatly impair the responsiveness of SII to cutaneous stimulation intact. This appears to be the primitive condition for eutherian mammals (Garraghty *et al*., 1991). In anthropoids, the cutaneous responsiveness of SII is the consequence of projections from area SI, lesions of which leave SII unresponsive (e.g., Pons *et al*., 1992).

There are interesting functional parallels in the organization of the cortical somatosensory and visual networks. The pathway from SI to SII is considered to be an important stage in a stream of somatosensory information processing that terminates in the hippocampus and provides the substrate for tactile recognition (Friedman *et al*., 1986). This constitutes an analogue of the ventral stream of visual processing – a somatosensory 'what' pathway. Information also flows from SI and neighboring areas in the central region posteriorly to area 5 and adjacent posterior parietal zones that control the movements of the limbs in space. This may be more than merely an analogue of the dorsal visual (vision-for-action) pathway, as the posterior parietal cortex is a locus of interaction of the somatic sensory and motor systems with the visual system.

4.01.3.4.3 Cortical motor systems Among the principal users of information processed through the somatosensory cortex are the areas of the motor cortex. Woolsey, as already noted, believed there were two cortical motor areas, MI and MII, and that these were present in many eutherian groups. Even in Woolsey's day, however, his conception of the MI as a single, large area, spanning the entire territory between the somatosensory and prefrontal region to include both areas 4 and 6 of Brodmann, was controversial. Other workers identified area 4 with the primary motor area, while area 6, which is less responsive to electrical stimulation and was thought to be involved in higher-order aspects of motor control, was termed 'premotor' cortex (reviewed by Wise, 1985; Preuss *et al*., 1996). Recent research has shown both these interpretations of the motor cortex to be overly simplistic, with modern researchers recognizing MI, which appears to consist of at least two major subdivisions, a dorsal and a ventral premotor subdivision (PMD and PMV, which, like MI, appear to include major internal divisions), an SMA (MII) located on the mesial wall of the hemisphere, a presupplementary motor area, located anteriorly to SMA, and several cingulate motor areas, buried within the cingulate sulcus (reviewed by Dum and Strick, 2002; Kaas, 2004; see The Evolution of Sensory and Motor Systems in Primates). Most of the evidence for these areas comes from New World and Old World monkeys, but recent work in the strepsirhine *Otolemur* suggests a similar set of areas, so it seems very likely that a similarly large complement of areas was present in stem primates. Among primates, there appears to be at least one notable difference in areal organization: in macaque monkeys, area PMV consists of two subdivisions, distinguishable on functional and anatomical grounds (Gentilucci *et al*., 1988; Rizzolatti *et al*., 1988), whereas in the strepsirhines and New World monkeys that have been examined, PMV appears to be a unitary field (Preuss *et al*., 1996; Wu and Kaas, 2003; Fang *et al*., 2005).

By contrast to primates, the motor region of the nonprimate mammals that have been investigated evidently consists of only a very few areas (see The Evolution of Sensory and Motor Systems in Primates). Of the areas present in primates, MI, at least, is probably a common feature of eutherian mammals. There is likely an additional, very small region of premotor cortex in nonprimate mammals, but there is very little indication of anything approaching the seven or more nonprimary motor areas present in primates, so presumably many of those areas are neomorphic in primates. The clearest case for a neomorphic motor area in primates can probably be made for area PMV. It includes an anatomically discrete field of corticospinal projections that is not seen in tree shrews or in other mammals (Nudo and Masterton, 1990). It also has a distinctive somatotopy, representing the face and forelimb virtually exclusively, and seems to play an important role in organizing grasping movements of the hands and mouth, which led Preuss (1993) to suggest that this area evolved to organize the visually guided reaching and grasping behaviors of stem primates in the fine-branch niche.

4.01.3.4.4 Corticospinal tract The cortex can affect motor activity via a number of routes, the most direct of which are the direct projections to cranial nerve nuclei in the brainstem (corticobulbar projections) and to the spinal cord (corticospinal projections). These projections arise from a broad territory of frontal and parietal cortex in primates and other mammals, spanning the motor and somatosensory regions.

The CST has attracted a great deal of attention from comparative neurobiologists. Early research noted that lesions of the cortex had profound and lasting effects on movement and motor control in primates, whereas in many other mammals the effects were minimal. Damage to the motor cortex in humans and other anthropoid primates results in paresis; with time, most aspects of motor control recover, but individuated movements of the digits are refractory to recovery (Kuypers, 1981).

Comparative anatomical studies of the CST reinforced the idea that the CST plays a special role in movement control in primates. The CST is reported to vary markedly across mammals in its location

within the brainstem and spinal cord, the number and diameter of fibers it contains, the levels of spinal cord to which it reaches, and the location of terminals with respect to the interneurons and motor neurons of the spinal cord.

In some mammals, the CST is reported to descend only to the level of the cervical or thoracic spinal cord (reviewed by Kuypers, 1981). In these animals, the CST can reach motor neuron populations controlling the forelimbs, but can have no direct effect on control of the hindlimb or tail, the motor neurons for which are located at lumbar and sacral levels of the cord. Tree shrews are said to have CST projections that reach no further than the mid-thoracic cord (Jane *et al*., 1965; Verhaart, 1966; Shriver and Noback, 1967). Other mammals, including primates, carnivores, bats, and rodents, are reported to have projections that extend into the lumbosacral cord (Kuypers, 1981). Primates, it has long been argued, are distinguished from most other mammals by having corticospinal fibers that terminate directly on the motor neurons that innervate the muscles of the hands and feet; among nonprimates, only raccoons were said to have comparable, monosynaptic innervation of hand muscle motor neurons (Kuypers, 1981). Moreover, it was argued that the extent to which the CST extends beyond cervical levels in the spinal cord, and the extent to which motor neurons receive direct, monosynaptic input from the cortex, determines the digital dexterity of animals, with primates and raccoons having these conditions and being the most dexterous animals (Heffner and Masterton, 1975, 1983; see also the review in Striedter, 2005).

One problem with the studies of corticospinal connectivity cited above is that for the most part they employed techniques for studying connections (lesion-degeneration methods) that are not considered very reliable today. It seems likely that the conclusions reached about the level of the spinal cord reached by the CST in a given species are trustworthy in most cases, but conclusions about whether or not CST projections reach the territory of spinal motor neurons in the ventral horn should be considered cautiously. A study by Bortoff and Strick (1993), using a modern tracer substance (WGA-HRP), reported a result quite in line with classical considerations. They compared the distribution of corticospinal terminations after injections of WGA-HRP into area M1 of two closely related New World monkeys: capuchins (*Cebus apella*), which are noted for their manipulative abilities, and squirrel monkeys (*Saimiri sciureus*), which are not. They reported that CST terminations are much more extensive in the ventral horn motor neuron territory of *Cebus* than *Saimiri*. Reliable conclusions about the evolution of the CST will require more investigation using modern tracing techniques. It is also necessary to apply better analytical techniques to multispecies data sets than have been used in the past. For example, the regression techniques used to relate CST anatomy to digital dexterity by Heffner and Masterton (1975, 1983) are no longer considered adequate; improved methods ('independent contrasts') make it possible to remove the confounding effects of phylogeny in correlations, such as the over-representation of primates in the sample. Using these techniques to re-analyze the Heffner–Masterton data set, Iwaniuk *et al*. (1999) concluded that the length of the CST was related to digital dexterity, but not the extent to which it invaded the territory of motor neurons controlling the distal extremities. This is certainly not the last word on the subject (as Iwaniuk *et al*., 1999, would likely acknowledge), but it does highlight the need for neuroscientists to apply the best methods of phylogenetic inference, as well as the best techniques for studying neural organization, to problems of nervous system evolution.

4.01.3.5 Auditory System

The auditory system has not been studied by comparative biologists with anything like the effort devoted to the visual system. There is presently little indication that the origins of primates were accompanied by marked modifications of the auditory sensory apparatus, although changes in central auditory systems are known and will be discussed below. Small, nocturnal strepsirhine primates, as well as tarsiers, use hearing along with vision to localize insect prey, and if stem primates were nocturnal predators this would likely have been true of them as well. It is noteworthy that some nocturnal primates (bush babies and tarsiers in particular) have large external ears (pinnae), that are ridged and can be moved and shaped to assist sound localization (Charles-Dominique, 1977). The orientation of the pinnae modulates sound frequency, and this may play an important role in sound localization, which in small mammals relies more on frequency differences than interaural timing differences (Heffner, 2004).

Glendenning and Masterton (1998) compared the volumes of auditory brainstem nuclei in a variety of mammals and reported that primates are fairly typical among mammals in terms of the sizes of the

different nuclei relative to each other. The dorsal cochlear nucleus, however, appears to have undergone a reduction of lamination in the origin of anthropoid primates, with further reduction in hominoids (Moore, 1980; Johnson *et al*., 1994).

As with other neocortical sensory systems, the cortical auditory system of primates is characterized by a large number of areas (see Organization and Correspondence of the Auditory Cortex of Humans and Nonhuman Primates). The areas are arrayed in a distinctive fashion: along the upper part of the temporal lobe (partly or completely buried within the lateral sulcus), there is a 'core' of areas that share similar histology, with a well-developed layer 4 densely packed with granular cells. The posterior-most core area is the primary auditory area (A1). A1 is bordered rostrally by the rostral (R) area, which can be difficult to distinguish from A1 cytoarchitectonically, and area R is replaced rostrally by the rostrotemporal (RT) area. The core is surrounded on all sides by a 'belt' of auditory areas; currently, there are thought to be eight belt areas. The lateral part of the belt is bordered by several 'parabelt' areas, which occupy the superior temporal gyrus. Posterior to the core and belt is an additional field involved in auditory processing known as area Tpt. Many of the core and belt areas are known to represent auditory frequencies in systematic fashion, that is, they are 'tonotopically' organized. As with other cortical sensory systems, the cortical auditory system exhibits a hierarchical organization, with areas in the core relaying auditory information to surrounding areas. The auditory system, however, exhibits a greater degree of parallel processing than the visual and somatosensory systems: the core and belt areas all receive inputs from the main auditory nucleus of the thalamus, the medial geniculate nucleus (MG), the three core areas receiving inputs from the ventral MG, and the belt areas from the dorsal MG (see Organization and Correspondence of the Auditory Cortex of Humans and Nonhuman Primates; see also Rauschecker *et al*., 1997). As the organization of primate auditory cortex has come to be better understood, it has been suggested that there are at least partly separate processing streams specialized for analysis of information about the identity of sound sources and about their spatial localization, that is, 'what' and 'where' systems (Romanski *et al*., 1999; see also Kaas and Hackett, 1999; Rauschecker and Tian, 2000).

The organization detailed above has now been documented in both Old World and New World monkeys (see Organization and Correspondence of the Auditory Cortex of Humans and Nonhuman Primates). Comparative architectonic studies indicate that an identifiable core is present in strepsirhine primates (Preuss and Goldman-Rakic, 1991a), and in chimpanzees and humans (Hackett *et al*., 2001). Also, Tpt has been identified architectonically in strepsirhines, Old World monkeys, and humans (Galaburda and Pandya, 1982; Preuss and Goldman-Rakic, 1991a). Area A1 is probably common to all mammals, and most nonprimate mammals have at least five or six additional auditory areas (see, e.g., Stiebler *et al*., 1997). It is not clear which of the primate auditory areas these are homologous to, nor is it clear that nonprimates exhibit the core-belt-parabelt system present in primates.

One of the major mysteries of human evolution is how auditory cortex was modified in relation to the evolution of language. One possibility is that humans evolved new areas to support language, but there is at present no evidence of this (Preuss, 2004b). Indeed, the cortical territory most strongly identified with Wernicke's area, the cortex of the planum temporale, posterior to area A1, contains architectonic area Tpt, which as discussed has also been identified in nonhuman primates (Galaburda and Pandya, 1982). Presumably, the cortex of Tpt must have undergone changes in its internal organization and/or its relationship to other cortical areas to support human language. Since humans tend to be left-hemisphere dominant for language, the fact that the planum temporale tends to be larger in the left hemisphere than the right might be seen as a language-related modification (Galaburda *et al*., 1978). This cannot be the entire story, however, as great apes exhibit a pattern of posterior temporal asymmetries at least qualitatively similar to those of humans (Gannon *et al*., 1998; Hopkins *et al*., 1998), although the possibility of quantitative species differences remain. To date, the only clear difference to have been documented between the posterior temporal region of humans and apes is an asymmetry of cortical minicolumns in area Tpt: humans have more widely spaced columns on the left than on the right, whereas no asymmetry is present in chimpanzees or macaques (Buxhoeveden *et al*., 2001).

4.01.3.6 Limbic System

The limbic system comprises a ring of cortex that makes up the lateral and medial margins of the cortex, including the hippocampus and parahippocampal cortex, retrosplenial and posterior cingulate cortex, the anterior cingulate and prelimbic areas (which are sometimes now considered portions of the medial prefrontal cortex), and orbital and

insular cortex (including regions of olfactory and taste representation), along with noncortical structures that are connected with these regions, such as the amygdala and portions of the hypothalamus. What unites these regions are their roles in motivation and emotion, mediated by connections with the autonomic system. One might think that the limbic system would be a hotbed of comparative neuroscientific investigation, if only because modern evidence indicates that the limbic system is critically involved in social cognition (see The Evolution of Human Emotion). There is also the very interesting question of how the specialized cortical systems of primates came to interact with the limbic region, which is composed of structures that for the most part have homologues not only in other mammals but in nonmammalian vertebrates (see, e.g., Rolls, 2004). Presumably, it is because these structures have been viewed as ancient (we apply terms like 'paleocortex' and 'archicortex' to the limbic cortex) that they have received so little attention from an evolutionary viewpoint, although of course old structures can undergo evolutionary changes just as new ones can.

In fact, there is good evidence that cortical components of the limbic system were modified in primate evolution (Figure 5). Primates possess divisions of posterior parahippocampal cortex (areas TH and TF) that have no obvious counterparts in other mammals (Preuss, 2006). These areas are important way stations between the neocortex and the memory systems of the hippocampus. In addition, the posterior cingulate region of primates contains a territory, area 23, that is not recognized in most other mammals (Preuss, 2006). Area 23 underwent further modification in anthropoid evolution, with the addition of a well-developed internal granular layer (Zilles *et al.*, 1986; Preuss and Goldman-Rakic, 1991a). Also, subdivisions of area 23 and retrosplenial area 29 identifiable in anthropoids are not distinguishable in strepsirhines, and strepsirhines possess divisions of retrosplenial area 30 that are not distinguishable in anthropoids (Zilles *et al.*, 1986). Tarsiers appear to share some features of anthropoid posterior cingulate cortex not present in strepsirhines (Zilles *et al.*, 1986). New World monkeys differ from other primates in the thickness and cell density of the outer layers of retrosplenial area 29 (Armstrong *et al.*, 1986). Evolutionary changes of the posterior cingulate and retrosplenial cortex are of special interest, as in humans these territories are believed to be part of the system for representing conscious self-reflection (Fink *et al.*, 1996; Vogt and Laureys, 2005).

There is also evidence of changes at finer levels of organization in the limbic system. Humans and great apes possess an unusual class of large, spindle-shaped neurons in layer 5 of anterior cingulate and orbitoinsular cortex (Nimchinsky *et al.*, 1999). These 'spindle cells' or 'Von Economo neurons' are especially large and prominent in humans, and they have been suggested to have a role in human social judgment (see Role of Spindle Cells in the Social Cognition of Apes and Humans). In addition to

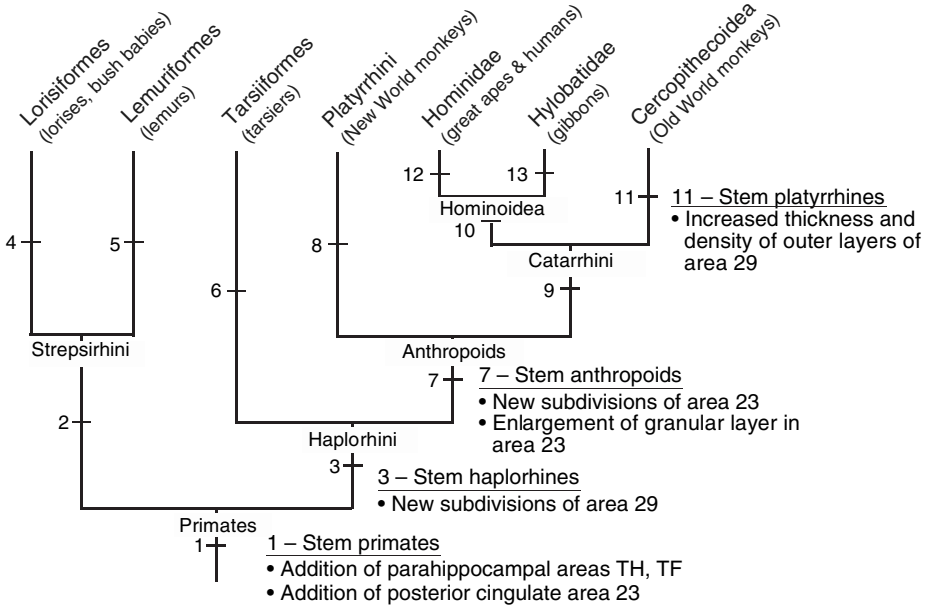

Figure 5 Interpretation of the shared derived features of the limbic system in primates and major primate subgroups.

spindle cells, great apes and humans have a specialized class of calretinin-containing pyramidal cells in anterior cingulate cortex (Hof *et al*., 2001).

4.01.3.7 Higher-Order Forebrain Systems

I include among the 'higher-order' regions of the forebrain the cortex of the STS, the posterior parietal cortex, and portions of the 'prefrontal' cortex (Figure 6). These are regions that receive information from the sensory cortical systems, integrating multiple inputs to perform specific, supramodal cognitive and behavioral functions. I also include in this discussion the dorsal pulvinar, a division of the thalamus that has extensive connections with higher-order cortical regions. These four anatomical regions are noteworthy, first, because they either have no homologues in nonprimate mammals or underwent major modifications in primate evolution (Preuss, 2006), and second, because they enlarged out of proportion to other brain regions in human evolution (Preuss, 2000b, 2004b).

4.01.3.7.1 Superior temporal sulcal cortex The STS of Old World macaque monkeys contains a series of anteroposteriorly elongated architectonically distinct zones that extend much of the length of the sulcus anterior to visual area MT. This region was dubbed the superior temporal polysensory (STP) area by Bruce *et al*. (1981), and it receives inputs from both the dorsal and ventral visual streams (Boussaoud *et al*. 1990; Cusick 1997). STP has attracted special attention since the discovery in macaques that its neurons are active in response to viewing biological motion (moving faces, eyes, limbs, and bodies) and that individual STP neurons can be very selective for particular actions (Perrett *et al*., 1985; see also the review of Puce and Perrett, 2003). Currently, STP is regarded as a key node in a network of cortical areas, also including posterior parietal and prefrontal cortex, involved in the analysis of conspecifics' social behavior (Frith and Frith, 1999; Puce and Perrett, 2003). Functional imaging studies strongly suggest that a homologue of STP exists in the posterior part of the STS of humans. Evidence from strepsirhine primates, however, suggests that the anatomy of the STS region differs markedly from monkeys: Preuss and Goldman-Rakic (1991a, 1991b) confirmed that the macaque STS was comprised of multiple architectonic zones, but could distinguish only a single zone in *Otolemur*. This implies that major changes in STS organization took place during anthropoid or catarrhine evolution. There is no evidence that a homologue of STP or its constituent areas exists in nonprimate mammals.

4.01.3.7.2 Posterior parietal cortex The posterior parietal cortex corresponds to areas 5 and 7 of Brodmann (1909) in monkeys and lemurs. In macaques, areas 5 and 7 occupy the superior and inferior parietal lobules (SPL, IPL), respectively. In humans,

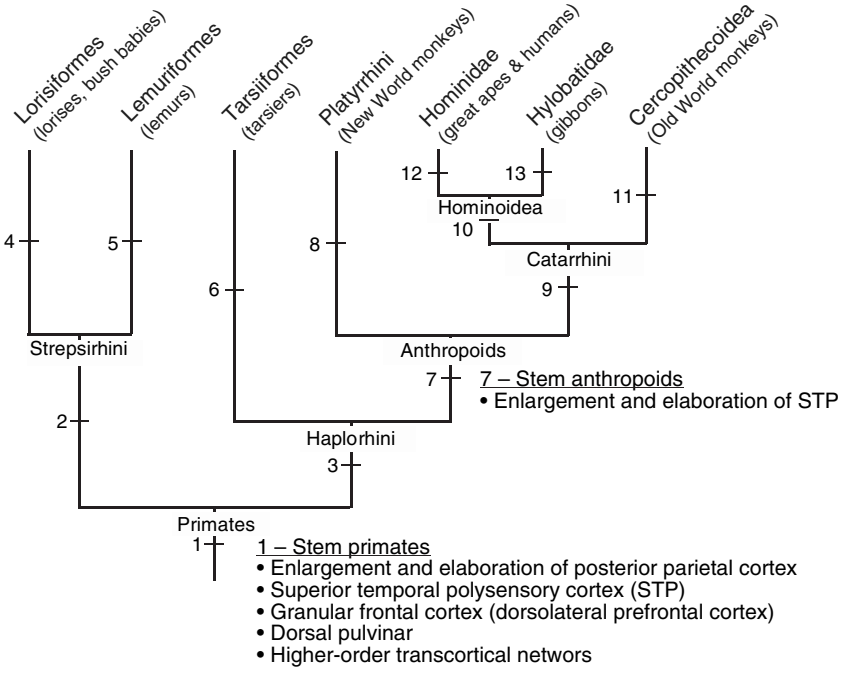

Figure 6 Interpretation of the shared derived features of higher-order forebrain systems in primates and major primate subgroups.

Brodmann maintained that the IPL was occupied by areas 40 and 39, for which he recognized no homologues in monkeys. Other authorities, however, have regarded areas 40 and 39 as homologous to the anterior and posterior parts of area 7, designated as areas 7b and 7a or as areas PF and PG, in nonhuman primates (see, e.g., Economo and Parker, 1929; Bonin and Bailey, 1947; Bailey and Bonin, 1951; Eidelberg and Galaburda, 1984). Neurologists have long appreciated that posterior parietal cortex plays an important role in spatial attention and in action control. In humans, these functions are strongly lateralized: lesions involving right parietal cortex result in hemispatial neglect (inattention to objects and events to the patient's left), while lesions involving left parietal cortex yield a bilateral manual apraxia, a deficit in the ability to produce skilled hand movements, such as those required to use tools or to gesture (see Neurological Specializations for Manual Gesture and Tool Use in Humans; Haaland *et al*., 2000; Muhlau *et al*., 2005). Modern studies of nonhuman primates indicate that posterior parietal cortex receives information from the dorsal stream of the visual system as well as information about eye movements and limb position, and furthermore, that the region is a patchwork of small, functionally specialized territories involved in controlling the direction of attention, eye movements, and reaching and grasping movements of the hands (Andersen *et al*., 1997; Colby and Goldberg, 1999; Grefkes and Fink, 2005). Results of functional imaging studies in humans are consistent with these studies, and reveal that posterior parietal subdivisions are involved in transcortical networks that mediate spatial attention (e.g., Corbetta *et al*., 1998; Kastner and Ungerleider, 2000) and attention to and control of action (e.g., Rushworth *et al*., 2001; Schluter *et al*., 2001; Johnson-Frey *et al*., 2005; see Neurological Specializations for Manual Gesture and Tool Use in Humans).

Although comparative studies indicate many commonalities among primates in the organization of posterior parietal cortex, it was by no means static in primate evolution. For one thing, there is no indication that any nonhuman primate exhibits the extreme degree of functional hemispheric specialization present in humans (see The Evolution of Hemispheric Specializations of the Human Brain; Corballis, 1991). Furthermore, functional imaging studies of human and macaque subjects viewing identical stimuli indicate that moving stimuli activate more discrete zones of parietal cortex in humans (Vanduffel *et al*., 2002; Orban *et al*., 2004; see also Orban *et al*., 2006). This might indicate that humans possess more parietal areas than macaques or, alternatively, that certain areas became more sensitive to certain classes of visual stimuli in humans than in macaques (Preuss, 2004a). At present, it is not clear whether these differences are true human specializations, or whether they evolved early in the history of the hominoid (ape-human) group, or (as could well be the case) specializations of macaques. It is noteworthy that the posterior parietal cortex of primates is very different than that of other mammals. In rats, for example, there is a small region of cortex between the visual and somatosensory regions that receives input from both and that been likened to posterior parietal cortex, but if this is the homologue of primate posterior parietal cortex, the region must have undergone extensive modification in primate evolution, with the addition of many new subdivisions specialized for primate-characteristic behaviors (Preuss, 2006).

4.01.3.7.3 Prefrontal cortex Prefrontal cortex in primates, as currently understood, includes several different territories: a large region with a well-developed granular layer 4 that occupies mainly the dorsolateral surface of the frontal lobe (i.e., granular frontal cortex, or more commonly, dorsolateral prefrontal cortex); an orbital region, the anterior parts of which are granular, but which grades off posteriorly into agranular cortex; and a medial region, which also is granular anteriorly but grades off posteriorly into agranular cortex. Classically, the medial agranular regions were classified as anterior cingulate cortex (Brodmann, 1909), rather than as prefrontal cortex, but for reasons to be discussed below, it has come to be thought of as 'prefrontal'.

Among mammals, only primates have a region of cortex with a well-developed granular layer on the dorsolateral surface of the frontal lobe (Brodmann, 1909). The region is present in all primates that have been examined, and is much larger in anthropoids than in strepsirhines (Brodmann, 1909; Preuss and Goldman-Rakic, 1991c). Owing in part to the influence of Brodmann, the granular dorsolateral prefrontal cortex initially came to be regarded as a hallmark of the primate brain. The fact that some neurologists in the early part of the twentieth century regarded this region as the seat of higher-order cognitive functions reinforced this view. Modern experimental studies in nonhuman primates (reviewed by Goldman-Rakic, 1988; Preuss and Goldman-Rakic, 1991b; Pandya and Yeterian, 1996; Barbas, 2000; Petrides, 2000) reveal it to have strong connections with the higher-order parietal and temporal areas discussed above, and functional studies in humans and nonhuman primates indicate

that different parts of the granular frontal cortex are involved in attention, working memory, and planning (Passingham, 1993; Goldman-Rakic, 1996; Fuster, 2000; Miller and Cohen, 2001; Tanji and Hoshi, 2001; Miller *et al*., 2002; Passingham and Sakai, 2004; Petrides, 2005).

The idea that dorsolateral prefrontal cortex is special to primates has, nevertheless, been challenged (see the reviews of Preuss, 1995a, 2006). With the introduction of the first generation of techniques for studying cortical connectivity (lesion-degeneration techniques), it became clear that the cortical regions differed in their patterns of connectivity as well as their histology. Early research on the forebrain connections of the cortex focused on connections with the thalamus because cortical lesions produce degeneration in thalamic nuclei that project to them; most other connections could not be reliably resolved until improved methods became available in the 1970s. Rose and Woolsey (1949) championed the idea that regions of cortex could be defined by the thalamic nuclei that projected to them. As the dorsolateral prefrontal cortex, the largest prefrontal region in primates, receives its major thalamic inputs from the mediodorsal thalamic (MD) nucleus, prefrontal cortex came to be defined as MD-projection cortex (Rose and Woolsey, 1948). As it happens, all mammals that have been examined have a MD nucleus and a cortical territory to which it projects, so by this reasoning, all mammals possess a homologue of dorsolateral prefrontal cortex, even though the MD-projection cortex of nonprimates lacks the well-developed granular layer that marks this region in primates (Rose and Woolsey, 1948; Akert, 1964). It was also reported that dopamine-containing nuclei of the brainstem project very strongly to MD-projection cortex in both primates and nonprimates, and this has also been used to identify homologues in different mammals (Divac *et al*., 1978). Attempts have also been made to refine this analysis by identifying homologues of specific subdivisions of primate dorsolateral prefrontal cortex in nonprimates (Akert, 1964). A region of special interest has been the cortex that lines the principal sulcus of macaques (principalis cortex), because lesions of this region impair performance on spatial working memory tasks, a set of cognitive tasks that have been adapted for use in a wide range of mammals. Using the criteria of MD projections, dopamine projections, and involvement in spatial working memory tasks, homologues of macaque principalis cortex have been proposed in nonprimate species, and most importantly in rats, which are the most widely used model animals in mammalian neuroscience. In rats, the principalis homologue has usually been localized to the medial surface of the frontal lobe, and some workers have identified it specifically with area 32 (the prelimbic area) (Brito *et al*., 1982; Passingham *et al*., 1988; Dalley *et al*., 2004; Vertes, 2004).

This might seem a satisfactory account of prefrontal homologies, but there are difficulties with both the evidence and the reasoning (Preuss, 1995a). For one thing, in primates, MD projects not only to the granular, dorsolateral prefrontal frontal cortex, but also to agranular regions, including orbital cortex, the classical anterior cingulate areas (areas 24 and 32 of Brodmann), and even to insular and premotor cortex. For another, while dorsolateral prefrontal cortex receives dopaminergic inputs, the strongest dopamine projections in primates are actually to the motor region and the orbital and medial cortex. Finally, in primates, lesions of the medial frontal cortex, involving the cingulate region and sparing the dorsolateral region, produce impairments on spatial working memory tasks. Thus, none of the features that have been used to identify homologues of granular prefrontal cortex in nonprimates are actually diagnostic of granular prefrontal cortex in primates. In fact, the medial frontal cortex of rodents very closely resembles the agranular parts of the medial frontal cortex of primates on a variety of structural and functional grounds – both are limbic regions, after all. It is true that the medial frontal cortex of rodents resembles primate granular frontal cortex in certain respects, but these are also the ways that the medial frontal cortex of primates resembles the dorsolateral prefrontal cortex of primates; the similarities are not diagnostic. Moreover, primate granular frontal cortex has additional features of areal organization and connectivity that do not match any known region of frontal cortex in any nonprimate mammal (Preuss, 1995a).

On present evidence, then, there are good grounds for concluding that dorsolateral prefrontal cortex is in fact one of the distinctive features of the primate brain. In addition, there is evidence that this region underwent extensive modification during primate history. A comparative study of the connectivity and histology of this region in galagos and macaques indicated that the latter have many more subdivisions of granular frontal cortex (Preuss and Goldman-Rakic, 1991b, 1991c). It was concluded that galagos share with macaques homologues of frontal eyefield and related areas, but that galagos lack homologues of the areas located within and surrounding the principal sulcus of macaques. This result suggests that the principalis areas are derived features of

anthropoid or catarrhine cortex, and reinforces the view that a principalis homologue is absent in nonprimates.

4.01.3.7.4 Tying it all together – the dorsal pulvinar As noted above, the higher-order temporal, parietal, and frontal regions of primates are strongly interconnected, making up a collection of distributed networks that subserve specific cognitive functions (Goldman-Rakic, 1988). In primates, these regions are all connected with a particular region of the thalamus known as the medial pulvinar, or more appropriately, the dorsal pulvinar (see Gutierrez *et al*., 2000, and the review of Preuss, 2006). Neuroanatomists have long noted the great size of the pulvinar in primates compared to other mammals (e.g., Le Gros Clark, 1959). The pulvinar consists of several different territories, however, and most attention has been paid to those regions of the pulvinar that are specifically related to the visual system, receiving input from the retina and SC and projecting to striate and extrastriate visual cortex. In primates, the visual pulvinar proper consists of the inferior pulvinar and part of the lateral pulvinar; the homologous territories in nonprimate mammals are referred to as the pulvinar or as the pulvinar-lateral posterior complex. The dorsal pulvinar is most likely neomorphic in primates, as there are no structures in a similar location or with similar connections in other mammals (Preuss, 2006). By virtue of its connections, the dorsal pulvinar is in a position to coordinate the activities of the distributed, higher-order cortical systems of primates, although the nucleus has received very little experimental investigation and its functions are presently unknown.

4.01.4 Conclusions and New Directions

This review documents numerous evolutionary changes in the nervous systems of primates, localizing them wherever possible to their period of origin in primate evolutionary history. It is clear that primate brain evolution cannot be understood simply as a matter of enlargement and general differentiation; rather, the record indicates that primate brains changed in very specific ways at particular time points in evolutionary history. Ultimately, one would like to see the evidence for evolutionary modifications of the nervous system woven into a broader account of primate evolution, an account that relates changes in the nervous system to changes in other aspects of anatomy and, of course, to behavior. We are a long way from realizing this goal. Without question, many of the nervous system specializations described here are, in a way, rather unremarkable in light of what we know about primate evolution generally. For example, given the emphasis placed in primate origins research on the importance of high-acuity nocturnal vision and visually guided grasping, it is not surprising that we find that stem primates underwent changes in central systems involved in vision, eye-movement control, and grasping. It is not possible at present, however, to make very strong claims about why primates evolved the particular nervous system features they possess, such as, why primate visual systems have such a strong hierarchical organization compared to other mammals, or why primates have CO-rich blobs in area V1. Current theories of structure–function relationships in the nervous system are not sufficiently well developed to provide us with much insight into issues at this level of detail.

How do we get better theories of structure–function relationships? In my view, we need more comparative research. Comparative studies have not been a high priority for the institutions that fund neuroscience, but there is some reason to think this will change. Driven by the need to make sense of data from dozens of different species coming from the various gene-sequencing projects, molecular biology has begun to incorporate the concepts and methods of evolutionary biology. Bioinformatics is, in a very real way, computerized evolutionary biology. We can expect the transformation of molecular genetics to affect the neurosciences. The demonstration of differences in the genomes and proteomes of different mammalian and primate species will naturally lead to the question: what are their phenotypic consequences? Already, we have seen how comparative genetic studies can inform our understanding of the evolution of primate sensory receptors (see The Vomeronasal Organ and Its Evolutionary Loss in Catarrhine Primates, The Comparative Biology of Photopigments and Color Vision in Primates, The Loss of Olfactory Receptor Genes in Human Evolution; Gilad *et al*., 2004). What's more, we can use the information provided by comparative molecular studies about changes in the sequences and tissue-specific expression patterns of specific macromolecules to drive 'phenotype discovery' (Preuss *et al*., 2004). For example, by knowing which molecules have undergone change, we can employ such routine methods as *in situ* hybridization and immunohistochemistry to localize those molecular changes in their anatomical context.

While we can expect comparative molecular biology to provide new insights into the primate brain evolution, the foregoing review draws attention to one really fundamental need: correlative comparative studies of the brain, behavior, and cognition. Consider this: many of the features that distinguish human brains from, say, those of rats, evolved very early in primate evolution. We possess them because our ancestors possessed them. To understand why our brains have these design features, we need to understand what they contributed to the behavior of early primates. To understand that, we need comparative studies of brain, behavior, and cognition in a variety of mammals, including especially anthropoid and strepsirhine primates, along with the animals to which primates are thought to be closely related, such as tree shrews, rodents, and rabbits. No such comparative science exists today.

In addition to sharing many design features with other primates and other mammals, humans presumably possess features that are uniquely human and that provide the basis for our distinctive cognitive and behavioral characteristics. We currently have very little reliable information about brain specializations of humans. One bright spot in this area is that imaging technologies have now developed to the point where we can examine humans and nonhuman primates on something like a level playing field (see, e.g., Orban *et al*., 2004). The problem is that most of these studies involve comparisons of humans and macaque monkeys. It is important to recognize that the demonstration of a human–macaque difference is not a demonstration of a human specialization. For example, a macaque–human difference could have arisen early in hominoid evolution, long before humans diverged from the African apes; this would be a hominoid specialization. Alternatively, an observed difference could be a macaque or a catarrhine specialization, with humans conserving the ancestral condition. Demonstrations of human specializations, therefore, require comparing humans to a wider range of primate species. Of central importance is the comparison of humans to chimpanzees: since chimpanzees are our closest relatives, any claim of human specialization requires the demonstration that humans differ from chimpanzees (Preuss, 2004b). Although there has recently been a renaissance of human–chimpanzee comparative psychological research (see Human Cognitive Specializations, The Evolution of Human Emotion) we know almost nothing of the differences in brain organization between humans and chimpanzees that would provide the basis for human cognitive and behavioral specializations. There is, therefore, a pressing need for comparative studies of the brain and cognition in humans and chimpanzees.

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4.02 The Evolution of Sensory and Motor Systems in Primates

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Glossary

area or nucleus — These are large, functionally significant subdivisions of neocortex and the brainstem. Each area (areas were the "organs of the brain" for Brodmann, 1909) or nucleus has a unique set of connections with other structures and uniquely contributes to the function of the system. Sensory and motor areas and nuclei, typically, topographically represent receptors of a sensory surface (skin or deep tissues) and/or body movements.

Eutheria — The mammalian clade stemming from the most recent common ancestor of placental mammals.

motor cortex — Subdivisions of cortex that are specialized to mediate and control body movements. Typically, movements can be evoked by electrically stimulating areas of motor cortex. Primary motor cortex (M1) is called agranular cortex because it has no obvious layer 4 of granule (sensory) neurons.

somatosensory cortex — Areas of cortex that are more specialized for processing sensory inputs than for motor control. While electrical stimulation of somatosensory areas may elicit movements, these areas have a well-developed layer 4 for receiving sensory inputs.

> And at last came the monkey, and anybody could see that man was not far off now. And in truth that was so. The monkey went on developing for close upon five million years, and then turned into a man – to all appearances (Mark Twain – *Letters from Earth*).

4.02.1 Introduction

Humans are known for their curiosity, and they are especially curious about themselves. Anyone that has viewed highly skilled athletes, musicians, or craftsmen must be impressed with their exceptional sensorimotor performances, which have been individualized and specialized by training and experience. These abilities depend on a massive sensorimotor network that has been gradually acquired during the course of human evolution. This network allowed our ancestors to make and use tools and weapons, craft garments, and process food. How did this system evolve and how does it work? Answers to these questions are incomplete, but we now have an outline of this system in humans and other mammals, information that allows the major steps in our evolution to be reconstructed, and a framework for understanding how the system works. This reconstruction of the course of brain evolution is informed by the fossil record, but it is largely based on comparative studies of extant primates and other mammals. Nevertheless, the fossil

record does indicate that early primates emerged as small, probably nocturnal (however, see Tan *et al.*, 2005), arboreal, small-brained mammals that likely fed on insects, fruit, small vertebrates, and buds (Ross, 1996). In some lines of primate evolution, the fossil record also indicates that brains got bigger in proportion to body size, and in the line leading to humans this transformation was remarkable, especially over the last 2 My. The extensive increase in the absolute and relative size of the brain suggests that major changes in brain organization occurred, but the fossil record provides little information about the nature of the changes.

In order to understand how sensorimotor systems evolved in primates, we need to compare the organizations of sensorimotor systems in primates and mammals most closely related to primates, and use distribution patterns across taxa to make inferences about when specific features of the human sensorimotor system emerged (see Eldredge and Cracraft, 1980; Wiley, 1981). While the focus is on the evolution of the human sensorimotor system, we also take note of a few interesting specializations that occurred in other lines of primate evolution.

4.02.2 The Somatosensory and Motor Systems of the Mammalian Ancestors of Primates: Inferences from the Systems of Rodents, Tree Shrews, and Other Mammals

It might seem strange to compare our nervous system with those of rats, rabbits, and tree shrews because rodents and lagomorphs seem so unlike us, and most of us know little about tree shrews. Yet these mammals turn out to be our closest living nonprimate relatives, and thereby these are the mammals to study if we want to know how the brains of our immediate nonprimate ancestors were organized.

Mammals emerged from mammal-like reptiles around 230 Mya and radiated into over 4500 surviving (extant) species of mammals. Because of recent advances in the use of molecular data to classify mammals (e.g., Murphy *et al.*, 2001, 2004), it has been possible to distinguish six major branches of mammalian evolution. Monotremes and marsupials constitute two early branches, while the placental mammals include Xenarthra (sloths, anteaters, and armadillos) and Afrotheria, Laurasiatheria, and Euarchontoglires as newly recognized clades (Figure 1). The Euarchontoglires clade emerged some 95 Mya and diverged into Glires, a line that gave rise to lagomorphs (rabbits, hares, and pikas) and rodents, and euarchontans, a line that gave rise to dermopteras (flying lemurs), scandentias (tree shrews), and primates. Of our closest living relatives – flying lemurs and tree shrews – we know very little about the brain of flying lemurs, but fortunately, tree shrew brains have been well studied, in part, because of early recognition by the great comparative anatomist Le Gros Clark that tree shrews resemble primates. Clark (1959) considered tree shrews to be primates, but this classification is no longer held tenable. In tree shrews, we look for traits that are shared by primates, rodents, and lagomorphs, as these traits may be those retained from a common ancestor of the Euarchontoglires clade. However, traits seen only in tree shrews need to be considered with caution, as they could be specializations of tree shrews rather than traits shared with the ancestors of primates (Kaas, 2002).

Euarchontoglires mammals also share a number of features of the sensorimotor system that were likely retained from early mammals. The basic features of the sensorimotor system of early mammals can be deduced by comparing components across members of the three major branches of mammalian evolution, monotremes, marsupials, and placental (eutherian) mammals (Kaas, 2004a). Some of the basic components of the somatosensory system of early mammals (Kaas, 2004b, 2004c) are illustrated in Figure 2. Starting with the inputs, low threshold cutaneous receptors project via peripheral nerves to the dorsal column–trigeminal complex in the lower brainstem, as well as to neurons in the dorsal horn of the spinal cord. Afferents terminate in the dorsal column–trigeminal complex in an orderly way so that ipsilateral tail, hindlimb, forelimb, and head are represented in a mediolateral sequence. Second-level neurons project to the ventroposterior nucleus (VP) of the opposite thalamus in a tail-to-tongue lateromedial somatotopic sequence. Third-level neurons in the VP in turn project to primary somatosensory cortex (S1) and to two smaller somatosensory areas just lateral (ventral) to S1, the second somatosensory area, S2, and the parietal ventral area, PV. Thus, VP neurons independently activate three somatosensory areas. Fourth-level neurons in S1 project to rostral (SR) and caudal (SC) somatosensory bands that border S1, and these areas and S1 all project to S2 and PV. Neurons in S2 and PV access neurons in perirhinal and parahippocampal areas that feed into the hippocampus for memory functions (Squire and Knowlton, 1994), and the amygdala for fear conditioning and other functions (LeDoux, 2000). Other

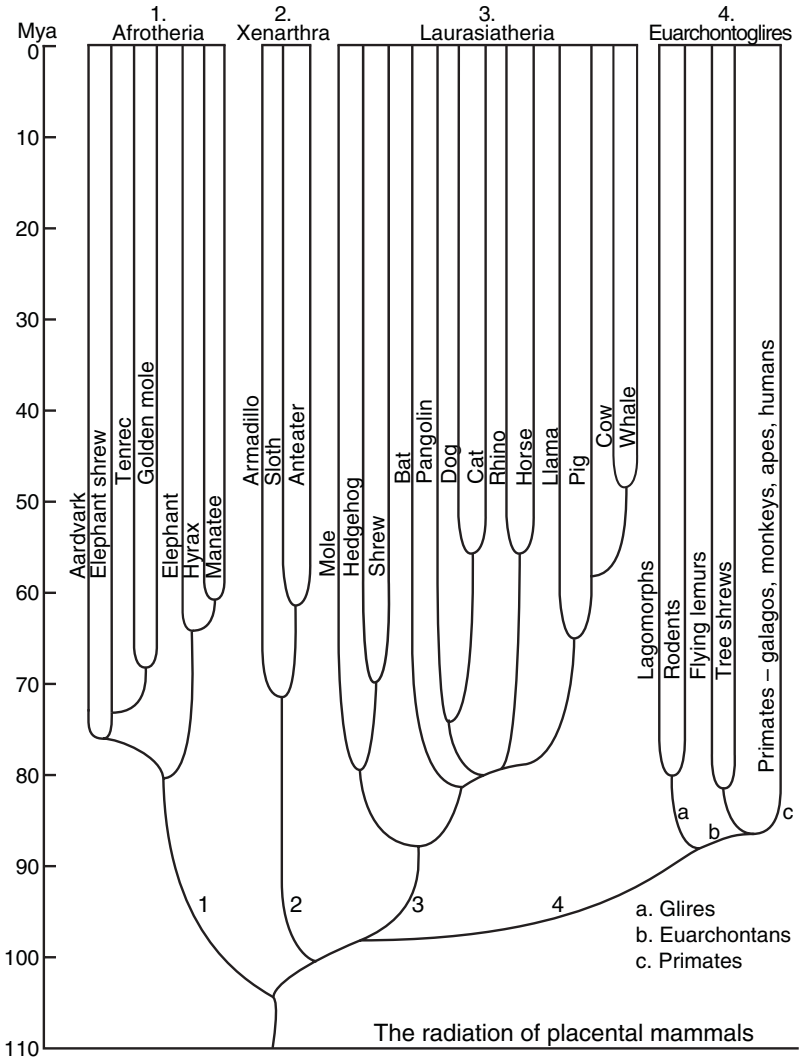

Figure 1 The emergence and radiation of the four major clades of placental (eutherian) mammals. In each clade, a few representative extant members are noted. (1) Afrotheria includes small insectivore-like mammals such as tenrecs and golden moles, as well as the very large elephants and manatees. (2) Xenartheria has the small-brained, specialized anteaters, sloths, and armadillos. (3) The diverse Laurasiatheria range from the widespread bats to carnivores, ungulates, and whales. (4) The Euarchontoglires include Glires – lagomorphs (rabbits) and rodents and euarchontans – primates, flying lemurs, and tree shrews. See Figure 6 for the primate radiation. Time in Mya is indicated on the left. Based on Murphy, W. J., Pevzner, P. A., and O'Brien, J. O. 2004. Mammalian phylogenomics comes of age. *Trends Genet.* 20, 631–639.

sensory inputs to the somatosensory thalamus include muscle spindle receptors for proprioception, which relay in parallel to the dorsal column pathway, first in the lower brainstem and then to the contralateral thalamus, where proprioceptive neurons in the posterior nucleus or a rostral subdivision of VP project to S1 and adjoining areas of somatosensory cortex. Muscle spindle and other somatosensory information are also sent to the cerebellum, and cerebellar deep nuclei project to the ventrolateral nucleus of the thalamus. Somatosensory information also reaches the thalamus via the spinothalamic path, which terminates in and around the VP. Early mammals apparently had no separate motor cortex, as somatosensory areas, especially SR, S1, and S2, had motor functions including those mediated by cortical projections to the brainstem and spinal cord. Other projections from the somatosensory cortex included those to the basal ganglia, and the somatosensory and motor thalamus. The zona incerta of the ventral thalamus is also an important part of this basic mammalian somatosensory system, with somatosensory inputs and inhibitory GABAergic projections to somatosensory cortex the brainstem, and the superior colliculus (Nicolelis *et al.*, 1992).

The most provocative component of the above summary of the sensorimotor system of early

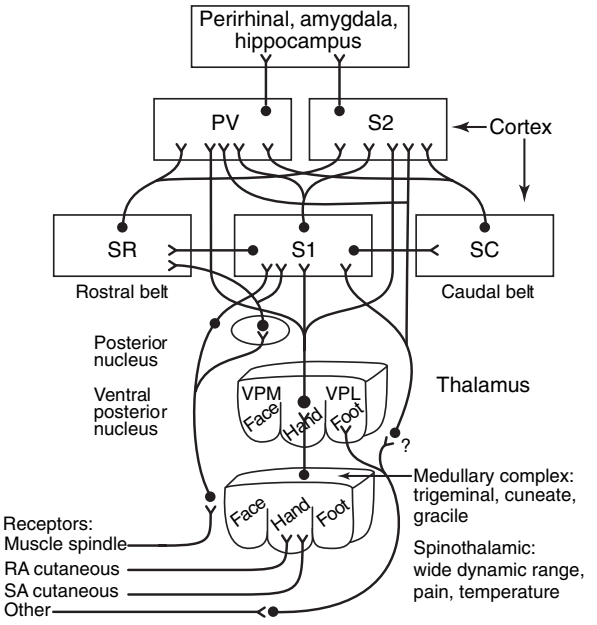

Figure 2 A block diagram of the proposed components of the somatosensory system of early mammals. Receptors and afferents in the skin, muscles, and joints projected to the brainstem and spinal cord, where branches contacted second-order neurons that projected to the contralateral thalamus, or formed fiber pathways to the medullary complex of the brainstem, where second-order neurons projected to the contralateral thalamus. Neurons in the ventral posterior nucleus (VP) received rapidly adapting (RA) and slowly adapting (SA) cutaneous receptor information and projected to primary somatosensory cortex (S1), the second somatosensory area (S2), and the parietal ventral (PV) somatosensory area. A posterior nucleus (a probable homologue of the primate ventral posterior superior nucleus) likely received a relay of muscle spindle receptor information while projecting to SR and S1. S1 projected to rostral (SR) and caudal (SC) somatosensory belt-like areas adjoining S1, and to PV and S2. All of these areas, but especially S1 and SR, were involved in motor control via subcortical projections (not shown) to the basal ganglia, the brainstem, and the spinal cord. PV and S2 interconnected with perirhinal cortex and thereby with the hippocampus and amygdala. The medullary complex, also known as the dorsal column–trigeminal complex, included the principal subnucleus of the trigeminal complex, and the cuneate and gracile subnuclei, to form a representation of the body from face (trigeminal) to forelimb (cuneate) to hindlimb (gracile). The medial subnucleus of VP (VPM) represents the face and mouth, while the lateral subnucleus (VPL) represents the lower body. Based on Kaas, J. H. 2004b. The evolution of the large, complex sensorimotor systems of anthropoid primates. In: Evolution of the Vertebrate Brain and Behavior (*eds.* S. Pellis and L. Marino), *Int. J. Comp. Psychol.* vol. 17, pp. 34–52.

mammals is the claim that there was no separate motor cortex. This is based on the evidence that at least some marsupials and monotremes lack motor areas of cortex. For example, the well-studied North American opossum has the four basic areas of somatosensory cortex (Figure 3) but no motor areas (Beck *et al.*, 1996). In opossums, there is no architectonic, electrophysiological, or connectional evidence for a separate primary motor area, M1, such as those found in all studied placental mammals. Instead, the projection of cerebellum to the motor thalamus, the ventral lateral nucleus, is relayed to somatosensory cortex (Killackey and Ebner, 1973). As a note of caution on this claim, some investigators have presented evidence for a motor region rostral to S1 in monotremes (e.g., Krubitzer *et al.*, 1995) and some marsupials (see Beck *et al.*, 1996), but the more compelling evidence suggests that M1 and other motor areas did not emerge until the advent of placental (eutherian) mammals.

Lagomorphs, rodents, and tree shrews represent the closest living relatives of primates that have been available for study (Figure 1). Together they share some features of sensorimotor system organization with most other mammals. All have S1 and S2 as subdivisions of somatosensory cortex. However, these areas are specialized in different ways. In rabbits, three-fourths of S1 and S2 are devoted to tactile receptors of the head, especially those of the lips and facial vibrissae (Gould, 1986). Little cortical tissue is devoted to the forepaw and less to the hindpaw. In rats (Figure 4), most of these areas represent the facial vibrissae, the buccal pad, and other parts of the face, but the representations of the forepaw is significantly larger than in rabbits (Remple *et al.*, 2003). This difference likely corresponds to the greater use of the forepaw in manipulating food objects by rats (Whishaw, 2003). In tree shrews, there is a large representation of the glabrous nose, as well as large representations of lips and mouth parts, but the forepaw has an even larger representation than in rats, and the arrangement of the forearm representation in S1 more closely resembles that of primates (Sur *et al.*, 1980, 1981). As representational features of S1 across species closely reflect their distributions of peripheral receptors and the specialized use of parts of the sensory surface (Johnson, 1990), these differences in representation, as well as the increased representation of the forepaw in rats and especially tree shrews, likely reflect independent evolutionary trends. Yet, it is safe to propose that the immediate ancestors of primates, as semi-arboreal grasping mammals, had emphasized the forepaw in their somatosensory representations, most likely to an even greater extent than in tree shrews, their close relatives. In rats (Remple *et al.*, 2003) and tree shrews (Remple *et al.*, 2006), there is evidence for a PV somatosensory area just ventral to S2. PV may exist in rabbits and other lagomorphs as well, as this area has been found in a wide range of mammals, but there has been no attempt to identify PV in

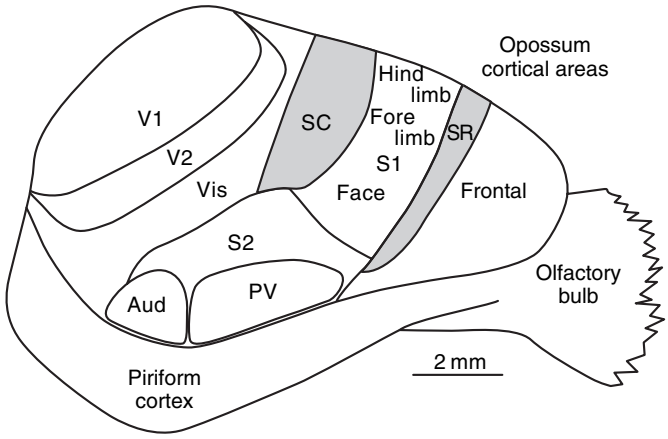

Figure 3 Subdivisions of somatosensory cortex in marsupial opossums shown on a dorsolateral view of the right cerebral hemisphere. Somatosensory areas include primary somatosensory cortex (S1), the adjoining rostral (SR) and caudal (SC) somatosensory belts, the second somatosensory area (S2) and the parietal ventral area (PV). Visual cortex (Vis) includes first (V1) and second (V2) visual areas, and auditory cortex (Aud) may include several areas. There is no evidence of a primary motor area or any premotor areas in frontal cortex. S1 represents the hindlimb to face in a mediolateral sequence, while S2, PV, and possibly SC and SR also contain topographic representations. Based on Beck, P. D., Pospichal, M. W., and Kaas, J. H. 1996. Topography, architecture, and connections of somatosensory cortex in opossums: Evidence for five somatosensory areas. *J. Comp. Neurol.* 366, 109–133.

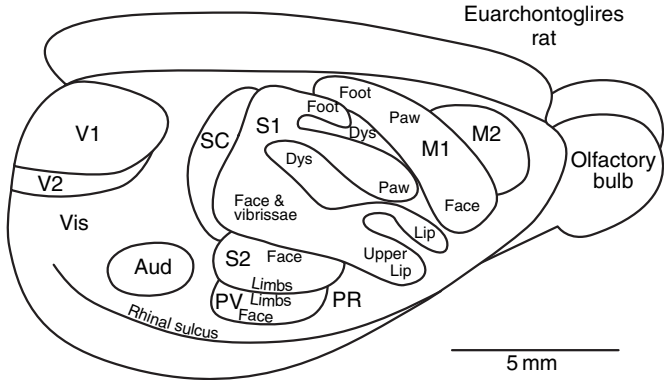

Figure 4 Subdivisions of sensorimotor cortex in rats on a dorsolateral view of the right cerebral hemisphere. The primary somatosensory area (S1), the second somatosensory area (S2), the parietal ventral somatosensory area (PV), and the caudal somatosensory (SC) belt are similar to those in other mammals. The rostral somatosensory area (SR) of other mammals (see Figure 3) forms a dysgranular (Dys) type of cortex that extends caudally to separate forepaw from face, and forepaw from foot representations of S1. A perirhinal (PR) region is indicated lateral to PV. Primary (M1) and secondary (M2) motor areas are found just rostral to S1 and Dys somatosensory cortex (SR). Auditory (Aud) cortex of the three areas, and the first and second visual areas (V1 and V2) are identified. Other visual areas (Vis) are lateral to V2. Based on Remple *et al.* (2003) and others.

lagomorphs. As in opossums (Figure 3), rats (Figure 4), and tree shrews (Figure 5) have caudal and rostral somatosensory bands with inputs from S1. Thus, they have at least five somatosensory areas. The rostral somatosensory belt has more connections with primary motor cortex, M1, and more motor functions, as judged by the thresholds for electrically evoked movements from their cortex. In addition, there is evidence for proprioceptive inputs from the posterior nucleus of the thalamus (Chapin and Lin, 1984; Hummelsheim and Wiesendanger, 1985; Gould *et al.*, 1989). The caudal somatosensory belt may be multisensory, with visual and perhaps auditory inputs (Wallace *et al.*, 2004; Remple *et al.*, 2006). Thus, the somatosensory cortex along the rostral border of S1 has motor and sensory features that resemble area 3a of primates, and the somatosensory belt caudal to S1 has features reminiscent of areas 1 and 2 of primates. A smaller adjoining posterior region resembles posterior parietal cortex of primates.

As in most other mammals, S1, S2, and possibly PV of tree shrews receive activating inputs from the VP of the thalamus (Garraghty *et al.*, 1991). Thus, VP projects in parallel to these areas. The cortical and VP activation is completely dependent on the

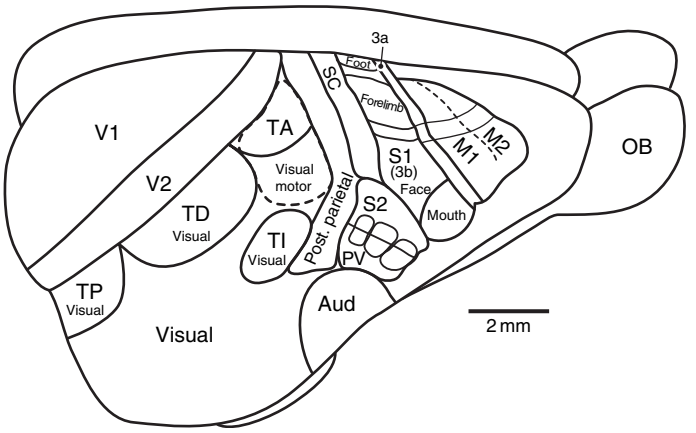

Figure 5 Subdivisions of sensorimotor cortex in tree shrews shown on a dorsolateral view of the right cerebral hemisphere. Somatosensory areas include primary somatosensory cortex (S1 or area 3b), a rostral somatosensory belt (SR in Figure 3) that is called area 3a here, a caudal somatosensory belt (SC) that has two divisions (SC1 and SC2), a second somatosensory area (S2), and a parietal ventral area (PV). In addition, a posterior parietal region has connections with somatosensory and motor areas, the anterior temporal area (TA) has visuomotor cortical connections, as does cortex just lateral to TA. The three ovals bridging the S2–PV border correspond to representations of the hindlimb, forelimb, and mouth in a caudorostral sequence. Motor cortex includes a primary motor area (M1) and a premotor area (M2). An auditory region (Aud) may contain several auditory areas, while most of the caudal half of the cerebral hemisphere is visual. The first and second visual areas (V1 and V2) are identified, as well as proposed temporal posterior (TP), temporal dorsal (TD), temporal anterior (TA), and temporal inferior (TI) visual areas. Scale bar: 2 mm. Based on Remple, M. S., Reed, J. L., Stepniewska, I., and Kaas, J. H. 2006. The organization of frontoparietal cortex in the tree shrew (*Tupaia blangeri*). I: Architecture, microelectrode maps and corticospinal connections. *J. Comp. Neurol.* 497, 133–154.

relay to VP of the dorsal column trigeminal brainstem complex via the medial lemniscus in rats (Jain *et al.*, 2003) and monkeys (Jain *et al.*, 1997), and it is not replaced after injury by the spinothalamic system.

As in other placental mammals, tree shrews (Remple *et al.*, 2006) and rats and other rodents (Wise and Donoghue, 1986) have a primary motor area, M1. In rodents, a popular proposal is that M1 partly overlaps S1, especially in the medial portion devoted to the hindlimb (Sanderson *et al.*, 1984). This premise largely stems from the observation that movements can be evoked from portions of S1 at current thresholds comparable to those for M1. This reflects the widespread role of S1 in motor functions. In rats (Li *et al.*, 1990), more of the corticospinal neurons originate in S1 than rostral somatosensory belt or M1, in marked contrast to primates (Wu *et al.*, 2000). In tree shrews (Remple *et al.*, 2006), S1 (3b), the rostral belt (3a), and M1 contribute nearly equally to corticospinal projections. M1 is an architectonically distinct area of agranular cortex in rats and tree shrews, with a complete or nearly complete representation of movements of the contralateral body. In rats, the emphasis is on facial whisker movements and in tree shrews, face and tongue movements; the evoked movements of the forelimb in these mammals are rather crude, with little evidence of individual digit control (Remple *et al.*, 2003; Sanderson *et al.*, 1984; Wise and Donoghue, 1986). As a reflection of the predominant origin of corticospinal axons from S1 rather than M1, the spinal cord terminations in most mammals are largely in the dorsal horn and intermediate zone of the spinal gray (e.g., Doetsch and Towe, 1981; Armand, 1982). The dense terminations of corticospinal axons of primates onto the motor neurons supplying distal limb muscles does not exist or is very limited in rodents and tree shrews. As an unusual feature, the spinal course of the corticospinal fibers in rodents and tree shrews is at the base of the contralateral dorsal columns rather than in the lateral spinal cord as in primates.

In addition to M1 (Neafsey *et al.*, 1986; Remple *et al.*, 2006), rodents and tree shrews have a second motor area, M2, along the rostrodorsal border of M1 (Figures 4 and 5). This second motor area has few corticospinal neurons, and higher currents are required to evoke movements than in M1. The overall pattern of somatotopy of evoked movements parallels that of M1. There are various possible interpretations of how this M2 compares to subdivisions of motor cortex in primates. As M1 in monkeys has rostral and caudal subdivisions (e.g., Stepniewska *et al.*, 1993), one possibility is that M2 is a subdivision of M1. A more likely possibility is that M2 is homologous to either the supplementary motor area (SMA) or the premotor cortex (PM) of

primates (Rouiller *et al.*, 1993). While premotor cortex has been divided into dorsal and ventral premotor areas in primates, and these areas have been further divided in monkeys (see below), the somatotopy of ventral premotor cortex, representing the head and forelimb, and the somatotopy of dorsal premotor cortex, devoted mainly to the hindlimb and forelimb, suggests that they may have differentiated out of a single premotor area bordering M1 (an area that might have resembled M2 of rats and tree shrews).

In conclusion, comparisons of the sensorimotor systems of the nearest available relatives of primates (lagomorphs, rodents, and tree shrews) suggest that the immediate ancestors of primates had a rather simple sensorimotor system. The basic subcortical components of the system were likely those found in many other mammals. In primary somatosensory cortex, a moderate enlargement and rearrangement of the forepaw representation was likely. Cortex caudal to S1 included a rostral somatosensory band (SC) bordering S1 and a more caudal zone of posterior parietal cortex with inputs from S1. The position of SC, the somatosensory region immediately caudal to S1, suggests that it subsequently differentiated into area 1 or perhaps areas 1 and 2 of primates. A band of dysgranular cortex on the rostral border of S1 had more pronounced motor functions and connections and possibly proprioceptive sensory inputs. This cortex appears to be the homologue of area 3a of primates. A separate and architectonically distinct agranular motor cortex, M1, was present as well as a premotor area, M2. However, the majority of the corticospinal projections emerged from S1, the rostral somatosensory area, RS, and M1 rather than M2. Furthermore, these corticospinal projections terminated in the dorsal and intermediate levels of the spinal grey and not in the motor neuron pools of the ventral spinal grey, thereby having a less direct effect on motor control. Finally, these cortical projections were focused in the brainstem and cervical spinal cord with little extension into the thoracic and lumbar spinal cord.

4.02.3 Somatosensory and Motor Systems of Early Primates: Inferences from Prosimians and Other Primates

Primates emerged as a branch of the Euarchontoglires superclade (Figure 1) over 7080 Mya (Block and Boyer, 2002; Murphy *et al.*, 2004). Subsequently, they separated into four main lines, including the plesiadapiforms, a semi-order of extinct archaic primates, and the euprimates. The stem euprimates led to the present-day lemurs, lorises, and galagos, the highly specialized tarsiers, and the greatly varied anthropoid monkeys, apes, and humans (Figure 6). Some of the lemurs and galagos closely resemble early primates in body type, brain size relative to body size, and brain shape. Most early primates were the size of cats or smaller, nocturnal, and fed in the fine branches of bushes and trees on insects, small vertebrates, fruits, and leaves (Ross, 1996; see The Role of Vision in the Origin and Evolution of Primates). This behavioral niche required exceptional sensorimotor abilities in that there was the need to stabilize the body in moving branches while reaching for food items (Block and Boyer, 2002). Whishaw (2003) suggests that visual control of hand movements is likely to be the distinguishing feature of primate behavior. To mediate improvements in visually guided reaching behaviors and other sensorimotor abilities, early primates were characterized by an increase in the complexity of visual cortex, the involvement of regions of posterior parietal cortex in visuomotor processing, and the emergence of premotor areas with visuomotor inputs.

Given that great effort is needed to reveal the organization of sensorimotor systems in any primate, and that there are over 200 species of primates (Purvis, 1995), with many of them protected or otherwise unavailable for study, research on primates has concentrated on a few useful and informative species (Kaas, 2002). Galagos are rat- to cat-sized, arboreal, nocturnal, African primates that feed on fruits, gums, and insects. They are easily bred and reared in the laboratory, and their nervous systems have been more extensively studied than those of other prosimian primates. Thus, much of what we know about the sensorimotor systems of prosimian primates is based on results of studies on galagos. In general, we assume that features of the sensorimotor system of galagos and other prosimians that are shared with other primates emerged early in primate or preprimate evolution. Traits present in prosimians and other Euarchontoglires mammals emerged early, with or before Euarchontoglires mammals.

As in other mammals, afferents from the skin and muscles of galagos and other primates enter the brainstem and spinal cord with one branch synapsing on cells in the dorsal horn or the brainstem and the other coursing to the dorsal column–trigeminal complex in the lower brainstem (Figure 2). A notable modification in primates is that the representation of the glabrous hand is expanded and differentiated in the cuneate nucleus of the complex. In galagos, this nucleus is larger than in rats

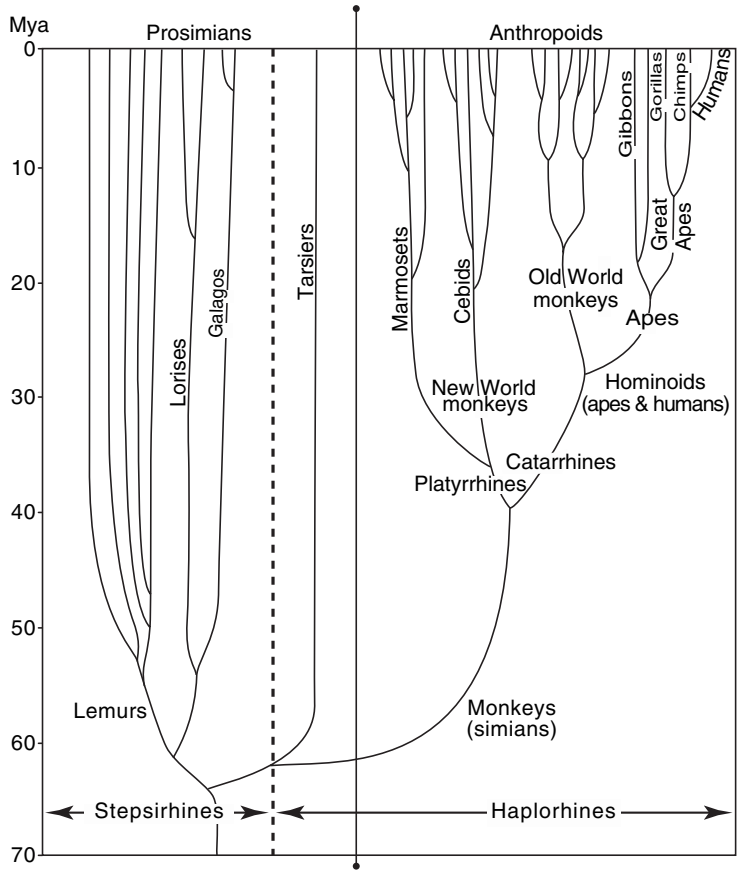

Figure 6 The evolution of extant primates. While archaic primates diverged from other Euarchontoglires over 80 Mya, the separation into prosimians, tarsiers, and anthropoids occurred between 70 and 60 Mya. Based on Purvis (1995) and others. Time in Mya is indicated on the left. Modified from Kaas, J. H. and Preuss, T. M. 2003. Human brain evolution. In: Fundamental Neuroscience (*ed.* L. R. Squire), 2nd edn., pp. 1147–1166, Academic Press.

and tree shrews, and the digits are represented from D1 (thumb) to D5 in a lateromedial sequence (Strata *et al.*, 2003). This nucleus receives the rapidly adapting and slowly adapting cutaneous afferents from the hand, while the muscle spindle (proprioceptive) receptors relay to a well-differentiated external cuneate nucleus. The dorsal column–trigeminal complex relays, as in other mammals, to the contralateral VP of the thalamus. VP is architectonically very distinct in Nissl (Figure 7), cytochrome oxidase, and other preparations (Kaas *et al.*, 2006), and the nucleus is subdivided by septa that separate subnuclei representing different body parts. Thus, a narrow lateral portion is devoted to the tail, an adjoining more medial portion represents the hindlimb, while a larger more medial sector is devoted to the forelimb, mainly the glabrous hand. An elongated medial portion has subdivisions representing various portions of the face and oral cavity (lips, tongue, teeth). VP of galagos and other primates differs from rats and tree shrews in that the nucleus is more elongated mediolaterally, with proportionally more of VP devoted to the limbs, especially the glabrous hand. Yet, the representations of the lips, tongue, and teeth are large. The VP projects to S1, S2, and PV, as in other mammals, thereby providing activating inputs in parallel to three areas of somatosensory cortex. Thus, lesions of S1 fail to deactivate S2 and PV (Garraghty *et al.*, 1991). In this way, galagos resemble their nonprimate ancestors and differ from anthropoid primates where VP no longer projects to S2 and PV, and lesions of S1 deactivate S2 (Garraghty *et al.*, 1990).

Three other nuclei of the primate somatosensory thalamus are apparent in galagos. Just ventral to VP, the ventroposterior inferior nucleus (VPI) contains small neurons that are pale-staining in Nissl preparations (Figure 7a). VPI receives inputs from the spinothalamic pathway and projects broadly to somatosensory areas of cortex, largely to the superficial layers. These VPI projections appear to modulate the activity of neurons in cortical areas, rather than provide an independent source of suprathreshold activation. VPI is not recognized as a

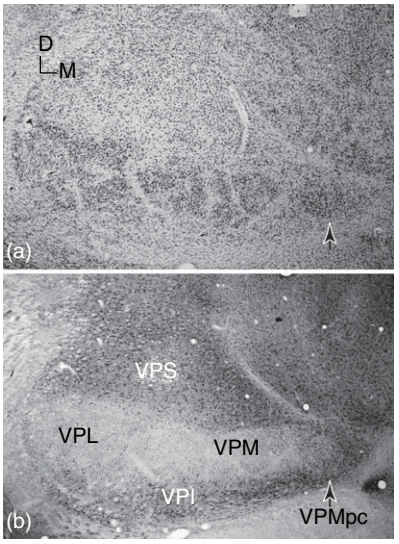

Figure 7 The somatosensory thalamus of a prosimian primate (galago). The thalamus has been cut into thin sections in the frontal (coronal) plane and processed with a Nissl stain for cell bodies (a) or for the expression of a calcium-binding protein, calbindin (b). In both sections, medial (toward the third ventricle) is right, and dorsal is up. In a Nissl preparation, the VP is apparent as a region of larger, darkly stained neurons (arrow), in contrast to the lightly stained, less densely packed neurons in the ventroposterior superior nucleus (VPS) just dorsal (superior) to VP and the ventroposterior inferior nucleus (VPI) just ventral (inferior) to VP. Medially, a 'taste' nucleus, the parvocellular ventroposterior medial nucleus (VPMpc), has more uniformly distributed, smaller cells. The VP is subdivided by a cell-poor septum into a large medial ventroposterior subnucleus (VPM) and a lateral ventroposterior subnucleus (VPL). Another septum separates VPL into a medial portion representing the hand and a lateral portion representing the foot. Other septa are apparent in VPM. In sections processed for calbindin, VP (VPL plus VPM) expresses little calbindin, while VPS, VPI, and VPMpc express more. Other histological preparations also demonstrate clear differences between these nuclei. These three somatosensory nuclei are well differentiated in primates compared to most other mammals. In many mammals (Figure 2), part or all of the region identified as the posterior nucleus may be a homologue of VPS, while a region comparable to VPI is seldom identified.

distinct nucleus in the nonprimate relatives of primates, but VPI is obvious in all primates. As spinothalamics also terminate in the septal zones of VP in primates, it is possible that VPI differentiated from a VP with both spinothalamic and medial lemniscus inputs. A VPI that closely resembles VPI of primates apparently differentiated independently in raccoons (Herron, 1983). The ventroposterior superior nucleus (VPS) is just dorsal to VP. This nucleus receives muscle spindle receptor inputs and projects to cortex termed 3a or SR (Figure 8) just rostral to S1 (area 3b). In Nissl preparations, VPS has less densely packed and less darkly stained neurons than VP, and these neurons express more calbindin and less cytochrome oxidase (Figure 7). VPS is apparent in all primates, but its homologue in nonprimates is uncertain. Possibly, the posterior nucleus of rodents or the proprioceptive rostral cap of VP is a homologue of VPS. Just medial to VPS, the anterior pulvinar (PA) projects widely to areas of somatosensory cortex, while receiving inputs from somatosensory cortex. This nucleus is more conspicuous in anthropoid primates, while having no obvious counterpart in rodents and tree shrews. Finally, a taste or gustatory relay nucleus, VPMpc, can be identified just ventromedial to VP in primates (Figure 7), as in other mammals.

In summary, the comparative evidence indicates that the somatosensory thalamus of early primates had been modified to reflect a primate pattern while retaining some primitive features. Most notably, the ventroposterior inferior and superior nuclei, together with the anterior pulvinar had differentiated to become identifiable structures. In addition, VP acquired more of a primate configuration, with a mediolaterally elongated shape and subdivisions separated by septa for the forelimb and hindlimb, as well as subdivisions of the face and oral cavity. The subdivision of VP for the forelimb, especially the glabrous hand, had become enlarged.

Somatosensory cortex of galagos has some, but not all of the features of somatosensory cortex in anthropoid primates. In some ways, anterior parietal cortex resembles that of tree shrews and rats. However, a primary somatosensory area, S1 (area 3b), is elongated mediolaterally and it contains an anthropoid-like somatosensory representation (Figure 8). The primary somatosensory area, S1, is bordered rostrally and caudally by parallel somatosensory representations. The rostral somatosensory field, SR, by position, architectonic features, responsiveness to stimuli, and inputs from VPS, seems to have most of the features of area 3a of anthropoid primates, and we use the term, area 3a, here. The caudal somatosensory area, SC, has the position, architecture, and the sparse inputs at least from the VP and the dense inputs from area 3b that characterize area 1 of anthropoid primates, but SC does not respond well to somatosensory stimuli in anesthetized galagos, and thus a detailed somatosensory representation has not been produced in microelectrode mapping studies. As such a map has been used to identify area 1 as a second systematic representation of the cutaneous receptors of the contralateral body surface in anthropoid primates (e.g., Kaas *et al.*, 1979), it remains uncertain if SC of

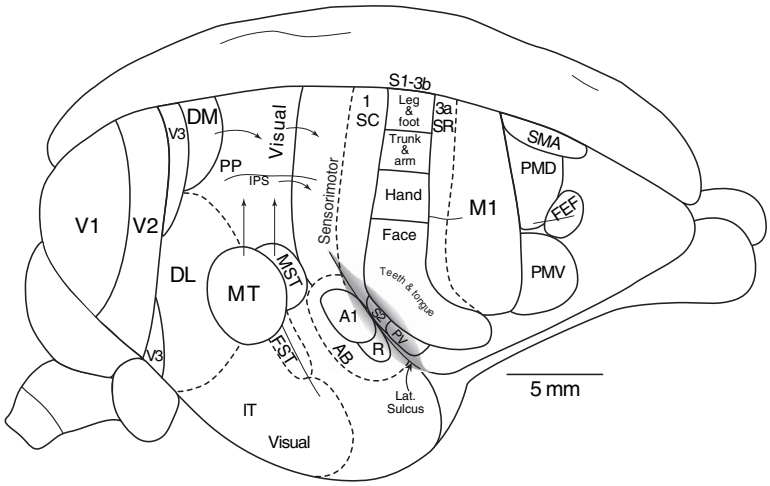

Figure 8 Sensorimotor cortex of a prosimian primate (galago) shown on a dorsolateral view of the right cerebral hemisphere. Somatosensory cortex includes S1 (area 3b), a rostral somatosensory strip (SR) that appears to be homologous to area 3a of other primates, and a caudal somatosensory strip (SC) that may be homologous to area 1 or areas 1 and 2 of other primates. On the upper bank of the lateral sulcus, adjoining S1, somatosensory areas S2 and PV are bordered deeper in the sulcus (not shown) by the parietal rostral somatosensory area (PR) and caudal and rostral divisions of the ventral somatosensory area (VSc and VSr), as well as a retroinsular somatosensory area (Ri). Posterior parietal cortex includes two major subdivisions: a rostral region with dense somatosensory inputs and projections to motor and premotor areas and a caudal region dominated by inputs from visual areas (arrows) and projections to the rostral somatosensory region of posterior parietal cortex (arrows). Both regions appear to contain several subdivisions or areas. Motor cortex includes primary motor cortex, M1, dorsal and ventral premotor areas (PMD and PMV), a frontal eye field (FEF), a supplementary motor area (SMA), a pre-SMA, and rostral and caudal cingulate motor areas (CMAr and CMAc) on the medial wall (not shown). The PMD has rostral (PMDr) and caudal (PMDc) subdivisions, differing in connections and architecture. For reference, first, second, and third (V1, V2, and V3), dorsomedial (DM), dorsolateral (DL), middle temporal (MT), medial superior temporal (MST), frontal superior temporal (FST) visual areas are shown. Auditory areas include the primary area (A1), rostral area (R), and the auditory belt (AB). IPS, intraparietal sulcus. Based on Wu *et al.* (2000), Wu and Kaas (2003), and Stepniewska *et al.* (2005).

galagos has all of the characteristics of area 1. Yet it seems reasonable to propose that SC in galagos, and perhaps other mammals, is the homologue of area 1 of anthropoid primates. Alternatively, SC could be the homologue of areas 1 and 2 combined (see below).

In cortex lateral to area 3b, galagos have the two areas, S2 and PV (Figure 8), with inputs from S1 (area 3b), SR (3a), and SC (area 1). S2 and PV in turn project to other areas located in the cortex of the lateral sulcus, including the insula (Wu and Kaas, 2003). Because these additional areas have been identified mainly by relative location, architecture, and connection patterns, their identities remain somewhat uncertain. Two of the locations appear to correspond to the recently defined caudal and rostral divisions of the ventral somatosensory complex, VSc and VSr of anthropoid primates (Coq *et al.*, 2004). Other locations with S2 and PV connections include the retroinsular cortex (Ri) and the parietal rostral area, PR, of monkeys (Krubitzer and Kaas, 1990). Thus, it appears that galagos have an array of somatosensory areas in cortex of the lateral sulcus that largely matches that described in monkeys, and includes at least two areas, PV and S2, also found in other mammals.

Posterior parietal cortex is more extensive and more complexly organized in galagos than in related nonprimates such as tree shrews and rats. The rostral half of posterior parietal cortex can clearly be identified as part of the somatosensory cortical network due to dense interconnections with somatosensory areas of anterior parietal cortex, especially area 1, and areas of the lateral sulcus, including S2, PV (Wu and Kaas, 2003), and adjoining areas (Fang *et al.*, 2005). The rostral half of posterior parietal cortex projects densely to subdivisions of motor cortex (see below). In addition, this cortex is clearly involved in motor functions. Electrical stimulation of sites throughout rostral posterior parietal cortex evokes movements of body parts, depending on the site stimulated (Stepniewska *et al.*, 2005). Overall, there is a tendency for the stimulation of medial locations in posterior parietal cortex to evoke hindlimb movements, middle locations to evoke forelimb movements, and lateral locations to evoke face and eye movements, but there is a more complex pattern imposed on this tendency. Subregions in this rostral half of posterior parietal cortex appear to relate to components of

ethologically relevant behaviors, so that there are regions where stimulation evokes defensive movements, reaching movements, and hand-to-mouth movements. All these evoked behaviors are mediated via other parts of the sensorimotor network, as movements are no longer evoked when the functions of primary motor cortex are blocked. Besides the direct somatosensory inputs to posterior parietal cortex, the rostral somatosensory half receives dense inputs from the caudal visual half, which is identified as predominantly visual in function by the presence of inputs from a number of previously defined primate visual areas (Fang *et al.*, 2005), including the middle temporal visual area (MT), the medial superior temporal area (MST), and the dorsomedial area (DM). Thus, visual and somatosensory inputs combine in posterior parietal cortex to provide guidance for the execution of reaching, retrieving, and defensive movements.

It is tempting to relate subdivisions of posterior parietal cortex in galagos to those proposed for anthropoid primates, especially the more fully studied areas of macaque monkey. Areas predominantly involved in reaching, retrieving, and eye movements have been described (Cohen and Andersen, 2002), and defensive movements have been evoked by electrical stimulation of one of these areas, the ventral intraparietal area, VIP (Cooke *et al.*, 2003). Unfortunately, not enough is known about these proposed areas in prosimians and simians to productively identify homologous areas and specify differences. Yet it is clear that galagos, as in anthropoid primates, have an extensive posterior parietal region with visual and somatosensory inputs, motor cortex outputs, and sensorimotor functions. This expanded region of cortex is larger and more complexly organized than in the extant relatives of primates.

Galagos also resemble anthropoid primates and differ from other mammals in the organization of motor cortex. In tree shrews and rats, there was evidence for only a primary motor area (M1), and a premotor field (M2). The primary motor area was not a major source of corticospinal projections, and representation of the forepaw was not proportionally larger. In galagos, M1 is not as architectonically differentiated as in simians, but a substantial sector of M1 represents forelimb movements, and this sector provides most of the corticospinal projections to the cervical spinal cord (Wu *et al.*, 2000), as in monkeys (e.g., Nudo and Masterton, 1990; Wu and Kaas, 1999). In both galagos and monkeys, additional corticospinal neurons are located in area 3a and in premotor cortex. However, M1 differs from monkeys (e.g., Stepniewska *et al.*, 1993) in that few locations in M1 of galagos evoke movements of digits. Thus, some aspects of galago M1 are intermediate between M1 of nonprimate relatives and M1 of simians.

Galagos have most of the premotor areas of anthropoid primates (Wu *et al.*, 2000). They have a dorsal premotor area, PMD, with a rostral division more densely connected to prefrontal cortex and a caudal division more densely connected to M1. They have a ventral premotor area, PMV, which is preferentially devoted to forelimb and face movements. Galagos also have a frontal eye field where electrical stimulation of sites evokes rapid, saccadic eye movements. On the medial lip of the dorsal cortex of the cerebral hemisphere, galagos have an SMA, and at least two cingulate motor areas, rostral (CMAr) and caudal (CMAc), exist in cortex of the medial wall, where there is evidence for a cingulate sensorimotor area. There is also evidence for a presupplementary motor area (pre-SMA). These motor fields are each characterized by a particular pattern of connections with other areas and thalamic nuclei in galagos and other primates (see Wu *et al.*, 2000; Fang *et al.*, 2005). Thus, PMD receives inputs from a broad band of mostly medial regions in posterior parietal cortex, PMV from more lateral and rostral parts of posterior cortex as well as from somatosensory areas, and SMA with the medial portion of posterior parietal cortex. Other connections are with frontal cortex, other motor areas including callosal connections between motor areas, basal ganglia, and the motor nuclei of the thalamus. All of these areas are also found in monkeys. As in macaques and other monkeys, these areas interconnect, and directly or indirectly influence the output of primary motor cortex, M1. In addition, most of these areas (PMV, PMDc, CSMA, CMAc, CMAr, SMA, and, of course, M1) contribute at least sparse corticospinal projections. Thus, both galagos and monkeys have highly similar systems of motor and premotor areas, as parts of a more extensive sensorimotor network.

In conclusion, it appears likely that the sensorimotor system of early primates resembled that found in present-day galagos. Many of the features of the system in galagos are shared with other primates, providing evidence that these features emerged early in primate evolution, perhaps in the immediate nonprimate ancestors of primates. To the extent that the sensorimotor system of galagos differs from that in anthropoid primates, galagos tend to resemble the nonprimate relatives of primates. Thus, M1 is less differentiated architectonically

and digit movements of the forepaw are poorly represented compared to M1 of monkeys. Posterior parietal cortex is less extensive and likely has fewer subdivisions in galagos. In addition, PMV appears to lack the rostral and caudal subdivisions that are apparent in macaque monkeys, and this would seem to be a more primitive condition. However, the important conclusion is that galagos share many features with other primates that are not found in the nonprimate relatives. The primate line emerged with an array of advances in the organization and connections of the somatosensory thalamus, anterior parietal cortex, lateral parietal cortex, posterior parietal cortex, and motor and premotor cortex.

4.02.4 New and Old World Monkeys: Similarities and Variations

While early primates were nocturnal visual predators in tropical rainforests, the line leading to haplorhine primates (tarsiers and anthropoids) were small, arboreal, insectivorous, but diurnal primates (Ross and Kay, 2004). The shift to diurnality led to a number of changes in the visual system (Kaas, 2003). The ancestors of tarsiers reverted back to a nocturnal niche, and specializations for nocturnal vision reemerged (Collins *et al.*, 2005). Tarsiers became the only primates who eat no vegetation. The early anthropoids modified their teeth and jaws to be able to add unripe fruits and leaves to their diet (Lockwood and Fleagle, 1999). Features of present-day platyrrhine (New World) monkeys and catarrhine (Old World) monkeys indicate that part of the anthropoid adaptation included modifications of the sensorimotor system. The radiation of New World monkeys was based on the chance immigration of early anthropoids from Africa to South America at least 30–40 Mya, probably by rafting (Flynn and Wyss, 1998). Several similar modifications of their sensorimotor systems probably occurred independently in both platyrrhine and catarrhine radiations.

In the somatosensory systems of monkeys, some modifications are apparent even at the levels of the brainstem and spinal cord. Because of an increase in the number of cutaneous receptors in the glabrous skin of the hand (forepaw) of monkeys, more of the dorsal horn of the cervical spinal cord is devoted to afferents from the digits and palm (Florence *et al.*, 1991) than in most other mammals. This selective enlargement is also apparent in the cuneate nucleus (representing the forelimb) of the dorsal column–trigeminal complex of the lower brainstem. In addition, there is at least one major variation in the somatotopy of the cuneate nucleus. In Old World macaque monkeys and humans, the distal phalanges of the digits are represented ventrally in the nucleus, and this appears to reflect the generalized mammalian pattern (Florence *et al.*, 1989). In New World squirrel monkeys, the pattern is reversed, with the representation of the digit tips dorsal in the nucleus (Florence *et al.*, 1991). This raises the possibility that different somatotopies in the cuneate nucleus characterize platyrrhine and catarrhine primates, with platyrrhines diverging from the ancestral pattern. However, more primates need to be studied to evaluate this hypothesis. While the somatotopic organizations are reversed in these two taxa, there is no obvious functional consequence of having either variation.

At the level of the thalamus, the ventroposterior (VP), ventroposterior superior (VPS), ventroposterior inferior (VPI), and anterior pulvinar (PA) nuclei are well differentiated in monkeys. However, in Old World monkeys, the marked histological differences between VP and VPS are reduced, with VPS becoming more similar to VP, suggesting an enhanced or altered role for VPS in the system. Cortical organization has also changed, as have patterns of thalamocortical connections. Most notably, in anterior parietal cortex, four strip-like, parallel somatosensory representations are found, corresponding closely to the classical architectonic fields 3a, 3b, 1, and 2 of Brodmann (1909). VP projects to layer 4 of area 3b and more superficially to area 1, and sparsely to parts of area 2 (e.g., Cusick *et al.*, 1985; Pons and Kaas, 1985; see Kaas, 2004c for review). A portion of neurons in VP, perhaps 20%, project to both area 3b and area 1. Nevertheless, evoked neural activity in area 1 depends on inputs from area 3b, as lesions of area 3b abolish the responsiveness of area 1 to cutaneous stimulation (Garraghty *et al.*, 1990). VPS projects to both area 3a and area 2, providing muscle spindle proprioceptive input, with perhaps 40% of the neurons projecting to both areas. Thus, some of the individual neurons in both VP and VPS project to areas 3a and 1, thereby providing the same information. Unlike other mammals, including prosimian galagos (Burton and Carlson, 1986), VP does not project to S2 and PV. Instead, VPI projects densely to these areas (Friedman and Murray, 1986; Krubitzer and Kaas, 1992). This VPI input apparently modulates the activity of S2 and PV neurons, as these areas depend on anterior parietal cortex for activation (Pons *et al.*, 1987). This is a modification from the ancestral condition seen in galagos and other mammals where VP activates S2 and PV independently. Thus, processing in anthropoid primates became more serial.

4.02.4.1 The Anterior Parietal Cortex of Simians

The representational features of the anterior parietal representations are somewhat variable across different New World and Old World monkeys. For example, the representations of the receptors of the glabrous digits are particularly large and distinct in the dexterous macaque and cebus monkeys, but the hand representation is not so pronounced in New World marmoset monkeys (Carlson *et al.*, 1986). Marmosets and other members of the family Callitrichidae are interesting as these very small monkeys have claws rather than nails on the digits of the hand. Although this has been considered a primitive feature, it appears to be a derived specialization (Sussman and Kinzey, 1984).

New World cebus monkeys and Old World macaque monkeys apparently evolved their greater hand representation and hand dexterity independently. Cebus monkeys are unusual in that they use their tail for active tactile exploration, and the ventral tip of the tail has a glabrous skin surface densely packed with cutaneous receptors. As one might expect from this arrangement, the somatosensory system devotes a large amount of tissue to these receptors in the prehensile tail, with over 10 mm^2 of area 3b activated by the tail (Felleman *et al.*, 1983). This is certainly a derived and remarkable feature of cebus and spider monkeys. Finally, certain aspects of the representation of the body in area 3b of monkeys are reversed in some taxa compared to others. In some monkeys such as cebus and squirrel monkeys, the dorsoventral dimension of the trunk is represented in a reverse order from that in other monkeys (Sur *et al.*, 1982; Felleman *et al.*, 1983). This reversal has no clear functional consequences or implications, but such features suggest that many details of brain organization will be found to vary in patterns that reflect phylogenetic relationships.

Area 1 is also variable in anthropoid primates. In most, area 1 stands out as an architectonically distinct strip of tissue along the caudal border of area 3b. In Nissl preparations, area 3b has a well-developed layer 4 of small cells (granular cells), area 1 has a notably less distinct layer 4, and area 2 has a more distinct layer 4. In addition, area 1 contains a systematic representation of cutaneous receptors, from tail to tongue in a mediolateral sequence, that forms a mirror reversal of the somatotopy of area 3b (see Merzenich *et al.*, 1978 for the first complete description). Neurons in area 1 have larger receptive fields and more complex response properties than those in area 3b. They constitute a second stage of cortical processing as they depend on area 3b projections for activation, although they also receive inputs from the VP of the thalamus. The strip of cortex caudal to area 3b in prosimian galagos also has most of these characteristics, as already discussed, but neurons in anesthetized galagos have not been responsive enough to demonstrate a second mirror image representation, as in monkeys. In addition, the cortex caudal to this strip in galagos is not architectonically very distinct from the strip. These differences suggest that some caution is needed in identifying the caudal somatosensory strip in galagos as area 1, although this does seem likely. This same need for caution applies to Callithricid monkeys, the small tamarins, and marmosets. Cortex just caudal to area 3b in the position of area 1 is not very responsive to tactile stimulation and a systematic representation in this cortex has not been demonstrated (Carlson *et al.*, 1986). Yet projection patterns from area 3b indicate that at least a crude parallel representation exists in this cortex (Krubitzer and Kaas, 1990). In this regard, the area 1 strip in marmosets resembles the SC strip in galagos. The reduced responsiveness of area 1 or SC in marmosets could reflect the retention of ancestral (prosimian) features, but more likely, a regression as the simian ancestors of marmosets evolved into the smallest of anthropoids.

Brodmann (1909) defined a strip of cortex just caudal to area 1 as area 2. In macaque monkeys, most of area 2 is highly responsive to tactile stimuli, and a representation of contralateral cutaneous receptors in area 2 roughly parallels the representation in area 1 (Pons *et al.*, 1985). Thus, the foot representation in area 2 is immediately caudal to the foot representation in area 1, and so on, to form a foot to face representation that parallels that in area 1. To some extent, this representation is a mirror reversal of the one in area 1, but the area 2 representation is not so simple, as it has further reversals of somatotopy within the field. In particular, glabrous digits and pads are represented twice. As area 2 has only been explored in detail in macaque monkeys, we do not know how or if it varies in organization across anthropoids, or even if it really exists in all anthropoids. The only microelectrode mapping study with evidence for an area 2 representation is New World monkeys provided only a brief description of a tail-to-hand representation of deep, possibly proprioceptive, receptors in a mediolateral sequence in parallel with the area 1 representation in owl monkeys (Merzenich *et al.*, 1978). More recently, Padberg *et al.* (2005) recorded from the lateral sector of the area 2 region of New World titi monkeys, and provided evidence for a forelimb representation in area 2 of New World monkeys. However, other interpretations are possible, especially in view of the lack of convincing architectonic evidence for an area 2 in New World monkeys.

Padberg *et al.* (2005) postulate that an area 2 does not exist in New World monkeys, but instead is a feature of the somatosensory system that emerged in catarrhine primates. A more conservative conclusion is that an area 2 representation of predominantly proprioceptive receptors (muscle spindle receptors) evolved in the early anthropoids and this representation became well differentiated in the early catarrhines, and perhaps independently in some of the platyrrhines such as cebus monkeys, although this is uncertain. In New World titi monkeys (Coq *et al.*, 2004), lateral area 1 has connections with the area 2 region, as expected for an area 2, and in New World squirrel monkeys (Cusick *et al.*, 1985), VPS projects to the area 2 region, as expected for area 2. Thus, there is some, but limited, evidence for the existence of an area 2 representation in New World monkeys.

4.02.4.2 The Posterior Parietal Cortex of Simians

The posterior parietal cortex of macaque monkeys has been subdivided in a number of ways into as many as 15–20 potential areas (Lewis and Van Essen, 2000b). The relative extent of posterior parietal cortex in some of the smaller New World monkeys is much less than in macaques, suggesting that more complexity evolved in catarrhine primates. Some of the proposed subdivisions of posterior parietal cortex of macaques are shown in Figure 9. These include the number of areas in the deep intraparietal sulcus of macaques, where the most studied region, the lateral intraparietal area (LIP), appears to be specialized for directing saccadic eye movements via connections with visual areas, the frontal eye field, and superior colliculus (Ben Hamed *et al.*, 2001). The adjacent medial intraparietal area (MIP) has been enlarged caudally by some investigators to become the parietal reach area (PRA), an area thought to be involved in the planning of visually guided reaching movements with the area via visual inputs and connections with dorsal premotor cortex (Marconi *et al.*, 2001; Cohen and Andersen, 2002). The ventral intraparietal area (VIP) receives visual and somatosensory inputs while projecting to premotor cortex (e.g., Lewis and Van Essen, 2000a). VIP appears to be important in guiding monkey locomotion, as well as defensive and avoidance movements to protect against collisions (Cooke *et al.*, 2003). The anterior intraparietal area (AIP) receives visual information from LIP, and projects to ventral premotor cortex, possibly to guide hand grasping and manipulation movements (Sakata and Taira, 1994; Nakamura *et al.*, 2001). The rostromedial bank of the intraparietal cortex, area 5ip or PEa, contains a systematic representation of cutaneous receptors of the hand and digits (Pons *et al.*, 1985) while receiving somatosensory inputs from areas 1, 2, and S2 and projecting to motor and dorsal premotor cortex (Pons and Kaas, 1986). A role in guiding reaching has been hypothesized for this region of cortex (Iwamura and Tanaka, 1996). Further subdivisions of posterior parietal cortex have been proposed for macaques, including subdivisions of large regions traditionally referred to as area 7a, 7b, 5a, and 5b. For example, the region of area 7 just lateral to the intraparietal sulcus has been recently divided into four areas with different patterns of connections to premotor cortex (Gregoriou *et al.*, 2006). At least some of these connection patterns and suggested functional roles are similar to those proposed for subdivisions of posterior parietal cortex in prosimian galagos (Stepniewska *et al.*, 2005), but too little is currently known to allow an area-by-area matching of homologous areas.

Another interpretation of the organization of the rostral half of posterior parietal cortex, including most of areas 5 and 7b, is possible. In monkeys, this broad band of cortex contains a crude somatotopic organization where medial parts relate to the shoulder, arm, trunk, and perhaps the forelimb, more lateral parts in the parietal sulcus relate to the glabrous hand, and the most lateral parts in area 7b relate to the face, lips, and oral structures (Krubitzer and Disbrow, 2006). Again, there is an overall similarity to the crude functional pattern in the rostral half of posterior parietal cortex of galagos. Thus, one could consider the complete band of cortex in both primates to be a single functional area. However, the bulk of the evidence suggests that this band of cortex contains several functionally distinct areas in both prosimians and simians.

In several parts of posterior parietal and parietal–temporal cortex of simians, both auditory and somatosensory inputs activate neurons. This includes VIP of posterior parietal cortex. More laterally, posterior parietal cortex adjoins the temporal lobe, where the caudodorsal portion of temporal cortex is subdivided into an array of three primary auditory areas, perhaps eight secondary areas, and at least two areas of a third level of cortical processing (Kaas and Hackett, 2000). At least one of the second-level areas, the caudomedial area (CM), has neurons that respond to auditory and somatosensory stimuli, with somatosensory inputs coming from somatosensory areas of the lateral sulcus and possibly the somatosensory thalamus (Schroeder *et al.*, 2003). Although cortical regions of somatosensory and auditory

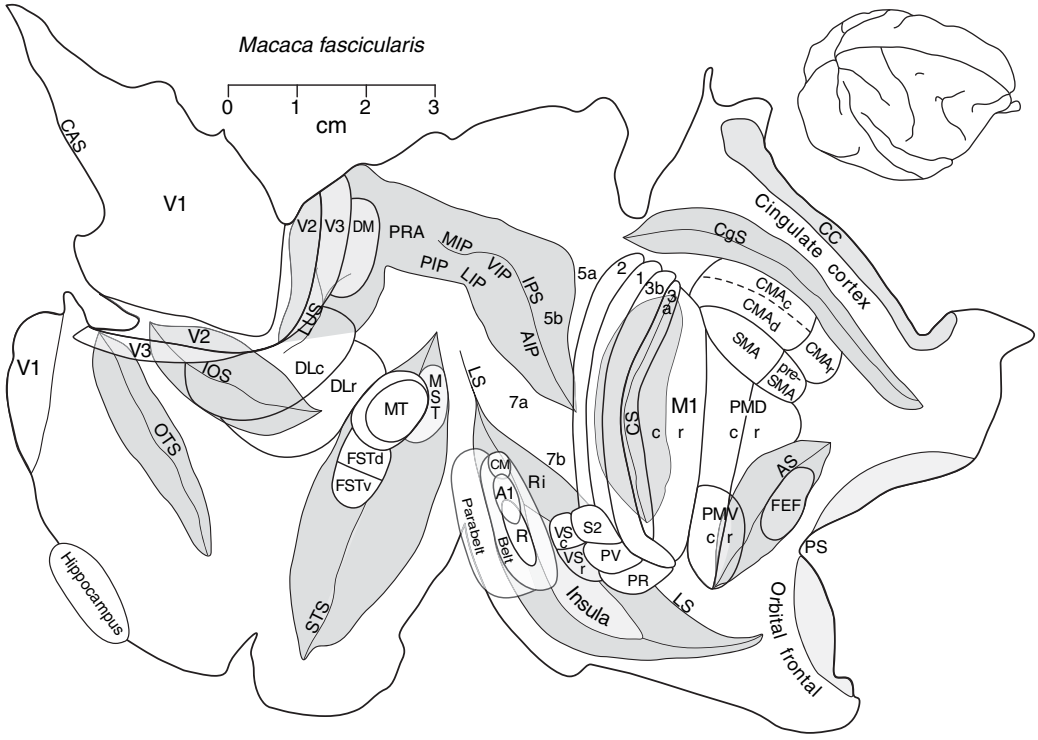

Figure 9 Some of the proposed subdivisions of neocortex of macaque monkeys. The neocortex of the macaque brain (upper right) has been separated from the rest of the brain and flattened (lower left) so that cortex buried in cortical fissures can be seen. To flatten the cortex as a single sheet, of course, requires some cuts and tears. In this view, the four large somatosensory areas of anterior parietal cortex, areas 3a, 3b, 1, and 2, are apparent as parallel, mediolateral strips. The parietal ventral (PV), second area (S2), rostral and caudal ventral somatosensory areas (VSr and VSc), the retroinsular area (Ri), and the parietal rostral area (PR) are in lateral parietal cortex, largely on the upper bank of the lateral fissure, but it is obvious that there is space for a number of other somatosensory areas. The lower bank of the lateral fissure contains auditory areas (primary, A1; rostral, R; auditory belt; and parabelt) with the caudomedial auditory area, CM, having both auditory and somatosensory functions. In posterior parietal cortex, several proposed areas are shown without boundaries, and many more areas have been proposed and may exist.

Areas of the intraparietal sulcus include anterior (AIP), lateral (LIP), medial (MIP), and posterior (PIP) intraparietal areas, along with the parietal reach area (PRA). The frontal motor areas include rostral and caudal divisions of primary motor cortex (M1r and M1c), rostral and caudal divisions of the ventral premotor areas (PMVr and PMVc), and the dorsal premotor areas (PMDr and PMDc), the frontal eye field (FEF), the supplementary motor area (SMA) with dorsal and ventral divisions, the presupplementary area (pre-SMA), and three cingulate motor areas (CMAr, CMAd, and CMAv). Several visual areas are denoted for reference: the first (V1), second (V2), third (V3), rostral and caudal dorsolateral (DLr and DLc), dorsomedial (DM), middle temporal (MT), medial superior temporal (MST), and dorsal and ventral divisions of the fundal superior temporal area (FSTd and FSTv). Cortex of the opened cingulate sulcus (CgS), the arcuate sulcus (AS), the lateral sulcus (LS), the principal sulcus (PS), the intraparietal sulcus (IPS), the lunate sulcus (LUS), the superior temporal sulcus (STS), the intraopercular sulcus (IOS), and the opercular temporal sulcus (OTS) are shaded, as is the corpus callosum (CC). See Kaas (1997) for a discussion of these visual areas.

1overlap in nonprimate mammals, there is no known homologue of CM in nonprimate mammals. Thus, CM appears to represent a primate elaboration of both auditory and somatosensory systems.

In summary, there is presently too little that is known about posterior parietal cortex in New World monkeys to allow detailed comparisons with Old World monkeys, but some overall features of shared organization are expected. Nevertheless, the large expanse of posterior parietal cortex in macaques, and the many proposed subdivisions, suggest that this region became much more complexly organized in catarrhine primates than in early anthropoids and most of their platyrrhine descendants.

4.02.4.3 The Motor and Premotor Cortex of Simians

Primary motor cortex varies in functional organization across taxa of simian primates in that representations of individual finger movements do not predominate in the forelimb portion of M1 in most New World monkeys (Gould *et al.*, 1986; Donoghue *et al.*, 1992; Stepniewska *et al.*, 1993), while larger parts of the forelimb region of M1 of

macaque monkeys is devoted to digit movements (e.g., Qi *et al.*, 2000). M1 of simian primates appears to have rostral and caudal subdivisions, differing in architecture and functions, with the caudal division more involved in digit movements (see Preuss *et al.*, 1996, 1997). M1 in humans may have similar subdivisions (Geyer *et al.*, 1996). Most New World monkeys, such as squirrel monkeys, differ from Old World macaques by having fewer direct projections from motor cortex to spinal motor neurons that control the movements of the hand, thereby allowing more independent movements of the digits (Nakajima *et al.*, 2000). The highly dexterous New World cebus monkeys have independently evolved similar direct cortical projections to spinal motor neurons (Bortoff and Strick, 1993).

The premotor region of cortex of macaque monkeys (Figure 9) also appears to have more functional subdivisions than premotor cortex of New World monkeys. Most notably, the ventral premotor region appears to be a single area in New World monkeys (Preuss *et al.*, 1996) and prosimian galagos (Wu *et al.*, 2000; Fang *et al.*, 2005), while ventral premotor cortex is subdivided into rostral and caudal areas (PMVr and PMVc) in Old World monkeys (Matelli *et al.*, 1985), termed PMVc or frontal motor area 4 (F4) and PMVr or frontal motor area 5 (F5) based on differences in histochemical (cytochrome oxidase) staining in the two fields. PMVc or F4 contains a motor representation of face and proximal arm movements, and neurons with tactile and visual receptive fields on (tactile) or near (visual) the face (Luppino *et al.*, 1999). PMVc (F4) appears to be involved in using information about nearby space to guide movements of the arm and face. Because PMVc resembles PMV of New World monkeys and prosimians, in that PMVc projects to primary motor cortex (M1) and to the spinal cord, PMVc of macaques likely corresponds to PMV of these other primates. PMVr (F5) has only been described in macaque monkeys, and it may be an area that is poorly differentiated or absent in New World monkeys. Stimulation of PMVr (F5) evokes movements of the hand and mouth. Neurons in PMVr (F5) respond when the monkey is performing hand and hand-to-mouth movements, and when the monkey is observing another performing such movements. Because the neurons respond both during an action or observing the same action performed by another monkey or human, they have been called mirror neurons (Gallese *et al.*, 1996). There is evidence that humans also have a mirror-neuron area, and human imitation may be based on a mirror-neuron system (Wohlschlager and Bekkering, 2002). Some have suggested that imitation is essential to language, and that PMVr (F5) is a monkey homologue of Broca's speech region. Petrides *et al.* (2005) postulate that part of dysgranular cortex just rostral to PMVr, is the monkey homologue of area 44 in humans. (Areas 44 and 45 are thought to correspond to Broca's speech region.) Electrical stimulation of this dysgranular cortex in monkeys evoked orofacial movements, and Petrides *et al.* (2005) suggested that Broca's area evolved from an area involved in controlling orofacial actions, such as area 44 of macaque monkeys (see also Preuss *et al.*, 1996). In addition, Nelissen *et al.* (2005) propose that F5 has a caudal region for the traditional mirror neurons, and an anterior region for neurons that are related to grasping, and these neurons for grasping respond to an isolated hand or a robot hand grasping, suggesting that the basics of grasping are coded in rostral F5. Thus, there is evidence for considerably more complexity in the PMV regions of macaques than in New World monkeys. Nevertheless, PMV of highly dexterous New World cebus monkeys has a well-developed representation of digits and dense connections with the hand portion of M1 (Dum and Strick, 2005), and further studies could reveal complexities of PMV organization in these New World monkeys.

In Old World monkeys, the region of the SMA has been divided into caudal SMA proper and rostral pre-SMA motor fields in macaques (reviewed by Tanji, 1994). A pre-SMA area may exist in New World monkeys, but the current evidence for such an area is limited (Sakai *et al.*, 2000). However, there is also some evidence for a pre-SMA in galagos (Wu *et al.*, 2000). Thus, pre-SMA may be an area common to primates.

4.02.5 Sensorimotor Systems in Apes and Humans

Compared to other primates, apes and humans are characterized by larger brains that take longer to mature and have higher energy requirements (Jablonski *et al.*, 2000). As a result, higher-quality foods are required, and reproductive rates are low. Success depends on a long life expectancy, and the ability to find and process high-quality foods. These requirements suggest that the larger brains of these primates function in many ways to allow food resources to be exploited and to extend the reproductive life span. However, only humans and some of their hominin ancestors with the largest of primate brains were able to expand their ranges from

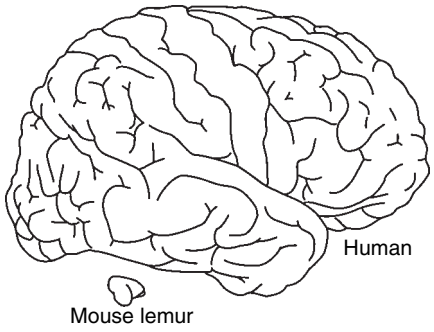

Figure 10 Primate brains vary greatly in size. The human brain is much larger than the brain of the mouse lemur, yet they share some sensorimotor areas. However, the large human brain has added a great number of additional sensorimotor and other areas, thereby becoming more modular and decreasing the problem of maintaining connections as the larger brain evolved. See text.

the favorable tropics where ripe fruit is regularly available to more challenging environments, where tool and weapon use allowed new food sources to be exploited, and resources to be defended. Changes in brain size were followed by changes in brain function, and brain organization, including alterations in the sensorimotor system (Figure 10).

As far as we know, the sensorimotor systems of apes and humans include the major components that have been proposed for their catarrhine relatives, the well-studied macaque monkeys. However, information is very limited, and there are many uncertainties. Yet early cortical stimulation (Hines, 1940; Leyton and Sherrington, 1917) and recording studies in chimpanzees (Woolsey *et al.*, 1943) indicate that at least one orderly somatotopic representation exists in anterior parietal cortex, and that an evoked movement map, M1, exists in architectonically defined area 4 (e.g., Bucy, 1935). Architectonic studies also indicate that anterior parietal cortex of apes and humans has the four fields of macaques, areas 3a, 3b, 1, and 2 (Grefkes *et al.*, 2001), and there is evidence for separate representations in areas 3a, 3b, 1, and 2 of humans (Young *et al.*, 2004). In humans, there is also evidence for areas PV and S2 of lateral parietal cortex (Disbrow *et al.*, 2000).

The organization of posterior parietal cortex in humans is generally modeled after proposals based on macaque monkeys, yet functional differences have been a focus of research. Clear evidence of the specialization of different functions in each cerebral hemisphere are evident in humans, where lesions of the right posterior parietal cortex typically produce a much more profound neglect of contralateral visual and tactile information than lesions of the left cerebral hemisphere (see Mountcastle, 2005). The general assumption, with some supporting evidence, is that the same frontal motor fields of macaques exist in humans, with, of course, specializations of ventral premotor fields of the left cerebral hemisphere to form Broca's speech region (e.g., Amunts *et al.*, 1999). Other specializations of motor fields of the left hemisphere are likely, given the greater role of the left hemisphere in motor planning and motor control (Kimura, 1993; Serrien *et al.*, 2006). Anatomical and functional asymmetries exist even in primary motor cortex. Further research is needed to determine what features of the sensorimotor cortex of humans are shared with apes and monkeys, and what features are new or perhaps lost.

For theoretical reasons, based on the major increase of brain size in humans, one would predict further evidence of differences in function between comparable regions of the two cerebral hemispheres, specialization of the larger somatosensory areas (3a, 3b, 1, and 2) and motor area (M1) for a fine-grain rather than global analysis of sensory information and motor control, and an increase in the number of cortical areas (Kaas, 2000).

Humans do have specializations of hand use that are reflected in the motor system. Humans are distinguished from other primates by their extremely dexterous use of the hands. In part, this was made possible by the evolution of upright posture, which freed the hand from a major role in locomotion, while allowing specialization that led to tool construction and use. Evidence of tool use pre-dates modern humans and extends back at least 2.5 My to our *Homo habilis* ancestors. The evidence suggests that the usual specialization of the right hand and the left sensorimotor cortex for dexterous functions began at least 2–3 Mya (Corballis, 1989, 1998; see The Evolution of Hemispheric Specializations of the Human Brain). Lethal intergroup violence, aided by the use of manufactured weapons, appears to have been an important selection factor in hominid evolution over the last 2–3 My (Kelly, 2005). Motor control for the making of tools and weapons would have likely depended on changes in the motor system. The neural basis for independent finger movements is thought to depend on the size and termination pattern of the pyramidal motor tract, which is exceptionally large in humans (Heffner and Masterton, 1983) and has the fullest monosynaptic linkage with spinal motor neurons in humans (see Wiesendanger, 1999; Lemon and Griffiths, 2005). These changes in the motor system were paralleled by changes in the hand, so a long, strong thumb could be apposed to fingertips in humans. The smaller thumb of early hominoids allowed a power grip, but possibly not a fully developed precision grip

(Napier, 1999), the hallmark of human hand use. These sorts of changes in the human motor system are well known, as they have been easy to measure, but they are likely to be only the tip of the iceberg. We expect that further research will reveal many additional hominoid and human specializations of the sensorimotor brain. Anatomical asymmetries of human cortex that are related to language functions are also expressed in the great apes (Gannon *et al.*, 2005), indicating that such hemispheric specializations initially evolved for functions other than language, and that hemispheric specializations have a long history.

4.02.6 Summary and Conclusions

The exceptional sensorimotor skills of humans reflect their massive and complexly organized sensorimotor system. While the details of the organization of this system are incompletely known for humans, and even more so for apes, we can deduce how the major features of the human system evolved by comparing features in extant mammals. Ideally, such an analysis would broadly survey extant mammals, consider features cladistically in a series of smaller clades leading to humans, and determine if conclusions are consistent with observations from the record of fossil brain endocasts on brain size and fissure patterns (e.g., Radinsky, 1975), as well as theoretical predictions based on brain size (Kaas, 2000). In practice, this approach is difficult to realize fully because understandings of the organizations of sensorimotor systems are fragmentary and incomplete. The sensorimotor systems of only a few taxa are well known. In addition, the fossil record is also fragmentary, and few cortical fissures, which often are apparent in endocasts, have an established significance in terms of marking functional boundaries in cortex. Finally, large brains would seem to need to be organized in specifically different ways than small brains, but comparative studies have only partially validated assumptions about how brains should change with increasing size. Nevertheless, enough is known to allow a broad outline of sensorimotor system evolution in primates, and this outline can be expanded and modified as further observations become available.

4.02.6.1 Early Mammals

Only a few subdivisions of the thalamus and cortex characterized the sensorimotor system of early mammals. The thalamus included a distinct VP for relaying slowly adapting and rapidly adapting cutaneous receptor information to primary (S1) and secondary (S2) somatosensory cortex, and possibly to the PV. Muscle spindle receptor information was segregated in a thalamic region dorsorostral to VP, either in the region generally referred to as the posterior nucleus, or in a cell group commonly included in rostral VP. This information relayed to the rostral belt of somatosensory cortex, SR, a region that is referred to as dysgranular cortex in rats. Spinothalamic inputs terminated in and around VP, and relayed broadly to somatosensory cortex. Just rostral to VP, the ventral lateral nucleus received input from the cerebellum and projected to somatosensory cortex. There were no separate motor and premotor areas in early mammals, and motor functions were mediated via projections from somatosensory cortex to the basal ganglia, brainstem, and spinal cord, as well as by subcortical sensorimotor circuits that functioned relatively independently of sensorimotor cortex.

4.02.6.2 Early Eutherian Mammals

In the line leading to eutherian mammals, a separate primary motor area of cortex, M1, emerged. M1 lacked a notable layer 4, while projecting to brainstem and spinal cord neuron pools, largely on neurons between those with sensory inputs and the motor neurons contacting muscles. Sensory inputs to M1 were from somatosensory areas S1, S2, PV, the caudal and rostral somatosensory belts (SC and SR), and an emerging but small posterior parietal cortex with somatosensory, visual, and perhaps auditory inputs. M1 also received inputs from a rostrally adjacent premotor area, M2, with posterior parietal and frontal lobe inputs, as well as from the motor thalamus (VL). M2 provided few corticospinal projections, while S1 and SR provided dense corticospinal projections. M2 may be the homologue of dorsal and ventral premotor areas, or of the SMA of primates.

4.02.6.3 Early Ancestors of Primates

The early members of the Euarchontoglires clade that emerged some 95 Mya did not differ significantly from other eutherian mammals, but the early archontan mammals, those that gave rise to flying lemurs, tree shrews, and primates, may have already developed some primate-like characteristics, judging from recent studies on tree shrews. Most notably, the posterior parietal region of cortex was expanded in size, and this cortex had considerable input from an array of visual areas, as well as from somatosensory areas of cortex. Posterior parietal cortex probably had several divisions projecting

differently to motor and premotor cortex. The SC belt may have had rostral and caudal subdivisions. More of S1 was devoted to sensory receptors of the forepaw, and motor cortex had more projections from a forelimb representation to the cervical spinal cord. The forepaw was used more for grasping and manipulating food items.

4.02.6.4 Early Primates

The most notable difference between primates and their nonprimate relatives is the presence of an array of premotor areas in the frontal lobe. Prosimian galagos and other primates have a dorsal premotor area, PMD, with a rostral division (PMDr) with connections with prefrontal cortex and sparse connections with primary motor cortex, M1, and a caudal division (PMDc) with dense connections with M1. Galagos also have a ventral premotor area, PMV, but without the distinct rostral and caudal divisions of anthropoid primates. Galagos have a frontal eye field (FEF), an SMA, a pre-SMA, and rostral and caudal cingulate motor areas (CMAr and CMAc). These are all likely features of the brains of early primates. Other changes occurred in posterior parietal cortex, which was more expansive in early primates and included a rostral zone, with subdivisions receiving different somatosensory inputs and projecting differently to motor and premotor areas, and a caudal zone with inputs from higher-order visual areas and with projections to the rostral divisions of posterior parietal cortex. The proportional representation of the glabrous hand (forepaw) was increased in somatosensory and motor fields, and the proportion of corticospinal projections from M1, compared to other fields, was increased, corresponding to an enhanced role of the forelimb in reaching and grasping small prey, fruit, and buds in the fine-branch niche of early primates.

4.02.6.5 Early Anthropoids

These primates gave rise to tarsiers and monkeys. As highly specialized primates, with little information about their sensorimotor system, not much can be said about tarsiers, except that VP, VPS, and VPI regions can be identified histologically in the somatosensory thalamus, and that a clear area 3b (S1) can be identified in cortex. Extant monkeys differ from prosimians by having four distinct subdivisions of anterior parietal cortex, areas 3a, 3b, 1, and 2, each with a separate representation of receptors of the contralateral body, a distinctive pattern of connections with other areas of cortex, and subcortical structures, and identifying histological features. Yet area 1 is poorly differentiated in one branch of New World monkeys, the small marmosets and other callitrichines, and the evidence for an area 2 has been questioned in these and other New World monkeys. As some features of somatosensory cortex may have regressed in the callitrichines, early anthropoids may have had a more differentiated area 1, and a poorly differentiated area 2. Area 2 became distinct in the line leading to present-day Old World monkeys, apes, and humans. In more recent anthropoids in the Old World line, posterior parietal cortex increased in size and complexity, ventral premotor cortex subdivided and motor and somatosensory areas increased their representations of the hand. Area 2 became a well-differentiated area, with inputs from VPS and interconnections with area 3a. At least some of these changes occurred independently in New World cebus monkeys, as well as their unique sensorimotor specialization for their tail.

4.02.6.6 Hominoids (Apes and Humans)

About 30 Mya or more, one line of Old World monkeys gave rise to apes, which are characterized by a longer gestation time, a longer time to first reproduction, and a generally larger size than monkeys. The longer gestation time, slower maturation, and larger size allowed ape brains to become bigger than monkeys', a trend that was accelerated in hominins, humans, and their bipedal ancestors. The brains of our early bipedal ancestors were about the size of those of modern-day chimpanzees (400–500 cm^3), but in the line leading to modern humans, the brain rapidly increased in size, especially over the last 1 My, to the range of 1200–1400 cm^3. This increase was accomplished by an increase in the surface area of each cerebral hemisphere from about 240 to 800 cm^2. Even the first hominids and their close ape relatives had brains much bigger than those of the well-studied Old World macaque monkeys with approximately 72 cm^2 of surface area for each cerebral hemisphere. Research and conclusions about brain organization in macaque monkeys has greatly influenced current concepts about how the human brain is organized, but because of scaling problems, it is extremely unlikely that human neocortex is simply a 10- to 15-fold enlarged version of macaque neocortex. Of course, there is clear evidence that this is not the case. Most notably, the easily identified primary sensory and motor areas are larger in the large human neocortex, but not as large as they would be if they maintained a monkey-like proportion of the total. The larger size implies that these areas

function differently than their homologue in smaller primate brains, allowing more emphasis on fine-grain discriminations and motor performances, but functioning less well on the global aspects of perception and movement control. However, if some areas of cortex did not enlarge proportionally, what happened to the rest of the neocortex? The obvious suggestion is that the number of cortical areas, the functionally distinct subdivisions of cortex, increased. The most compelling evidence for this is that humans have abilities not found in monkeys or apes, and that brain regions responsible for some of these abilities do not have symmetrical counterparts in each hemisphere. A large part of the temporal lobe and parts of the frontal lobe of the left cerebral hemisphere are specialized for language in humans, and a large part of posterior parietal cortex of the right hemisphere is specialized for spatial reasoning and functions that allow an appreciation of music and mathematics (Corballis, 1998). For purposes of reducing the connection problems of large brains alone, there should be a considerable increase in modularity, reflected in an increase in numbers of cortical areas, perhaps in the range of 150 distinct fields for human neocortex. How this impacts on the sensorimotor system is largely uncertain at this time, but the expectation is that posterior parietal cortex, lateral parietal cortex, and frontal lobe motor regions of humans have more functional divisions than Old World monkeys have or our early hominin ancestors had. The evidence for this, at least for monkey–human comparisons, is starting to emerge from ongoing fMRI studies that have great capacity and potential for revealing the functional subdivisions of human neocortex.

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4.03 The Role of Vision in the Origin and Evolution of Primates

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Glossary

Anthropoidea	Monophyletic group (clade) consisting of living monkeys, apes, and humans, their last common ancestor, and all fossil taxa more closely related to living anthropoids than other primates.
APA	Arcuate premotor area.
cathemeral	Active during the day and at night.
CMAd and CMAv	Dorsal and ventral cingulate motor areas, located in the cingulated sulcus on the medial aspect of the cerebral hemisphere.
corticospinal tracts	Fiber bundles consisting of axons from cell bodies in the cerebral cortex that connect to motor neurons and interneurons in the spinal cord.
crepuscular	Active in the evening and morning.
diurnal	Active principally or solely during the day.
euprimates or primates of modern aspect	Monophyletic group consisting of Haplorhini, Strepsirrhini, Omomyiformes and Adapiformes. The taxon was erected to distinguish primates of modern aspect from archaic primates, or plesiadapiforms.
frontation	Caudal angle between the nasion–inion chord and the intersection of the midsagittal plane with the orbital plane.
hallux	Big toe, first toe
Haplorhini	Monophyletic group (clade) consisting of Anthropoidea and tarsiers, their last common ancestor, and all fossil taxa more closely related to living haplorhines than to other primates.
MI	Primary motor cortex.
nocturnal	Active principally or solely at night.
orbital convergence	Degree to which orbits face in the same direction, or converge on each other. Convergence is the caudal dihedral angle between the plane of the orbit and the midsagittal plane.
PMv	Ventral premotor area
pollex	Thumb; first, or radial digit
SMA	Supplementary motor area, motor cortex located on the medial aspect of the cerebral hemisphere, dorsal to the cingulate sulcus.
stereopsis	Seeing objects as solid, or three-dimensional.

Strepsirrhini Monophyletic group (clade) consisting of living lemurs, lorises, and galagos, their last common ancestor, and all fossil taxa more closely related to living strepsirrhines than to other primates.

4.03.1 Introduction

The visual system features prominently in adaptive explanations for the divergence of primates from other mammals and the origin of anthropoid or simian primates from their prosimian ancestors. However, the origin and radiation of primates was associated with modification of a number of other sensory and motor complexes, including the auditory, feeding, and locomotion systems. The integrated nature of these modifications demands that considerations of the role of the visual system in primate evolution include changes in these other systems. Many current explanations for primate origins do not take this integration into account. Here, we consider the role of vision in association with other functional systems. After briefly reviewing the taxonomy of extant primates, we begin by enumerating the features distinguishing primates from other mammals, especially their close relatives. We then review the hypotheses advanced to explain the evolution of these features, evaluating those hypotheses with special reference to the neuroscience literature dealing with the visual system, motor control of hand movements, and eye–hand coordination.

4.03.2 What Is a Primate?

Living primates are classified into three universally accepted groups (Figure 1): Anthropoidea (monkeys, apes, and humans), Tarsiiformes (tarsiers), and Strepsirrhini (Malagasy lemurs together with lorises and galagos) (Martin, 1990; Fleagle, 1999; Hartwig, 2002). (Many publications spell this with one 'r', i.e., Strepsirhini. However, two 'r's are preferable because, although the Zoological Code of Nomenclature does not codify spelling of taxonomic names above the family level, the original spelling of the term was 'Strepsirrhini' (Geoffroy Saint-Hilaire, 1812a) and this is also the correct derivation from the Greek (Jenkins, 1987).) The phylogenetic position of *Tarsius* is controversial, with some researchers placing it as the sister taxon of anthropoids, making a clade Haplorhini (e.g., Cartmill, 1980; Martin, 1990; Kay *et al.*, 1997, 2004; Ross *et al.*, 1998) and others placing it as the sister taxon of strepsirrhines, making a clade Prosimii (e.g., Eizirik *et al.*, 2001). Many researchers accept Haplorhini as a valid clade, but use the term 'Prosimii' to refer to the paraphyletic group consisting of strepsirrhines and tarsiers. Here we follow the classification of Fleagle (1999).

Living strepsirrhines include the Malagasy primate families united in the Lemuriformes (or Lemuroidea) and the African and Asian strepsirrhines, grouped together in the Lorisiformes (or Lorisoidea). The Lemuriformes includes Cheirogaleidae, Daubentoniidae, Indriidae, Lemuridae, and Lepilemuridae (or Megaladapidae). Lorisiformes includes the African Galagidae, and the African and Asian Lorisidae.

Anthropoids (also known as simians) are divided into two major clades: the Platyrrhini, or New World monkeys; and the Catarrhini, including Old World monkeys, apes, and humans. There is general agreement on the family or subfamily groupings of most of the platyrrhines – Callithrichidae (marmosets and tamarins), Atelinae (spider, woolly, and woolly spider monkeys), Alouattinae (howler monkeys), Pitheciinae (sakis, bearded sakis, and uacaris), Cebinae (including *Cebus* and *Saimiri*), Aotinae (owl or night monkeys), and Callicebinae (titi monkeys) – but the relationships among these groups are debated. The Catarrhini are divided into two major clades, the Cercopithecoidea, including cercopithecines and colobines, and the Hominoidea, including the apes and humans (see The Comparative Biology of Photopigments and Color Vision in Primates, Visual Cortex: Evolution of Maps and Mapping).

It is generally believed that the closest living relatives of primates are scandentians (tree shrews) and dermopterans (flying lemurs), although the phylogenetic relationships of these animals to primates continue to stimulate debate. The hypothesis of a grouping of dermopterans, scandentians, primates, bats, and elephant shrews in a superorder Archonta (Gregory, 1920) is not supported by recent analyses. Bats instead are included with carnivores, ungulates, and whales in a clade Laurasiatheria, while primates group with tree shrews, dermopterans, and the rabbit–rodent clade, Glires, in a larger clade, Euarchontoglires (Springer *et al.*, 1997; O'Brien *et al.*, 1999). Recent molecular trees for mammals either place Scandentia and Dermoptera in a clade that is the sister taxon to primates (Springer *et al.*, 1997; Murphy *et al.*, 1999; Eizirik *et al.*, 2001, 2004) or group Scandentia and Primates in a clade with Dermoptera as the sister taxon (Liu *et al.*, 2001). The long-term robustness of these phylogenetic groupings remains to be seen.

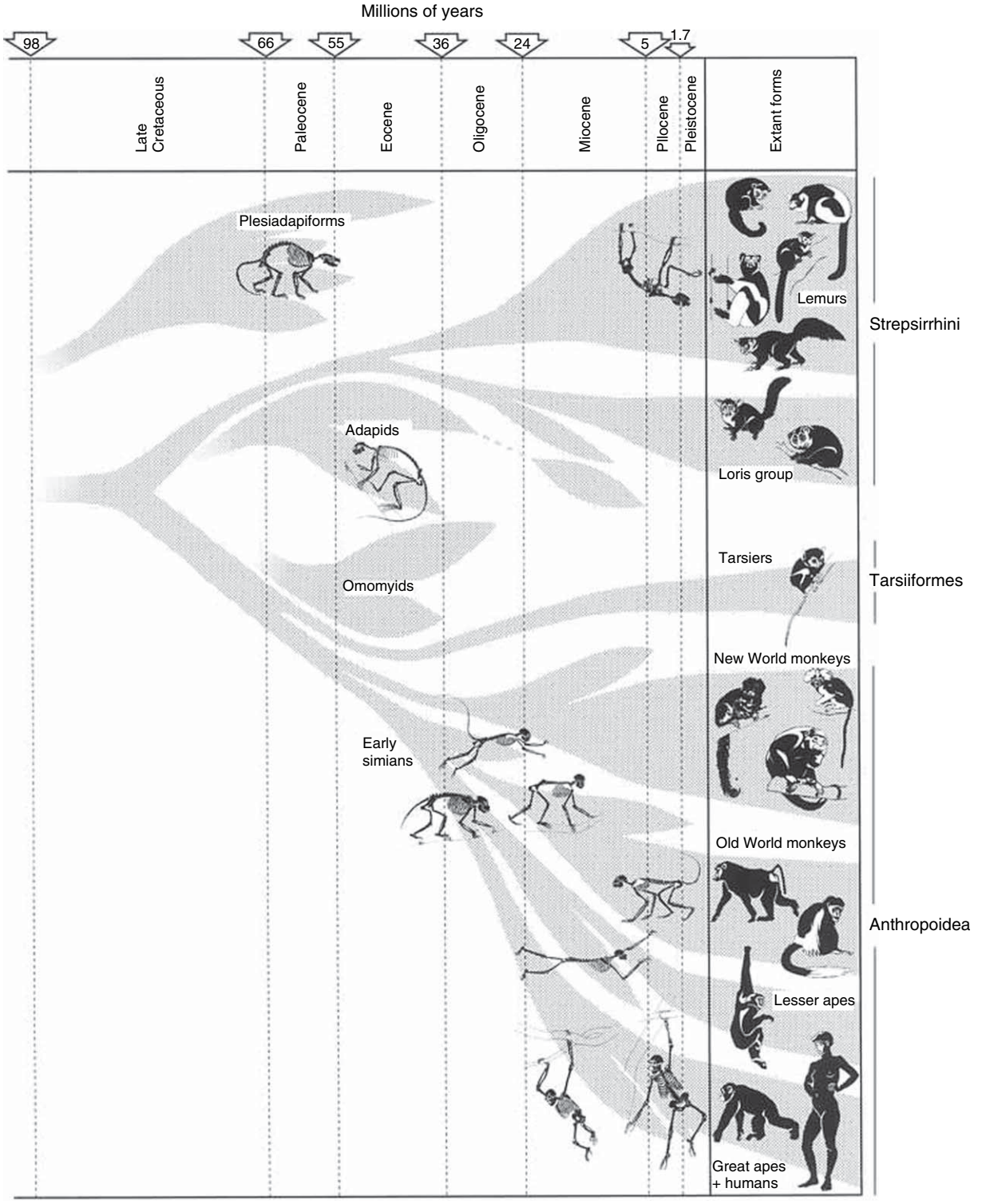

Figure 1 Outline phylogenetic tree of primates (modified from Martin, 1993). The generally accepted groups of living primates are shown on the right. Two groups of fossil primates that appear and radiate in the Eocene – adapids and omomyids – are of uncertain affinities to living primates (Martin, 1993). Plesiadapiforms and their relatives, including carpolestids (Bloch and Boyer, 2002), are also of uncertain affinities to primates. When plesiadapiforms are included in Primates, living primates, omomyids, and adapids are grouped together as Euprimates. Reproduced from Martin, R. D. 1993. Primate origins: Plugging the gaps. *Nature* 363, 223–234, with permission from Nature Publishing Group.

4.03.3 Early Explanations for Primate Origins

Primates have long been distinguished from other mammals by their grasping hands and feet, various enhancements of the visual system, and their relatively enlarged brains (Geoffroy Saint-Hilaire, 1812a, 1812b; Elliot Smith, 1924). Like many of the explanations to follow, Grafton Elliot Smith's early explanations for primate origins invoked functional benefits of these features in an arboreal habitat, but Elliot Smith also emphasized that changes in primate locomotion and grasping were integrated with changes in the somatic, auditory, and visual sensory systems. In an address to the Anthropological Section of the British Association for the Advancement of Science delivered in 1912 (Elliot Smith, 1924, chapter 1), Elliot Smith identified the neopallium (later termed neocortex) as the most salient feature distinguishing mammalian brains from those of nonmammals. The neopallium of mammals not only receives input from the visual, auditory, tactile, and kinesthetic senses, providing a substrate for merging and associating of the information streaming in from the periphery, but also contains the motor areas that put into effect the decisions made on the basis of these associations. Thus, Elliot Smith saw the neopallium as the organ that made it possible for mammals to learn and adapt to their surroundings.

The adaptability conferred on basal mammals by the neopallium was lost by many descendant lineages when they became specialized for cursorial, flying, aquatic, or burrowing environments. Primates, in contrast, retained their primitive adaptability, plasticity, and flexibility, primarily because they were arboreal. Arboreal mammals, Elliot Smith argued, require a balanced emphasis of the senses, with enhancement of vision, hearing, and touch. The agility of movement required in the trees "necessitates an efficient motor cortex to control and coordinate such actions as an arboreal mode of life demands . . . and also a well-developed muscular sensitivity to enable such acts to be carried out with precision and quickness" (Elliot Smith, 1924, p. 30). This general enhancement of the special senses, as well as the somatic sensory and motor systems used in locomotion, accounted for the general enlargement of the brain characteristic of primates.

Elliot Smith also emphasized the integrated nature of changes in the visual and tactile senses. The integrated nature of the neopallium meant that enhancement of the visual system in primates affected the whole neopallium, not just the visual areas.

> The sense of touch also shared in the effects, for tactile impressions and the related kinaesthetic sensibility, the importance of which to an agile tree-living animal is obvious, assist vision in the conscious appreciation of the nature and the various properties of the things seen, and in learning to perform agile actions which are guided by vision (Elliot Smith, 1924, p. 32).

This correlated development of visual and tactile senses led to integrated development of improved eye–hand coordination, linking up the tactile, kinesthetic, and visual cortical areas. Thus, for Elliot Smith, primate arboreality was not the only factor responsible for their adaptability, plasticity, and ability to learn, but it also resulted in enhanced development of their visual and grasping abilities, and in the integration and co-evolution of the two systems.

Wood Jones's theory of primate evolution included many of Elliot Smith's conclusions, but he also discussed specific features that are the focus of current explanations for primate origins. Wood Jones (1916) explained forward-facing eyes and postorbital bars as secondary consequences of a shift to arboreality, not specializations for it. He argued that, with progressive adoption of arboreal habits, the hindlimb became specialized for supporting the body weight during climbing, liberating "the fore-limb from any such servile function as supporting the weight of the body: it becomes a free organ full of possibilities," a process Wood Jones referred to as "emancipation of the fore-limb" (Wood Jones, 1916, p. 17). The emancipated forelimb could then take over from the jaws the role of food acquisition, allowing the snout to be reduced in size. As the snout recedes, the orbits are dragged around toward the front of the face, and postorbital ossifications (bar and septum) develop between the orbit and the temporal fossa (Wood Jones, 1916, p. 99). Echoing Elliot Smith, Wood Jones noted that one incidental benefit of the combination of a dextrous forelimb with forward-facing eyes is the ability to simultaneously manipulate and view an object in front of the face, making it advantageous to merge tactile and visual information in the newly expanding cortical association areas created by the expanding brain.

Wood Jones and Elliot Smith's arboreal theory of primate evolution (Howells, 1947) was adopted by Le Gros Clark (1934, 1959) as the explanation for general trends in primate evolution. The sensorimotor integration integral to Wood Jones' and Elliot Smith's theory was embodied in Le Gros Clark's total morphological pattern, "the integrated combination of unitary characters which together make up the complete functional design of a given anatomical structure" (Le Gros Clark, 1959, p. 13). The lack

of specialization and the retention of adaptability were attributed by Le Gros Clark to:

> . . . an arboreal habitat, a mode of life which among other things demands or encourages prehensile functions of the limbs, a high degree of visual acuity, and the accurate control and coordination of muscular activity by a well-developed brain (Le Gros Clark, 1959, p. 43).

Overlapping visual fields and high visual acuity were argued to confer the ability to judge distances necessary for leaping in an arboreal environment. Le Gros Clark also re-emphasized the importance of eye–hand coordination for primate evolution identified by Elliot Smith, arguing that the enhancement of the tactile senses that accompanied the changes to the visual system were related to improved ability for manual manipulation and appreciation of the environment.

4.03.4 Primates in the Fine-Branch Niche

Le Gros Clark's theory of primate evolution was promulgated to the next generation of primatologists and became the received view (Cartmill, 1982). In the 1960s and 1970s, field research on behavior and ecology of nocturnal strepsirrhine primates in Madagascar and West Africa by R. D. Martin and P. Charles-Dominique suggested to them some refinements of the arboreal theory. Their fieldwork revealed similarities between cheirogaleids and galagids in a number of features, including nocturnality, small body size, hindlimb-dominated locomotion utilizing grasping extremities in the fine-branch and creeper niche, and an omnivorous diet including fruit, insects caught with the hands, and gum obtained with the help of the toothcomb (Charles-Dominique and Martin, 1970; Martin, 1972, 1973). They interpreted these commonalities as retentions from the common ancestor of strepsirrhines at least, and possibly primates as a whole, suggesting that occupation of the fine-branch niche might be the adaptive shift that characterized primate origins.

The advantages of the distinctive features of the primate visual system to an occupant of the fine-branch niche were not precisely articulated, although Martin addressed them briefly in 1979:

> Occupation of the "fine branch niche" by a relatively small-bodied ancestral primate would hence explain the emphasis on the grasping foot characteristic throughout the order Primates and at the same time provide a reason for the emphasis on vision and replacement of the primitive prehensile function of the snout by mobile, grasping hands. (Leaping between adjacent fine branches and grasping of small animal prey on nearby supports with the hands would explain the relatively large eyes, the universal possession of a postorbital bar, and the reduction of the snout and anterior teeth among primates.) (Martin, 1979, p. 64).

Martin subsequently argued that forward rotation of the orbits enhances stereoscopic vision that would be advantageous for "[a]ctive locomotion in a network of fine arboreal supports" (Martin, 1990, p. 657). This fine-branch niche hypothesis for primate origins included only very general explanations for the origins of orbital convergence and a postorbital bar in stem primates, made no mention of eye–hand coordination, and emphasized the importance of locomotion in terminal branches over predation or food acquisition (Martin, 1979).

4.03.5 Orbital Convergence, Postorbital Bar, Manual Grasping, and Visual Predation

Cartmill (1970, 1972) took issue with the arboreal theory of primate evolution on the grounds that arboreality alone cannot explain the origins of grasping extremities, convergent orbits, and nails on the digits, because a variety of active, leaping arboreal animals, such as squirrels, lack these features altogether. He argued:

> If the primate evolutionary trends have not been characteristic of other lineages of arboreal mammals, we may conclude that there is something wrong with the arboreal theory in its received form and any explanation of the primate trends must involve a more detailed description of the habitus of the ancestral primate (Cartmill, 1972, pp. 102–103).

Cartmill (1974) noted that many nonprimate animals with forward-facing eyes, such as cats, owls, and hawks, are "visually directed predators," and many nonprimate animals with grasping extremities, such as chameleons and small marsupials, engage in "prolonged and stealthy locomotion on slender terminal branches in pursuit of insects." Cartmill's hypothesis was significant in that it demonstrated that arboreality alone could not explain the evolution of optic convergence and grasping extremities in primates; something more specific was needed. Cartmill invoked adaptation to visual predation in the fine branches of the shrub layer of tropical rainforests to explain both grasping hands and convergent orbits. An integral component of the hypothesis was the importance of eye–hand coordination originally identified by Elliot Smith:

> The prehensile forelimbs necessary for stalking insects along thin branches serve also, among living insectivorous prosimians, as prey-seizing organs analogous to the tongue of a chameleon. The importance to primates of hand–eye coordination, which [Elliot] Smith was the first to stress, can be plausibly traced to an ancestral habitus in which the hand was used for striking prey (Cartmill, 1972, p. 116).

Cartmill (1970) formally defined orbit orientation in terms of two variables (convergence and frontation) (Figure 2), which he measured in a wide sample of arboreal mammals. Subsequent morphometric work by Ross (1995), Noble *et al.* (2000), Heesy (2003, 2005), and Ravosa and Savokova (2004) has expanded the available data and the most extensive data set (Heesy, 2003, 2005) is shown in Figure 3. Primates certainly have more convergent orbits than do dermopterans and scandentians, but many other mammals overlap with primates in their degree of orbital convergence, including a number of carnivorans, bats, and marsupials. However, few mammals share the combination of high degrees of frontation and convergence seen in primates, and when allometric factors are taken into account, primates have more convergent orbits for their relative orbit size than other mammals (Noble *et al.*, 2000; Heesy, 2003).

Refinements to Cartmill's visual predation hypothesis were necessary. Pettigrew (cited by Allman, 1977, p. 29; Pettigrew, 1978) and Allman (1977) pointed out that orbital convergence has advantages for nocturnal animals that are not applicable to diurnal animals. The

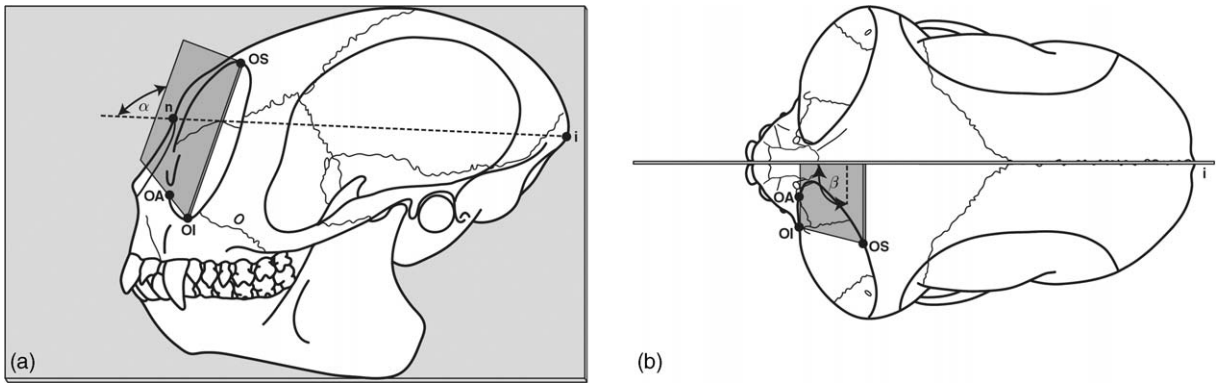

Figure 2 Diagram illustrating definitions of orbital convergence and frontation from Cartmill (1970, 1972) and subsequently used by Ross (1995), Noble *et al.* (2000), Heesy (2003, 2005), and Ravosa and Savakova (2004). The midsagittal plane is lightly shaded, the orbital plane heavily shaded. a, Frontation is the caudal angle between the nasion–inion chord and the intersection of the midsagittal plane with the orbital plane (i.e., $180^\circ - \alpha$). b, Convergence is the caudal dihedral angle between the plane of the orbit and the midsagittal plane (i.e., $180^\circ - \beta$).

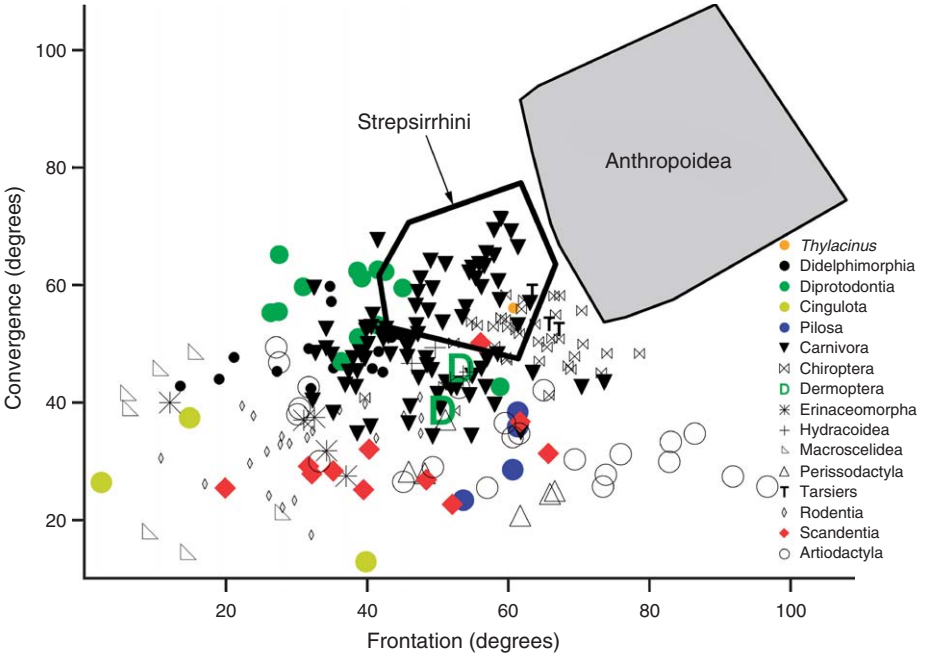

Figure 3 Bivariate plot of orbital convergence angle (degrees) against orbital frontation angle (degrees) in mammals. Individual data points for primates are excluded and replaced by minimum convex polygons (*sensu* Jerison, 1973). Data are from Heesy, C. P. 2005. Function of the mammalian postorbital bar. *J. Morphol.* 264, 363–380.

Allman–Pettigrew model notes that orbital convergence is associated with convergence of the optic axes on the visual axes, a means of improving retinal image quality that is necessary for nocturnal animals but not for diurnal ones. Whereas diurnal animals can ensure high retinal image quality by decreasing pupil diameter, thereby restricting incoming images to the paraxial region of the dioptric apparatus, nocturnal animals must maintain large pupil apertures in order to preserve image brightness. Consequently, nocturnal animals must improve image quality in the area of visual field overlap by optic and orbital convergence. This suggested to Allman (1977) that, if the first primates had high degrees of orbital convergence, they were probably nocturnal (Figure 4).

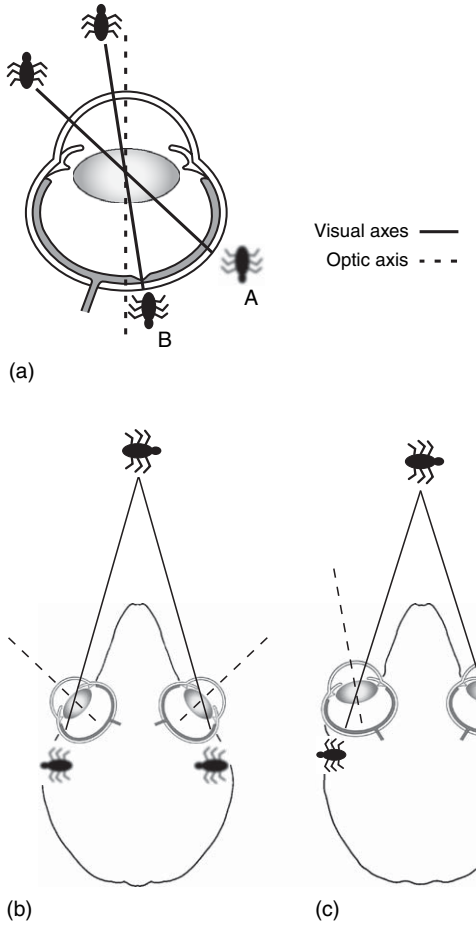

Figure 4 Diagrams illustrating functional significance of orbital convergence in nocturnal primates. a, Diagram of eye illustrating the effect of relative orientation of optic and visual axes on image quality. Image quality is best when the visual axis is more closely aligned with the optical axis. b, Diagram of visual and optic axis orientation in an animal with laterally facing orbits. c, Diagram of visual and optic axis orientation in an animal with convergent orbits. The quality of the image of the area in front of the animal is lower in (b) than in (c) because the optic and visual axes are less closely aligned.

4.03.6 The Primate Postorbital Bar

Primates all have postorbital bars which, while not unique to primates, do serve to separate them from their nearest putative fossil relatives, the plesiadapiforms. Cartmill (1970) and Heesy (2003) list a variety of other mammals with postorbital bars and processes. Dermopterans have postorbital processes (i.e., incomplete bars), while tree shrews have complete postorbital bars. Cartmill (1970, 1972) hypothesized that the primate postorbital bar functions to protect the orbital contents against movements originating from the chewing muscles in the temporal fossa. These movements might occur in all chewing animals, but Cartmill hypothesized that they were particularly problematic in animals with convergent orbits. Orbital convergence brings the plane of the orbit out of the plane of the temporal fossa, such that distortions of the postorbital ligament caused by contraction and bulging of the temporal muscles impinge upon the orbital contents (Figure 5).

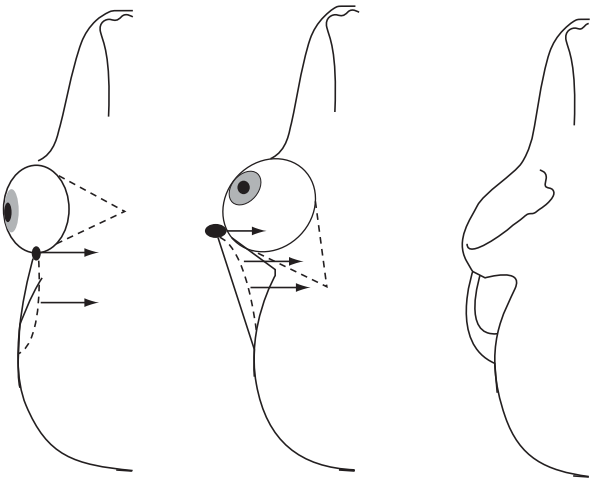

Figure 5 Diagram illustrating the function of the postorbital bar according to Cartmill (1972). In the left figure, the orbit is laterally directed and contractions of the temporalis muscle that pull the temporalis fascia and postorbital ligament medially do not impinge upon the eye (medially directed arrows). In the middle figure, the effects of moderate orbital convergence are illustrated. Convergence of the orbits is achieved by anterolateral displacement of the postorbital ligament. This brings the ligament lateral to the eye so that medial displacement of the ligament moves the eye around. As shown in the right figure, to prevent unwanted eye movements, the ligament is ossified into a postorbital bar to stiffen the lateral orbital wall. Adapted from Heesy, C. P. 2003. The Evolution of Orbit Orientation in Mammals and the Function of the Primate Postorbital Bar. PhD thesis, Stony Brook University, with permission of the author.

This hypothesis receives support from recent comparative morphometric analyses of orbit orientation in nonprimate mammals. Increased orbital frontation (roughly equivalent to verticality) in animals with moderate degrees of orbital convergence also causes the orbital and temporal planes to diverge, necessitating evolution of a postorbital bar (Noble *et al.*, 2000; Ravosa and Savokova, 2004). Heesy (2003, 2005) showed that the degree of postorbital ossification across a wide range of mammals is correlated with the degree to which the planes of the orbital aperture and of the temporal fossa diverge, regardless of whether that divergence is caused by increased orbital convergence, frontation, or displacement (Figure 6). This suggests that the evolution of the postorbital bar in primates represents an instantiation of a general principle identified by Cartmill that applies across all mammals: when the orbit and temporal fossa are not coplanar, movements in the temporal fossa are more likely to disturb the orbital contents and some kind of postorbital ossification is necessary to insulate the orbit.

The precise source, magnitude, and nature of the eye movements originating in the temporal fossa are unknown. Lemme *et al.* (2005) measured deformation in the postorbital ligament of pigs during stimulation of the temporalis and masseter muscles, and during mastication. They found that deformation of the ligament was primarily caused by contraction of the ipsilateral superficial masseter. In nonanthropoid primates, the chewing muscles, including the superficial masseter, are recruited much more vigorously on the working side than the balancing side, producing higher bone strain magnitudes on the postorbital bar of the working side than that of the balancing side (Ravosa *et al.*, 2000). Together, these results suggest that any disturbances suffered by the eyes during chewing would be asymmetrical. It might be difficult to offset or tolerate this asymmetry (Ravosa *et al.*, 2000), although this would depend on the nature of the movements. If the eyes were primarily protruded, the resulting diplopia would be less than if the eyes were abducted or adducted. Heesy *et al.* (2006) measured eye movements in anesthetized cats and galagos during stimulation of the masticatory muscles and found varying amounts of protrusion and abduction. Whether these movements occur in awake, chewing primates has not been established.

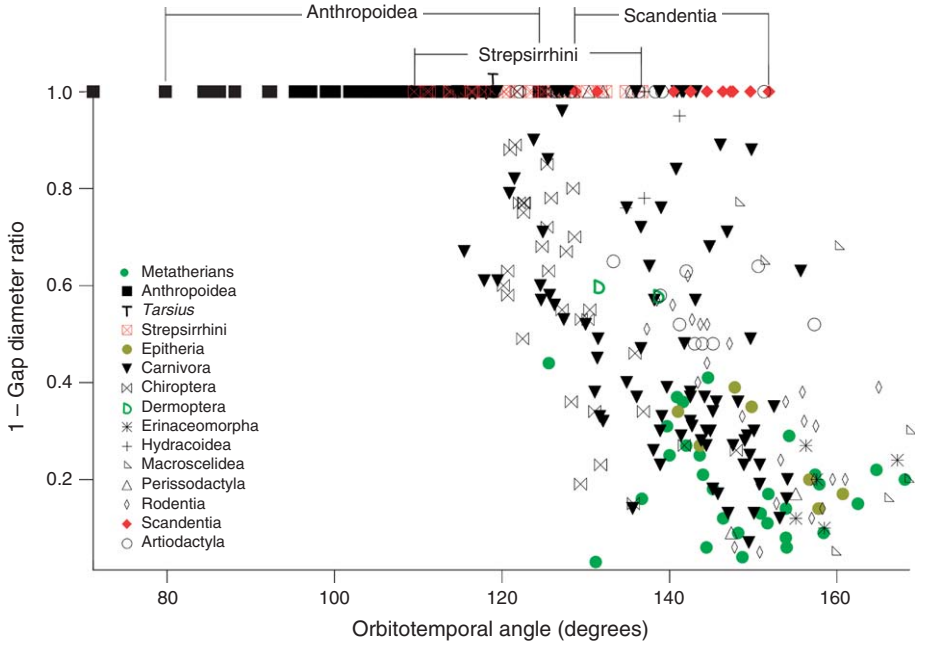

Figure 6 Bivariate plot of 1– postorbital gap:orbit diameter ratio against orbitotemporal angle. The gap:diameter ratio is the distance between the tips of the postorbital processes divided by orbit diameter. This value is subtracted from 1.0 so that animals with longer processes or bars are higher on the *y*-axis. Animals with values of 1.0 at least have complete postorbital bars. Orbitotemporal angle is the dihedral angle between the plane of the orbit and the plane of the temporal fossa. This angle quantifies the internal angle between the plane of the orbit and the plane of the temporal fossa. This plot shows that as the orbit becomes less coplanar with the temporal fossa (i.e., as the orbitotemporal angle decreases), the length of the gap between the postorbital processes decreases. Only animals with postorbital bars can have orbital planes that are strongly divergent from the plane of the temporal fossa. Note that only animals with postorbital septa (i.e., tarsiers and anthropoids) have extreme values of orbitotemporal angle. The data are from Heesy, C. P. 2005. Function of the mammalian postorbital bar. *J. Morphol.* 264, 363–380.

4.03.7 Criticisms of the Nocturnal Visual Predation Hypothesis

The visual predation hypothesis was the most widely accepted explanation of primate origins until counter-arguments began to appear in the 1990s. Critiques of the nocturnal visual predation (NVP) hypothesis can be grouped into three categories of argument: that the ancestral primates were not nocturnal; that the predatory adaptations of the ancestral primates were not visual; and that the visual adaptations of the ancestral primates were not predatory.

4.03.7.1 Ancestral Primates Were Not Nocturnal

Several researchers have argued against the NVP hypothesis on the grounds that basal primates were not nocturnal. Tan and Li's (1999; Li, 000) hypothesis that the ancestral primates were trichromatic and diurnal is unparsimonious in the context of a more comprehensive analysis of the data (Heesy and Ross, 2001). More recently, Ni *et al.* (2004) reported the discovery of a skull of the basal omomyiform primate *Teilhardina asiatica* from the earliest Eocene deposits of the Lingcha Formation, China (Figure 7). On the basis of the relative orbit size of this specimen, Ni *et al.* suggested that *T. asiatica* was diurnal. The use of relative orbit size as an indicator of activity pattern in fossil primates was pioneered by Walker (1967), but fully developed by Kay and Cartmill (1977; Kay and Kirk, 2000). This work showed that, in living primates with skull lengths below approximately 75 mm, nocturnal species generally have larger orbits than diurnal species. This separation of nocturnal and diurnal species in relative orbit size makes it possible to discriminate activity pattern in fossil species by plotting orbit size against body size to see whether the fossil resembles living nocturnal or diurnal primates. Applying this technique to interpret the activity pattern of the tiny *T. asiatica* necessitates extrapolation below the range of skull lengths exhibited by living primates. Ni and colleagues used a least-squares regression model to estimate the orbit dimensions of nocturnal and diurnal taxa at the skull length of *T. asiatica*, and argued that the relative orbit size of *T. asiatica* suggests that it was diurnal. Optimizing activity pattern onto a phylogenetic tree of primates and their relatives, Ni *et al.* reconstructed diurnality at the stem primate node, hence calling the NVP hypothesis into question.

Ni *et al.*'s analysis suffers from the difficulty of extrapolating the relationship between relative orbit size and activity pattern below the body size range of living primates (Martin and Ross, 2005). The

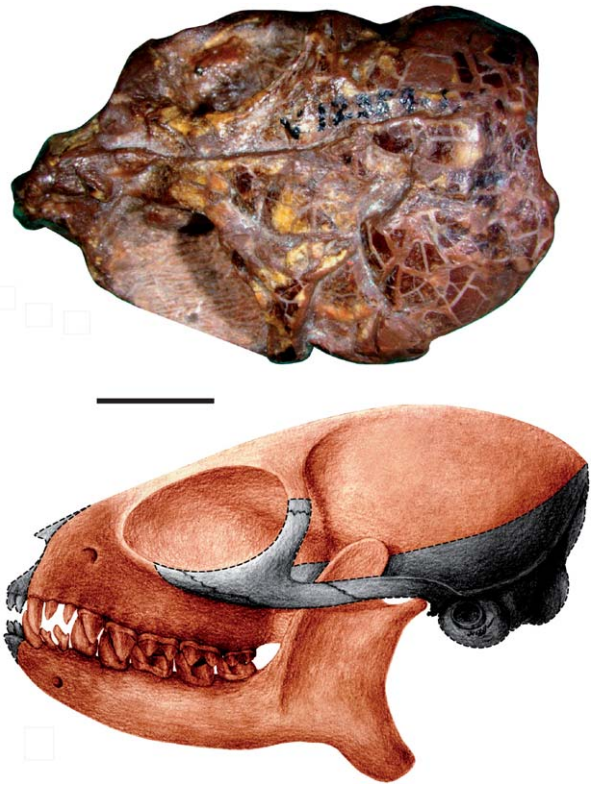

Figure 7 Skull of *T. asiatica* in dorsal and reconstructed lateral view; (IVVP V12357), earliest Eocene Lingcha Formation, Hengyang Basin, China (Ni *et al.*, 2004). Scale bar: 5 mm. Reproduced from Ni, X., Wang, Y., Hu, Y., and Li, C. (2004). A euprimate skull from the early Eocene of China. *Nature* 427, 65–68, with permission from Nature Publishing Group.

relationship between eye size and body size in mammals has been claimed to be nonlinear, such that eye size declines rapidly at body sizes below the range of extant primates (Ross, 2000; Kiltie, 2000; Martin and Ross, 2005). In Figure 8, corneal diameter of the eye is plotted against head-and-body length in mammals. The line that best fits the data is a fourth-degree polynomial, and a quadratic explains the data better than a linear least-squares line, but none of these lines is significantly different from any others, making it difficult to determine what kind of regression line should be used at small body sizes. This calls into question the hypothesis that *T. asiatica* was diurnal and raises the thorny issue of how to reconstruct activity pattern in fossil primates at body sizes below those of extant forms.

Various lines of evidence point to a nocturnal origin for basal euprimates (reviewed by Ross *et al.*, 2006). Charles-Dominique and Martin (1970) argued that the ancestral primate was probably nocturnal because nocturnality characterizes galagids, cheirogaleids, and lorisids, and these animals are probably the most primitive members of their respective lineages. Later, Martin (1973)

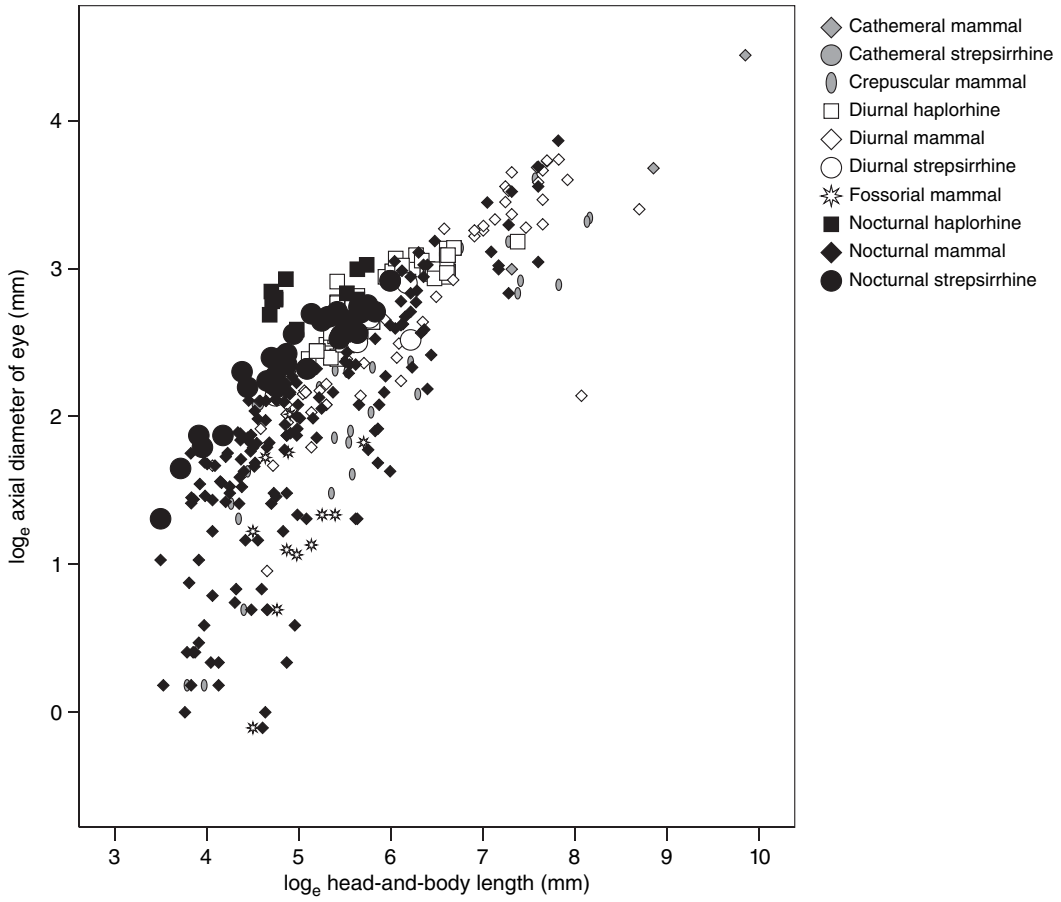

Figure 8 Plot of axial diameter of the eye against head-and-body length in mammals. Data from Ritland, S. 1982. The Allometry of the Vertebrate Eye. Unpublished PhD dissertation, Department of Biology, University of Chicago and Ross (unpublished data).

bolstered this argument by pointing out that the presence of the tapetum found in diurnal lemurids is best explained as a primitive retention from a last common ancestor of strepsirrhines that was nocturnal. Ross (2000) hypothesized that the earliest primates also probably possessed a tapetum. Explicitly cladistic reconstructions of the evolution of activity pattern in primates and their relatives corroborate the hypothesis that nocturnality characterized the first euprimates (Heesy and Ross, 2001). The possibility that certain early primates were extremely small, even around 10 g in size (Gebo *et al.*, 2000; Gebo, 2004), also suggests that these animals were nocturnal, as most living mammals in this size range are nocturnal. The possibility that basal primates were smaller than any living primates is not universally accepted, but some of them certainly were. It is therefore important to ask what the visual systems of such animals would have been like. Most extant mammals in this size range are olfactory-dominated animals. What kind of eye could a 10 g primate have carried and how would its brain organization have been affected (Kaas, 2000)? Theoretical investigations of such issues, combined with future fossil discoveries, promise to provide important clues as to the visual adaptations of early primates.

4.03.7.2 Predatory Adaptations of the Ancestral Primates Were Not Visual

The most common criticism of the NVP hypothesis is that primates use nonvisual senses to locate prey (Rasmussen, 1990; Sussman, 1991; Crompton, 1995). Sussman (1991) reviewed relevant data, pointing out that *Galagoides demidoff* and *Tarsius* can localize prey using hearing (Charles-Dominique, 1977; Niemitz, 1979), whereas lorises localize prey with olfaction. However, as has been noted previously, the fact that primates use nonvisual senses to localize prey does not necessarily mean that their visual sense is not important for prey localization (Dominy *et al.*, 2004; Ross *et al.*, 2005). Both galagos and lorises have been reported to use visual cues to localize moving prey (Charles-Dominique, 1977, p. 39; Schulze and Meier, 1995).

Moreover, it is also clear that, among extant mammals, increases in sound-localization acuity are associated both with increases in width of the binocular visual field and with narrowing of the field of highest visual acuity (Heffner and Heffner, 1985, 1992): animals with the highest auditory acuity also have large binocular visual fields and narrow fields of high-acuity vision. These data led Heffner and Heffner to suggest that the function of sound localizing is "directing the attention of other senses toward the sound-producing object" (Heffner and Heffner, 1992, p. 711). Primates notably have increased sound-localizing ability, increased binocular field width, and narrow fields of high visual acuity, and the work of Heffner and Heffner suggests that these features are interrelated. It cannot therefore be argued that use of auditory information for prey localization falsifies the hypothesis that visual cues are used as well (Sussman, 1991; Crompton, 1995). On the contrary, it suggests that if early primates were indeed nocturnal visual predators, they were probably auditory predators as well, and vice versa.

4.03.7.3 Visual Adaptations of Ancestral Primates Were Not Predatory

The most important criticism of the NVP hypothesis proposes alternate explanations for the origins of the high degrees of orbital convergence characteristic of primates. Two alternate reasons for orbital convergence have been suggested: localizing small fruits in terminal branches and locomotion in the fine-branch niche.

Sussman (1991) agreed with Cartmill that the divergent hallux and pollex and flattened nails are grasping organs, noting:

> It is generally agreed that these adaptations would have allowed Eocene prosimians far greater access to fruits and flowers, as well as plant-visiting insects, making them much more efficient at locomoting and foraging in the small terminal branches of bushes and trees than were the plesiadapoids (Sussman, 1991, p. 219).

But Sussman went on to suggest that the evolution of orbital and optic convergence is not explained either by locomotion or by predation on small insects, which he saw as being captured using hearing and olfaction. Instead, Sussman notes that fruit bats also appear to have convergent orbits, like primates, and implicitly suggests that in primates this might be related in some way to eye–hand coordination:

> these nocturnal animals [i.e., fruit bats and primates] were feeding on and manipulating items of very small size (e.g., fruits, flowers and insects), at very close range, and under low light conditions. This might require acute powers of discrimination and precise coordination (Sussman, 1991, p. 219).

Rasmussen's (1990) study of the feeding and locomotor behavior of *Caluromys* led him to suggest that there might be elements of truth to both Cartmill's and Sussman's models. He suggested that the stem primates were lured out onto the terminal branches by:

> . . . fruit and flowers with associated coevolving insect faunas . . . Once up into the swaying terminal branches, those individuals that could best meet their arthropod requirements by visual predation probably had a selective advantage over those whose visual, locomotor and manual coordination abilities were less suited for such a complex task (Rasmussen, 1990, p. 274).

Thus, Rasmussen argues that early primates were lured out into the terminal branches for the reasons advocated by Sussman, but the visual specializations were adaptations for the NVP suggested by Cartmill.

Crompton (1995) argued that stereopsis in the fine-branch niche "cannot readily be ascribed to the need to detect cryptic, immobile insects, since they are not the typical prey" (Crompton, 1995, p. 25). Instead, in a modified version of the fine-branch niche hypothesis, Crompton argued that foraging, leaping, and climbing among the dense supports of the fine-branch niche would benefit from stereopsis and grasping hands because this environment:

> . . . provides a visually complex, confusing background against which to distinguish a variety of mobile and immobile targets, both dietary items (fruit, as well as insects) and locomotor substrates (Crompton, 1995, p. 25).

In the end, Crompton invoked a multifactorial explanation for the origins of the orbital convergence.

> Orbital frontality is more likely to have first appeared as a consequence of the more general benefit that accrues, for a small-bodied primate similar to *Microcebus*, in the fine branch niche. This is provision of scotopic acuity and depth perception for the location of diverse targets, fruit and branches as well as insects in a complexly shaded environment (Crompton, 1995, p. 26).

The importance of the grasping hand for Crompton lies not only in climbing and manipulation of food, but also in securing a safe landing after short leaps. Once again, eye–hand coordination is implicit in Crompton's argument, although the relevance of this coordination for landing after a leap is not clear.

Thus, the adaptive significance of the distinctive features of the primate visual system is debated. Cartmill (1972, 1974) and Rasmussen (1990) agree that orbital convergence facilitates NVP on insects, captured with the hands in the fine-branch milieu; Sussman (1991) argues that orbital convergence is linked to manipulating small fruits, flowers, and insects under low light levels; Martin (1990) links orbital convergence to locomotion in a fine-branch niche, and Crompton (1995) invokes both

feeding on small food objects and locomotion to explain the evolution of orbital convergence.

These debates over the ecological significance of increased orbital convergence stimulated additional comparative morphometric research on orbit orientation in mammals. Heesy (2003) measured orbit orientation in a large sample of metatherian and eutherian mammals, and found strong effects of locomotor substrate, activity pattern, and diet on orbital orientation. Orbital convergence and frontation are higher in arboreal taxa than terrestrial or aerial taxa, and frontation and verticality are higher in faunivorous and omnivorous taxa than in opportunistic and nonpredatory animals. When these analyses were performed on eutherians exclusive of primates, nocturnal and cathemeral/crepuscular animals were found to have more convergent orbits than diurnal animals, and faunivorous taxa to have more convergent orbits than nonpredators. When all possible categories of locomotor substrate, activity pattern, and diet were considered, arboreal, nocturnal faunivores were ranked as having the highest degrees of orbital convergence. Heesy's analyses suggest that, across a wide range of mammals, nocturnal, arboreal faunivores tend to have more convergent orbits than other ecological categories. In a similar study, Ravosa and Savakova (2004) showed that, when allometric factors are taken into account, pteropodid bats do not have orbits that are as convergent as those of primates, negating one of Sussman's criticisms of the NVP hypothesis. Moreover, felid carnivorans (which are predominantly nocturnal) have primate-like degrees of orbital convergence, while nocturnal visual predatory tree shrews (*Ptilocercus*) and nocturnal procyonid carnivorans have more convergent orbits than diurnal predatory close relatives.

Both of these studies (Heesy, 2003; Ravosa and Savakova, 2004) corroborate the NVP hypothesis, but neither study explicitly evaluates the hypothesis relative to the fine-branch niche locomotion hypothesis. Ravosa and Savokova show that felids – NVPs not living in the fine-branch niche – have primate-like levels of orbital convergence, suggesting that NVP is sufficient to produce orbital convergence, but they do not exclude the possibility that fine-branch living also would produce this effect, even in the absence of NVP. Similar issues emerged from Lemelin's (1999) comparison of hand morphology in didelphid marsupials and primates. Although he confirmed that locomotion on fine terminal branches is associated with convergent similarities in hand and foot anatomy and proportions in marsupials and primates, the animals concerned also fed on small fruits and insects in the terminal branches. This makes it difficult to factor out the relative importance of feeding versus locomotion and of insectivory versus frugivory for hand and foot morphology.

To demonstrate that NVP is necessary and sufficient to explain orbital convergence and the unique hand morphology of primates, but fine branch locomotion or fruit feeding are not, NVPs living in the fine-branch niche need to be compared with non-NVPs living in the fine-branch niche. Variation in degrees of predation, hand morphology, and orbital convergence within primates provides one source of appropriate comparisons. Lemelin (1996, p. 173) reports preliminary results of analyses that demonstrated "significant and positive covariation between amount of insectivory, selection to catch styles, and relative lengths of the digits among closely related prosimians."

4.03.8 Comparative Neuroscience

In parallel with these developments in primatology, comparative neuroscience has revealed a series of distinctive features of the primate nervous system, which, judging by their common occurrence in most primates, can be hypothesized to have evolved along the primate stem lineage, after the divergence of any sister group, such as tree shrews and dermopterans.

4.03.8.1 Visual System

The high degree of orbital convergence characteristic of primates increases the size of the binocular field (Ross 2000; Heesy, 2004) and improves the potential and actual quality of the image falling on the central retina. These changes make it worthwhile increasing relative eye size to increase image size (Ross *et al.*, 2006), increase the density of photoreceptors and ganglion cells in the central retina to increase sampling frequency, and increase representation of the central retina in the visual structures of the brain (Allman, 1977). Barton (2004) has shown that, while controlling for body size, increases in relative orbital convergence are associated with increases in the relative volume of the lateral geniculate nucleus, relative area of the primary visual cortex, and relative neocortex size in general. Barton also shows that these increases are primarily attributable to increases in parvocellular rather than magnocellular pathways, suggesting that they reflect adaptations for improved fine-grained stereopsis, rather than increased sensitivity to movement. He suggests that:

> . . . the increase in visual brain size in primates with more convergent orbits might reflect enhancements of stereo-acuity and vergence-control mechanisms specifically related to the visually guided grasping and close-range manipulation of food items (Barton, 2004, p. 10115).

Barton's analysis treats variation in the visual system within primates, not across mammals, as a whole, so caution must be exercised when extending the results back into the primate stem lineage. To the extent that such extrapolation is valid, many of the changes to the visual system that occurred in the primate stem lineage can be hypothesized to have been not only integrated with each other, but also associated with improvements in fine-grained stereopsis and visual acuity in the center of the visual field. However, it is important to remember that the visual systems of stem primates were also characterized by an array of changes related to other functions, including improved sensitivity to movement, and improved ability to locate movements and sounds in space. Primates exhibit extensive projections from each retina to its ipsilateral lateral geniculate nucleus and superior colliculus, and both the visual cortex and superior colliculus contain representations of only the contralateral visual field. The superior colliculus provides the substrate for the visuomotor response, in which the eyes are directed to novel objects entering the visual field (Schiller and Stryker, 1972) and the unique arrangement of the projections to the superior colliculus in primates removes ambiguity regarding the position of those objects (Allman, 1999). As noted above, increased overlap of the visual fields across mammals is also associated with increased ability of the auditory system to localize sounds in space, suggesting that such abilities also characterized basal primates (Heffner and Heffner, 1982, 1992; Heffner, 2004). Primates are characterized by expansion and multiplication of their extrastriate visual areas, including not only areas that process information on fine-grained stereopsis and acuity (the ventral information stream in temporal cortex), but also areas such as the middle temporal (MT) area devoted to analysis of movement in the contralateral visual field (see reviews in Allman, 1977, 1999; Allman and McGuinness, 1988; Kaas, 2002). Thus, there is evidence that the basal primate visual system was modified not only to enhance fine-grained stereopsis (Barton, 2004), but also to improve the ability to detect and localize sources of movement and sound in the visual field. These latter attributes would be of particular benefit to NVPs, but of little obvious use for finding fruits and berries.

4.03.8.2 Hand Motor Control

In vertebrates, control of voluntary limb movements is mediated by descending pathways from the brain to the motor neurons in the spinal cord. All vertebrates possess reticulospinal, rubrospinal, tectospinal, and various other pathways from the brain to the spinal cord (Nudo and Masterson, 1988), but corticospinal tracts (CSTs) are found only in mammals. Simian primates and carnivores have larger CSTs than other mammals (Phillips and Porter, 1977; Figure 9), and the lateral CST of primates is unusual in both the degree to which it penetrates to caudal spinal cord segments and in the directness of its connections with motor neurons of the muscles of the distal extremities (Phillips and Porter, 1977; Heffner and Masterson, 1983). Across mammals and within primates, increased CST penetration down the spinal cord and increasingly ventral termination of CST connections within the cord are correlated with progressive increases in the degree of digital dexterity (Heffner and Masterson, 1975). This suggests that the emergence of these features in basal primates was associated with increased manual digital dexterity. Extant primates use their hands for many things, including grasping branches during locomotion, acquiring food, and social grooming (Bishop, 1964). Precisely which of these functions originally demanded enhanced dexterity is not immediately obvious from the anatomical data.

One question arising from these data is why there is a relationship between manual digital dexterity and CST penetration beyond those cervical spinal cord segments that supply the muscles of the forelimb (Heffner and Masterson, 1975). The answer to this conundrum may lie in a Wood Jonesian emancipation of the forelimb accompanying increased coordination of the hindlimbs and forelimbs. One benefit of this is illustrated in Figure 10, a photograph of a *Mirza coquereli* cantilevering from a

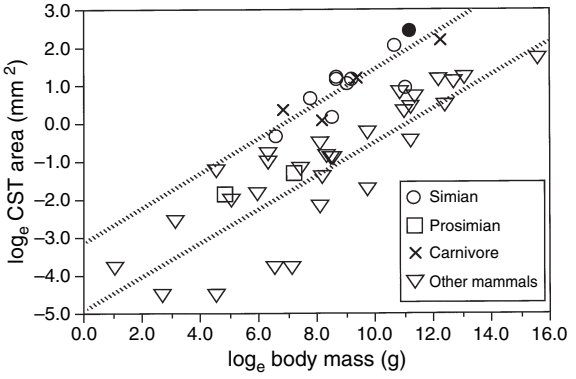

Figure 9 Bivariate plot of the natural log of CST area against the natural log of body mass in mammals. The least-squares regression lines for simian primates and for rodents are shown. Humans are shown by the solid circle. Simian primates have larger CSTs than other mammals, a feature in which they resemble carnivores. Data from Heffner, R. and Masterton, B. 1975. Variation in form of the pyramidal tract and its relationship to digital dexterity. *Brain Behav. Evol.* 12, 161–200.

Figure 10 *M. coquereli* adopting a cantilever posture. Image of Coquerel's dwarf Lemur, *M. coquereli*, Kirindy Forest, Madagascar, © Manfred Eberle, www.phocus.org.

vertical branch to grasp something out of the air. Various prosimians (cheirogaleids, galagos, and tarsiers) have been reported to manually acquire flying prey while holding onto branches with their feet (Crompton and Andau, 1986; Gebo, 1987; Martin, 1990). We hypothesize that extension of the CST down to lumbar and sacral spinal cord segments provides the anatomical connections necessary for arboreal mammals to coordinate a secure hold on the substrate with their hindlimbs or tail while they use their hands for catching insects, harvesting fruits, or other tasks requiring manual dexterity.

Another distinctive aspect of the primate corticomotor (CM), system is the degree of multiplication of premotor areas in frontal cortex. Macaques, for example, exhibit at least six separate premotor areas that project not only into primary motor cortex (MI), but also give origin to corticospinal neurons. In the three areas in which this has been studied, these corticospinal neurons include CM fibers that run directly from the cortex to the motor neurons in the ventral horn of the cervical and lumbar regions of the spinal cord (Dum and Strick, 2002). Five of the six premotor areas have distinct projections to both upper and lower cervical spinal cord segments. Three of these areas (supplementary motor area (SMA), dorsal cingulate motor areas (CMAd), ventral cingulate motor areas (CMAv)) project to lower cervical spinal cord segments, specifically to the intermediate zone and ventral horn, the latter of which contains the motor neuron cell bodies for the hand muscles. Each premotor area receives inputs from a different combination of posterior parietal and prefrontal cortical areas, "each participates in distinct loops with the basal ganglia and cerebellum" (Dum and Strick, 2002, p. 681), and each projects in parallel to the spinal cord. Just as the multiplicity of prestriate visual areas serves as the substrate for a multiplicity of diverse visual functions, so each of these multiple premotor areas is argued to be "a functionally distinct efferent system that differentially generates and/or controls specific aspects of motor behavior" (Dum and Strick, 2002, p. 677). The anatomical and physiological relationships between these areas and the control of hand movements suggests that the increased dexterity characteristic of primates is related to the multiplication and increased functional diversity of these cortical premotor areas.

Nudo and Masterson (1990b) showed that the size of CST cortex is highly correlated with body mass, brain mass, and the area of the neocortex, with the strongest relationship between CST cortex area and overall neocortex area. After they factored out the effect of increased cortex size, they found primates to show a constant proportion of CST cortex to overall cortex area, while raccoons show relative increases in CST cortex compared to other carnivorans. They attributed the enlargement of the CST cortex in primates to overall neocortical enlargement. Whatever the mechanism of enlargement, the size of the cortical areas giving rise to CSTs increases along the lineages leading to humans and raccoons from basal mammals in parallel with their dexterity (Nudo and Masterson, 1990b).

4.03.8.3 Eye–Hand Coordination

Elliot Smith noted that eye–hand coordination is an important component of the basal primate adaptations, but most explanations for primate origins in the literature have neglected to emphasize this basal attribute. Recent studies in comparative neuroscience have revealed distinctive anatomical features of the primate brain that are involved in mediating this coordination.

Although nonprimate mammals have premotor areas that give origin to CSTs, primates are unique in having CSTs arise from a distinct subregion of ventral premotor cortex not found in other mammals: region C of Nudo and Masterson (1990a) or the arcuate premotor area (APA) of Dum and Strick (2002). Allman (1999) synonymized region C with the ventral premotor region, PMv, but region C in macaques at least is only the rostral part of PMv lying within the posterior bank of the inferior limb of the arcuate sulcus. Regardless of terminology, primates are unique in possessing areas PMv and APA/region C, both of which appear to be important in the control of visually guided reaching and grasping movements (i.e., eye–hand coordination).

APA is unusual among the six premotor areas discussed above in that it exhibits very dense and numerous connections to the hand representation in MI, and to upper cervical segments supplying the muscles crossing the shoulder and elbow joints, but does not project to lower cervical spinal cord segments where hand motor neurons are located. Nevertheless, stimulation of this area commonly elicits movements of the fingers and thumb, but less commonly movements of more proximal joints, such as the wrist, elbow, and shoulder (Martino and Strick, 1987; Dum and Strick, 1991; He *et al.*, 1993). Dum and Strick (2002, p. 681) suggested that APA/region C "is primarily involved with control of distal forelimb movements" and the anatomical data presented above suggest that this control involves coordination of the movements in joints of the upper arm as well.

Preuss (1993) reviewed the evidence available at that time that PMv plays "a role in visually guided reaching and prehension." The work of Rizzolati *et al.* had revealed that neurons in PMv respond not only to tactile stimuli applied to the hands and face, but also to visual stimuli, especially to stimuli within reaching distance. Neurons in this region are active, "specifically during purposive, prehensive movements of the face and forelimbs" (Preuss, 1993). Preuss argued that integrated use of the mouth and the hand may have been important components of early primate feeding adaptations, whether for visually guided manual predation on insects as suggested by Cartmill (1970) or "visually guided grasping and manipulating fruits and flowers" as advocated by Rasmussen and Sussman (Preuss, 1993, p. 355). Preuss' hypothesis receives support from more recent observations that when PMv caudal to the inferior limb of the arcuate sulcus – close to the origin of the CST – is stimulated, coordinated movement of the hand to the mouth is elicited, accompanied by opening of the mouth (Graziano *et al.*, 2002a). This suggests an important role for PMv in visually guided movements of the arm and hand during feeding. However, PMv also functions in the integration of tactile, auditory, and visual information in the control of arm movements (Graziano and Gandhi, 2000; Graziano *et al.*, 1999, 2002a, 2002b). Graziano's work has revealed a polysensory zone that integrates visual, auditory, and tactile information into the planning of hand movements in space (Graziano *et al.*, 1999; Graziano and Gandhi, 2000). Integration of visual, auditory, and tactile information is plausibly related to capturing flying or moving prey, whereas auditory information is not obviously necessary for coordination of movements associated with locomotion or grasping fruits.

Improved sensorimotor coordination in control of primate hand movements is also indicated by expansion and elaboration of somatosensory areas (ventral somatosensory area (VS), the parietal rostral area (PR), and the retroinsular area (Ri)) and areas in the posterior parietal cortex that are important for visual and visuomotor processing (Wu *et al.*, 2000; Kaas, 2004). The latter areas connect forward into the array of new premotor areas in the frontal lobe, including the multiple premotor areas controlling hand and digit movements (e.g., PMv, SMA). Stimulation of the rostral half of posterior parietal cortex in *Otolemur* (Stepniewska *et al.*, 2005) and macaques (Their and Andersen, 1998; Cooke and Graziano, 2003) elicits complex movements that "seem to be components of ethologically meaningful behavioral patterns such as feeding and defense" (Stepniewska *et al.*, 2005, p. 4882). To the extent that these attributes and connections of PMv characterized stem primates, PMv was probably an important component of a neural system adapted not only for foraging for small fruits and berries, but also for NVP. Unfortunately, the available data do not allow definitive statements as to the original function of PMv. Graziano's research was carried out on macaques, and it is not clear to what extent nocturnal primates such as galagos and lorises possess a polysensory zone in PMv. Moreover, although the origin of PMv may have been more important for mediating eye–hand coordination used in feeding than in locomotion, the other premotor areas distinctive of primates (Kaas, 2004) may well have had locomotor-related functions originally, and the precise order in which they arose cannot currently be discerned. Indeed, it may be that the neurological adaptations associated with eye–hand coordination are either interchangeable with or so extremely similar for both NVP and fine-branch locomotion as to make it impossible to discriminate between these competing hypotheses regarding primate origins. However, both scenarios imply that improved eye–hand coordination was a fundamental adaptation in basal primates.

4.03.9 Locomotor System

As reviewed above, it was F. Wood Jones who suggested that specialization of the hindlimb for supporting body weight during climbing emancipated the forelimb from supportive functions, freeing it for specialization to perform other tasks. Several attributes of the primate locomotor system suggest that Wood Jones' hypothesis contains some nuggets of truth. The feet of primates are adapted for grasping as part of a distinctive grasp-leaping

pattern of locomotion (Szalay and Dagosto, 1988). Changes to the joint between the first metatarsal and the entocunieform allow the hallux (big toe) to be held in an abducted position, divergent from the rest of the digits, and to be stable under high forces during grasping. The proximal end of the first metatarsal manifests a robust process that not only buttresses the enotcunieform joint but also provides a hypertrophied area of attachment for the powerful peroneus longus muscle that plantarflexes and everts the foot at the ankle. The primate upper ankle joint is adapted for stability across an enhanced range of flexion and extension, as would be encountered during leaping, and the tarsal elements are elongated to enhance the length of the hindlimb, facilitating a more powerful leap (Martin, 1979). The lower ankle joint evinces adaptations for increased inversion and eversion of the foot necessary during climbing (Dagosto, 1988).

All digits of primates typically bear nails, rather than claws, although some species have re-evolved claw-like nails. Callitrichids and *Daubentonia* have claws on all digits except the hallux, and prosimians have a toilet claw on one pedal digit. The skin of the distal digits is expanded into pads sporting cutaneous ridges for increasing friction on arboreal supports. These ridges are also associated with Meissner's corpuscles for enhanced tactile sensitivity (Martin, 1986). The phalanges of the hands and feet are lengthened relative to the metapodials to improve grasping abilities on fine branches, an adaptation evolved convergently with didelphid marsupials (Lemellin, 1999).

Primate locomotor gaits are also distinctive (Martin, 1990; Schmitt, 2003). Primates typically employ diagonal sequence gaits in which the footfall of the forefoot always follows the contralateral hindfoot, ensuring a secure grasp of the substrate with the hindfeet before moving the forefoot (Cartmill *et al.*, 2002). Primates also walk with a compliant gait, characterized by more elbow flexion, less vertical displacement of the center of mass, and longer stride lengths than other mammals. These traits are hypothesized to have arisen as adaptations to locomotion on small compliant branches (Cartmill *et al.*, 2002; Schmitt and Lemelin, 2002). Convergent evolution of diagonal sequence gaits in the arboreal woolly possum, *Caluromys*, corroborates the hypothesized link between this trait and locomotion on fine supports (Lemelin *et al.*, 2003). Primates also have greater peak reaction forces at their hindlimbs than their forelimbs and they display a more protracted forelimb at touchdown than other mammals (Larson *et al.*, 2001). Convergent evolution of this complex of features in the arboreal kinkajou (*Potos flavus*), a carnivore that also possesses a prehensile tail, provides support for Wood Jones' suggestion that the forelimb function becomes more diverse when the hindlimbs bear the majority of the body weight. Kinkajous not only support more of their body weight with their hindlimbs than their forelimbs, and exhibit highly protracted forelimbs at touchdown, resembling primates (Larson *et al.*, 2001), but they also possess CM connections to the ventral horn of the spinal cord, and relatively dextrous forelimbs (Petras, 1969).

4.03.10 The Fossil Record of Primate Origins

Although it may not be possible to determine from studies of extant primates alone whether the visual and grasping adaptations of early primates originally functioned as adaptations for locomotion or for feeding on insects and small fruits in light-limited environments (Allman, 1977; Pettigrew, 1978; Martin, 1990; Cartmill, 1982; Crompton, 1995), more direct evidence from the fossil record can provide insight.

The lineage leading to extant primates is traditionally thought to have diverged from other mammals close to the Cretaceous/Tertiary boundary. The first members of this primate lineage were long thought to be the plesiadapiforms, a radiation of fossil mammals that thrived in the Paleocene and Early Eocene of the northern continents. The traditional interpretation is that plesiadapiforms, or archaic primates, gave rise to a single stem lineage for euprimates, which quickly divided into two lineages, the omomyiforms and adapiforms, which appear in northern continents at the beginning of the Eocene (*c.* 55 Mya). Compared with plesiadapiforms, these latter two clades manifest closer anatomical similarities and phyletic affinities with extant primates and are grouped with them as Euprimates. In the 1980s and 1990s, many researchers excluded plesidapiforms from Primates because they are not adaptively similar to euprimates, and because cladistic analyses identified at least some plesiadapiforms as basal dermopterans (Beard, 1990; Kay *et al.*, 1992). Recent fossil discoveries and reinterpretation of old fossils have called into question the possibility of dermopteran relationships for plesiadapiforms and have once again identified them as the fossil group most closely allied with euprimates, placing them even closer to extant primates than tree shrews (Silcox, 2001; Bloch and Boyer, 2002).

The best-preserved plesiadapiform skull, that of the paromomyid, *Ignacius graybullianus*, is illustrative of plesiadapiform skulls in general (Figure 11). The braincase is relatively small and the orbits are small, superiorly facing, and completely confluent with the temporal fossa. The infraorbital foramen, like that of *Palaechthon* (Kay and Cartmill, 1977), is relatively large, suggestive of the importance of the vibrissae in detecting prey (Kay *et al.*, 1992). Skulls of *Plesiadapis* are similar, suggesting that the pronounced visual adaptations of euprimates were not shared by plesadapiforms. In contrast, postcranial fossils of many plesiadapiforms display a range of adaptations for arboreality (Szalay and Dagosto, 1988). Recently, a grasping foot with a nail on the hallux was reported from the carpolestid plesiadapiform *Carpolestes*, a putative close relative of primates (Bloch and Boyer, 2002). If *Carpolestes* is indeed representative of the stem lineage of euprimates, it suggests that the manual and pedal grasping abilities of primates evolved prior to their visual specializations, potentially supporting Rasmussen's hypothesis that early primates originally ventured into the small terminal branches in search of small fruits and only subsequently developed the visual adaptations characteristic of living primates (Bloch and Boyer, 2002).

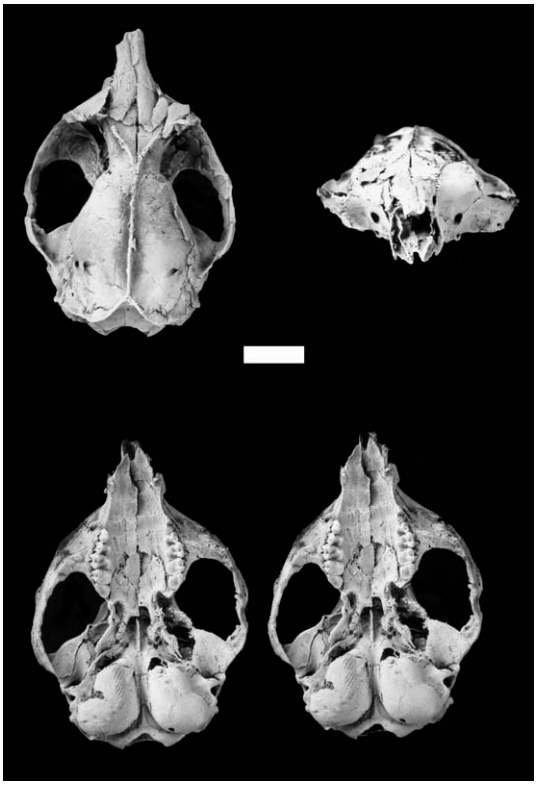

Figure 11 Skull of *I. graybullianus* in dorsal, rostral, and ventral (stereopair) view. Scale bar: 1 cm. Images courtesy of R. F. Kay.

Although *Carpolestes* may have resembled the antecedents of the ancestral primates in some respects, several problems dictate caution in basing interpretations on a direct reading of the fossil record. First, the fossil record is notoriously incomplete. Tavaré *et al.* (2002) have estimated that less than 7% of the species in the primate crown clade have been recovered such that major gaps are present. Hence, even when fossil evidence of extinct species of primates and their relatives is available, these species can be separated from events of interest by significant lengths of time, diminishing their relevance as direct indicators (Ross *et al.*, 2002). These issues are particularly relevant to *Carpolestes*. Statistical analysis of the primate fossil record suggests that the branching points for the origins of extant primates are significantly older than the earliest known fossil representatives currently available. Tavaré *et al.* (2002) estimated the age of primates to be approximately 82 Mya, whereas *Carpolestes* lived at the very end of the Paleocene (*c.* 56 Mya), 26 Mya later. This problem is compounded by the phylogenetic position of *Carpolestes*, which Bloch *et al.* (2001) have argued is nested deep within the carpolestids, removing the species morphologically as well as temporally from developments in the origin of primates. Indeed, from a temporal and phylogenetic perspective, the relevance of *Carpolestes* to questions surrounding primate origins is comparable (at best) to the relevance of living gibbons for hypotheses surrounding human origins. Future fossil discoveries will be needed to address these issues more directly.

4.03.11 Conclusions

The origin of primates of modern aspect was associated with the evolution of a suite of changes to the visual system in concert with changes in other functional systems. We contend that understanding the role of vision in primate origins and evolution requires an understanding of the integration between these systems. Changes to the visual system producing increased sensitivity to low light levels, improved fine-grained stereopsis, and increased visual acuity and motion sensitivity were accompanied by improved abilities to localize sounds or movements in space, increased dexterity, and changes to the somatosensory and somatic motor systems that provided for improved control of visually guided reaching and grasping movements. These changes were accompanied by modifications in gait and musculoskeletal anatomy of the hands and feet related to arboreal

locomotion, including leaping and grasping on fine-branch supports. These changes were manifest not only in the musculoskeletal periphery, but also throughout the central nervous system, including the origins and terminations of the CSTs, the premotor areas controlling limb movements, the visual cortex, and the primary and secondary sensorimotor areas.

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4.04 The Comparative Biology of Photopigments and Color Vision in Primates

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Glossary

dichromacy	A variant form of color vision. The defining character of dichromatic color vision is that a dichromat can match the appearance of a test light of any color by adjusting the relative intensities of two different colored lights.
LWS, SWS1	Abbreviations for two of the four families of vertebrate cone opsin genes.
monochromacy	An absence of color vision. Technically, a monochromat can match the appearance of test light of any color by adjusting the intensity of any other colored light.
opsins	Photopigment proteins.
S, M, L	The three types of cone photopigment found in primates. The letters are shorthand for short-, middle-, and long-wavelength sensitive, respectively.
spectrally opponent neurons	Cells in the visual system that transmit information used to support color vision.
trichromacy	A variant form of color vision that is the norm for catarrhine primates. The defining character of trichromatic color vision is that a trichromat can match the appearance of a test light of any color by adjusting the relative intensities of three different colored lights.

4.04.1 Evolution and Distribution of Primate Photopigments

Photopigments are sequestered in photoreceptors (in vertebrates, the familiar rods and cones) and serve to convert photon energies to neural signals, thus forming the indispensable first step on the path to seeing. Photopigment molecules consist of an apoprotein, an opsin, which is covalently bound to a chromophore. The genes specifying opsins have been the subject of intensive investigation and the opsin sequence information emerging from this work has in turn been used to produce photopigment phylogenies. Since many relationships between photopigments and vision have been elucidated in studies of contemporary species, it is possible to cautiously infer from these phylogenies something of the nature of vision in ancestral species.

Variations in opsin gene sequences serve to spectrally tune photopigments so that they preferentially absorb light most efficiently in a restricted portion of the spectrum (Nathans *et al*., 1986; Nathans, 1999). Although exceptions can exist, a basic feature of most visual systems that support color vision is the presence of two or more classes of photopigments, each having spectrally discrete absorption patterns. This requirement seems to have been achieved early on, perhaps by the date noted above (i.e., 800–1000 Mya), and by the time jawed vertebrates appeared (estimated as at least 540 Mya) there were already four separate classes of cone photopigments (rod photopigments were apparently added later) (Collin and Trezise, 2004). The implication drawn from the presence of multiple classes of cone photopigments is that rather elaborate color vision could also have been present by this time (see Evolution of Color Vision and Visual Pigments in Invertebrates).

A direct reflection of this early arrangement is that across contemporary vertebrates all cone photopigments are specified by genes drawn from four cone opsin gene families. Some groups of animals, for example, many birds, fishes, and reptiles, have retained representatives from each of these four families, and consequently have four types of cone photopigments

(Yokoyama and Radlwimmer, 1999). Other lineages have lost representation of one or more of these gene families and have correspondingly fewer types of cone photopigments. Among the latter are the mammals. It is now believed that sometime early in mammalian evolution, perhaps associated with the nocturnality of mammals during this time, two of these gene families were lost. The consequence of this loss is that contemporary mammals, at least all eutherian mammals, utilize opsins that are products of genes from only two families (Jacobs, 1993). Representatives from these two gene families, usually identified as short- and long-wavelength sensitive (respectively, SWS1 and LWS), produce pigments that, depending on the details of the gene sequence, can be spectrally tuned to have maximum sensitivity across the respective ranges running from the ultraviolet to the short wavelengths of visible light (SWS1 genes) and from the middle to long wavelengths (LWS genes) (see Figure 1a).

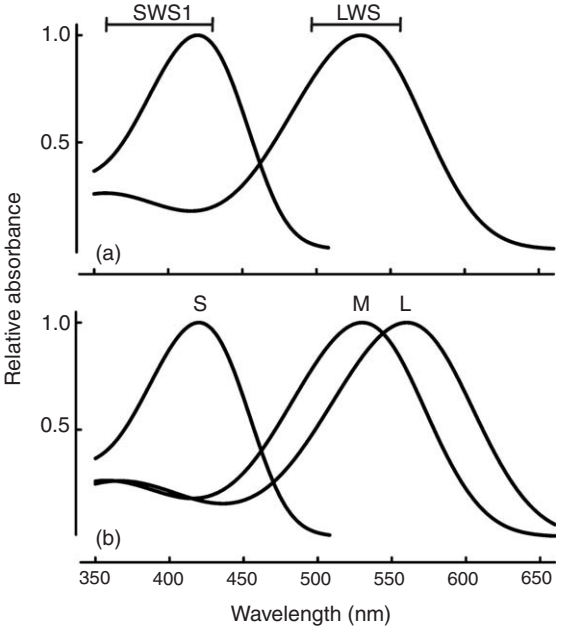

Figure 1 Spectral absorption curves for cone photopigments. a, The basic mammalian arrangement features two types of cone photopigment. These are respectively specified by representatives from two opsin gene families, SWS1 and LWS. In different species these genes spectrally tune the pigments across a range of wavelengths. That range is indicated for each of the two gene families by the horizontal bar shown above each pigment type. Thus, pigments specified by SWS1 opsin genes have λ_{max} from ~360 to 435nm while those produced by LWS opsin genes span the range from ~493 to 562nm. b, Spectral absorption curves for cone photopigments in catarrhine primates. These are typically designed as S, M, and L. The former is specified by a gene from the SWS1 family while the M and L pigments reflect LWS gene action. The separate M and L pigments arose as a result of an early gene duplication occurring at the base of the catarrhine radiation.

Primates are similar to other mammals in having a single rod opsin gene (located on chromosome 7) that encodes a rod photopigment with maximum sensitivity around 500nm. The SWS1 gene in primates is located on chromosome 3. This gene specifies a so-called S-cone pigment (S means short-wavelength sensitive, as before). There is some variation across primates in the absorption properties of the S-cone with peak sensitivities (λ_{max}) varying from ~415 to 435nm. In primates LWS genes are X-chromosome linked. From studies of human color vision, both its character and its inheritance, it has long been appreciated that humans with normal color vision must have two versions of these genes that specify spectrally discrete cone pigments. Although these were initially often called 'green' and 'red' cone pigments it is now conventional, and much less misleading, to refer to these pigments as M and L (respectively, middle- and long-wavelength sensitive).

In a landmark study 20 years ago, the human LWS opsin genes were cloned and sequenced (Nathans *et al.*, 1986). The genes specifying the L- and M-cone pigments are positioned in a head-to-tail tandem array on the X-chromosome and are very closely similar in structure having a 97% nucleotide identity. The implication drawn from this fact is that the two genes arose as a result of a duplication of an ancestral LWS gene. In humans, the resulting M and L pigments have λ_{max} ~530 and 562nm (as illustrated in Figure 1b). Subsequently, it has been learned that all catarrhines show evidence for the occurrence of the same gene duplication and thus the duplication event is believed to have occurred near the base of the catarrhine lineage, perhaps about 30Mya. This duplication was a hallmark in the evolution of primate vision as it set the stage for primates to acquire an additional dimension of color vision (Hunt *et al.*, 2005).

Although the cone pigment array in all catarrhines is similar to that illustrated in Figure 1b, there are some additional variations that can impact the character of catarrhine color vision. The chromosomal arrangement of the M and L opsin genes and their great sequence similarities allows mispairing and unequal crossing-over at meiosis. Depending on the detail of these two possibilities, this may result in either gain or loss of an opsin gene on one chromosome or the production of the so-called hybrid genes, that is, genes containing sequence from both M and L prototypes. One result of this process in humans is the widespread presence of multiple copies of the M opsin gene so that most individuals have, in total, more than two

X-chromosome opsin genes with, typically, a single L-cone opsin gene and multiple copies of the M-cone opsin gene. Another is that changes in the gene array can alter both the presence and the spectral absorption properties of the M and L pigments. These latter changes result in alterations in color vision, the most dramatic of which are the red–green color vision defects that are present in up to 10% of all males in some human populations. Whereas both these variations are commonplace in humans, they appear to be effectively absent in other catarrhines. This fact is puzzling and an explanation for it remains elusive. Differences in selective pressures against such variations in human and nonhuman primate lineages are often invoked as a possible explanation although there still seems to be no direct evidence on the matter one way or the other.

The photopigments and opsin gene arrays of platyrrhine primates differ dramatically from those of catarrhines. Like the catarrhines, platyrrhines have an S-cone opsin gene mapped to chromosome 7. However, unlike the catarrhines, most platyrrhine monkeys have only a single X-chromosome opsin gene. This gene is polymorphic with multiple allelic forms coding for photopigments with differing λ_{max} values (Jacobs, 1998). Most species have three such alleles. Since males have only a single X-chromosome, this means that all male monkeys have only two cone pigment types, but with variation in the λ_{max} of their single M/L cone pigment over the range from ~535 to 562nm. Females that are homozygous at the X-chromosome opsin gene site are like the males, but heterozygous females inherit genes that code for two spectrally discrete M/L pigment types. Through the agency of X-chromosome inactivation, the two pigments are expressed in different sets of photoreceptors and thus these females, like the catarrhine prototype, have a total of three cone types. The result is that these platyrrhine populations typically include three phenotypes characterized by two cone photopigments (all of the males plus the homozygous females) and three phenotypes having a total of three cone pigments (heterozygous females). These variations in turn underlie striking individual differences in color vision (Mollon *et al*., 1984).

There are some important variations on this opsin gene theme that have been detected in species from two genera of platyrrhine monkeys. One of these, the night monkeys (*Aotus*), show no evidence of polymorphism having only a single X-chromosome opsin gene that specifies an M-cone pigment (λ_{max}=543nm). Further, the *Aotus* S-cone opsin gene, although highly homologous in structure to other primate S-cone opsin genes, contains a number of insertions and deletions that introduce a premature stop codon in the gene sequence. As a consequence, these monkeys produce no viable S-cone pigment and thus have only a single cone pigment (Jacobs *et al*., 1996). The howler monkeys (*Alouatta*) provide a second interesting exception (Jacobs and Rowe, 2004). Like the catarrhines, these monkeys have two X-chromosome opsin genes that yield photopigments with peaks of ~535 and 562nm. Also similar to the catarrhines, the routine presence of two X-chromosome opsin genes in howler monkeys reflects the occurrence of an earlier opsin gene duplication. Although these X-chromosome opsin gene duplication events yield similar functional outcomes, they apparently occurred independently, once at the base of the catarrhine radiation and again in the line leading to modern howler monkeys. The howler monkeys have two sister taxa (*Ateles* and *Lagothrix*) that are similar to other platyrrhine monkeys in their X-chromosome opsin gene arrangements and that fact suggests that the gene duplication characteristic of *Alouatta* must have occurred subsequent to the divergence of these lineages, that is, <20Mya.

Our view of opsin genes and photopigments in animals from the third major group of primates, the more primitive strepsirrhines, remains incomplete. Originally, it was believed they were similar to the mammalian standard in routinely having two types of cone pigments as is illustrated in Figure 1a. That is apparently the case for some species. However, more recent work shows that other species have an opsin gene/photopigment arrangement similar to that described for *Aotus* with only one type of cone pigment (an M pigment) while still others have a polymorphism of the M/L opsin gene. These latter animals are thus similar to the polymorphic platyrrhines in that males and homozygous females routinely have only two cone pigments while heterozygous females have a total of three cone pigment types (Tan and Li, 1999). The distribution of these three patterns is not completely understood. Presently, it appears that: (1) the polymorphic strepsirrhines include lemurs and indrids from two families (*Varecia* and *Propithecus*), (2) those species with an opsin gene/pigment arrangement similar to *Aotus* include the Galagonidae (bush babies) and *Nycticebus* (slow loris), and (3) animals with two operational cone pigments and no evidence of polymorphism include both some common lemurs (*Eulemur* and *Lemur*) and at least one tarsier (*Tarsier bancanus*). There is some correlation between these opsin gene patterns and

the photic lifestyles of these species in that the polymorphic strepsirrhines are diurnal while those with only a single cone pigment are nocturnal. That relationship seems to break down for the third group that includes nocturnal and diurnal representatives as well as some species whose photic rhythms fall between these two patterns (usually termed 'cathemeral'). There is clearly more to be learned about opsin genes and cone pigments in the strepsirrhines.

4.04.2 Color Vision Variations in Primates

Multiple types of photopigment provide a necessary but not sufficient biological substrate for color vision. In addition, the nervous system must be so organized as to provide a means for contrasting signals that originate in cones containing different types of photopigment. A standard (perhaps universal) way to accomplish the latter requirement is to have the so-called spectrally opponent neurons in the visual system, cells in which signals originating in one or more of the cone types are effectively subtracted from signals originating in other cone types. Neural interconnections sufficient to produce spectral opponency between cones containing SWS1 specified pigments and cones containing LWS specified pigments seem common to all mammalian retinas (Wassle, 2004). In addition to this basic arrangement, primate retinas also contain spectrally opponent mechanisms that allow for signal contrasts between the LWS pigment subtypes (i.e., between the M and L cone types).

Extensive studies of human color vision, and particularly its variant forms, made clear that it is possible to infer much about the nature of color vision from knowledge of the cone pigment complement alone. Thus, any retina containing only a single type of cone pigment cannot support a color vision capacity (a condition termed monochromacy). Similarly, individuals with two types of cone pigment and associated spectral opponency derive a single dimension of color vision (dichromacy), whereas those with three classes of cone pigment get yet an additional dimension of color vision, becoming trichromatic. Within these broad classes the spectral positioning of the cone pigments can greatly influence the nature of color vision, so multiple forms of dichromacy and trichromacy are recognized. It is routine to assume the same sorts of linkages for nonhuman species, that is, the presence of two cone pigment types predicts dichromatic color vision, and so on.

Since it is generally easier to measure photopigments and examine opsin gene arrays than it is to do actual studies of animal color vision, much of what is assumed about nonhuman color vision reflects inferences drawn from studies of photopigments and their opsin gene precursors. Although such inferences are generally well supported, it should be kept in mind that extrapolations from cone pigment complement to color vision are just that, inferences. An important additional point, often ignored, is that knowledge about the cone complement *per se* cannot capture everything that is interesting about color vision. In particular, the acuteness of color vision, as opposed to its dimensionality, depends heavily on the numbers and spatial distributions of cones within the retina. Such information is typically not obtained from studies of photopigments and opsin genes. Thus, useful as they may be, inferences about color vision drawn strictly from studies of photopigments and opsin genes necessarily have some built-in limitations. With those caveats in mind the following summary statements about color vision among the primates can be offered.

4.04.2.1 Catarrhine Color Vision

The inference from comparisons of cone photopigments and their opsins is that all catarrhines would be expected to have very similar trichromatic color vision. Based particularly on a number of careful behavioral studies of various types of macaque monkeys, it seems clear that this assumption is correct. Although there may be some minor differences in color vision capacity among the catarrhines, including those suggested above, all these primates are predicted to share those basic color vision capacities that have been so exhaustively documented for normal human trichromats. Perhaps particularly important for primate success in the task of food harvesting, this includes the ability to make very discerning color discriminations across the red/green portion of the spectrum and thus, for example, allowing foraging primates to evaluate the ripeness of fruits or the potential palatability of foliage.

4.04.2.2 Platyrrhine Color Vision

The platyrrhine theme is polymorphism of the X-chromosome opsin genes and the M/L pigments. There are good studies of color vision in a number of these polymorphic species, particularly for squirrel monkeys (*Saimiri*), marmosets (*Callithrix*), and tamarins (*Saguinus*). The results indicate that the photopigment polymorphisms map directly into

corresponding color vision variations such that, in these species, all males and homozygous females have dichromatic color vision while heterozygous females gain an additional dimension of color vision, becoming trichromatic. Further, within each of these broad categories, subtypes can be detected reflecting the variations in the spectral positioning of the M/L cone photopigments. Although many species that have photopigment polymorphisms have not been subject to direct studies of color vision, the good predictability from photopigment to behavioral capacity in those that have been studied makes it quite likely that similar correspondences exist across all the polymorphic platyrrhines. It is a striking fact that within any group of such platyrrhine monkeys a variety of distinctive color vision phenotypes can be found. That observation has in recent years energized the search to understand the utility of color vision in natural settings (Surridge *et al*., 2003).

It was noted that monkeys of two platyrrhine genera do not have photopigment polymorphism. One of these, *Aotus*, has only a single type of cone photopigment. As predicted from this arrangement, these monkeys fail to show evidence of a color vision capability. Howler monkeys are alone among the platyrrhines in routinely having three types of cone photopigment. This predicts that they should be trichromatic. Although that seems likely to be the case, there are as yet no behavioral studies to verify that prediction. In view of the uniqueness of these monkeys, it would be very useful to make sure that prediction is in fact borne out.

4.04.2.3 Strepsirrhine Color Vision

Although there is at least an outline view of opsin gene and cone pigment variations among the various strepsirrhine species, there is at the same time very little in the way of compelling studies of how these arrangements map into color vision capacities. In accord with the linkages outlined above, one would predict some of the strepsirrhines to lack color vision, some would be expected to be routinely dichromatic, while still others may show color vision polymorphisms of the type known to characterize the platyrrhine monkeys. Whereas it seems reasonable to suppose these predictions are correct, there is at the same time some reason for caution. Unlike all catarrhine and platyrrhine species (save *Aotus*), the retinas of the strepsirrhines generally lack foveal specializations and the very high cone densities associated with these arrangements. The eyes of many strepsirrhines also retain a retinal tapetum, an anatomical adaptation classically associated with nocturnal vision. These facts would suggest, at the least, that color vision is apt to be a less acute capacity in strepsirrhines than in catarrhine and platyrrhine primates (Jacobs, 2004). It would be very useful to have new studies of strepsirrhine color vision conducted using modern techniques. For the present, one can do no better than to assume that the cone pigment arrangements at least predict the dimensionality of strepsirrhine color vision.

4.04.3 Conclusions and Continuing Issues

The comments above make clear that considerable headway has been toward an understanding of primate color vision and its biology and that, at least to a good first approximation, the distribution of color vision among the primates is known. Figure 2 is a phylogeny of primate color vision that summarizes

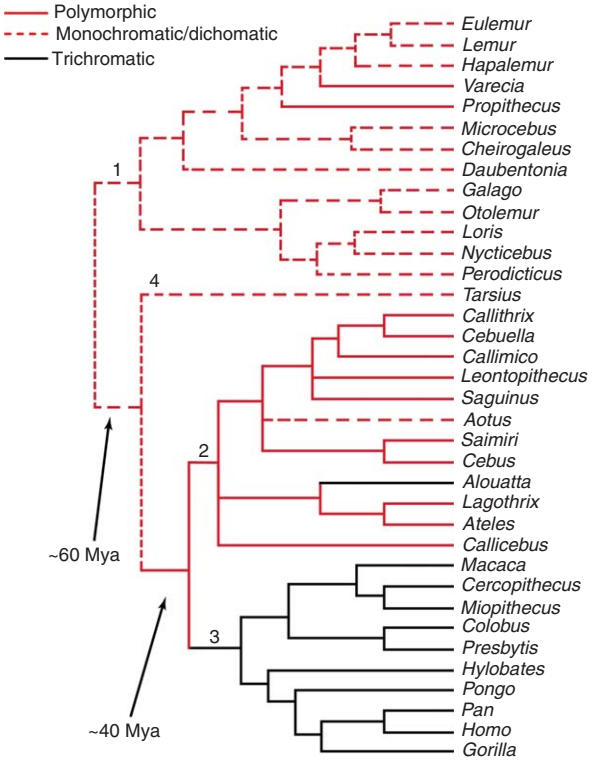

Figure 2 Distribution of primate color vision. Three categories of color vision capacity are indicated: routinely monochromatic or dichromatic, polymorphic, routinely trichromatic. The nature of these categories is further detailed in the text. The numbers on this primate phylogeny identify three taxonomic groups: 1, strepsirrhines; 2, platyrrhines; 3, catarrhines. *Tarsius* (#4) belongs to the same suborder as platyrrhine and catarrhine monkeys (Haplorhini). Reproduced from Jacobs, G. H. and Rowe, M. P. 2004. Evolution of vertebrate colour vision. *Clin. Exp. Optom.* 87, 206–216, with permission from Optometrists Association Australia.

the story. Identified there are three dimensional categories of color vision: routine monochromacy and routine dichromacy, polymorphic color vision that includes a rich mixture of dichromatic and trichromatic phenotoypes, and routine trichromacy. As noted, the picture is reasonably complete for the vast majority of catarrhines and platyrrhine species, but is much less well established for strepsirrhines.

There is some uncertainty about the staging of the changes that led to color vision in contemporary primates. Given the broad distribution of dichromacy across mammals, it seems likely that also represents the condition for ancestral primates. Accordingly, the loss of a functional S-cone pigment observed in a number of nocturnal primates would represent a secondary change. In addition to these primates, an analogous loss of function of S-cone opsin genes has been observed in a number of nonprimates, including both rodents and carnivores. In some of these lineages the loss is sporadic across species (e.g., nonhuman primates and rodents), suggesting that it probably occurred fairly recently. In others, however, S-cone loss appears universal (e.g., the pinnipeds and the cetaceans) and seems therefore to reflect an event that occurred near the base of the radiation. As yet the events triggering such loss are unknown, but since they reflect a variety of structural changes in the S-opsin gene that are different in different lineages they appear to have occurred independently a number of times. A traditional view has been that the ancestral primate was nocturnal (Martin and Ross, 2005). A counter view, recently offered, is that the ancestral primate was diurnal and that trichromacy originated early in primate history (Tan and Li, 1999). If that is so, there has been considerable subsequent loss of color vision dimensionality.

The appearance of three cone pigments in a handful of strepsirrhines might suggest that polymorphic trichromacy was present early on, well prior to the divergence of catarrhines and platyrrhines. If so, routine trichromacy in catarrhines could then have originated by unequal crossing-over between two chromosomes carrying different M/L alleles to produce different versions of the M/L opsin genes on a single X-chromosome. Alternatively, if polymorphism was not an ancestral condition, catarrhine trichromacy could have arisen from an X-chromosome gene duplication with subsequent structural divergence of the two genes to yield genes coding for spectrally discrete M and L pigments. In the latter view, then, opsin gene and photopigment polymorphism arose independently in the platyrrhines and in the small number of strepsirrhine lineages. Examination of the opsin genes of the howler monkeys supports the view that their routine trichromacy reflects events that also occurred independently from those that gave rise to catarrhine trichromacy. The origin and staging of photopigment polymorphism in the platyrrhines remains to be fully understood.

The remarkable variations in color vision across contemporary primates contrast sharply with what is seen in other mammalian orders where color vision is generally much less well developed and where there is greater uniformity of cone photopigments and color vision across species (Jacobs, 1993). Why has more elaborate color vision evolved in primates than it has is in other mammals? In recent years that question has been much debated. Typical answers point to the exploitation by primates of highly colored food sources such as fruits and leaves and to the evolution of a retinal organization in primates that supports high spatial resolution and thus provides a favorable substrate for the addition of new dimensions of color vision. The study of how particular primate color vision phenotypes map into natural behaviors is a topic of much current interest and the results of those studies may be expected to illuminate this issue in the coming years.

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4.05 The Evolution of Parallel Visual Pathways in the Brains of Primates

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Glossary

analogy	Functional similarity between parts of different organisms due to parallel evolution, without common ancestral origin.
Brodmann	Brodmann (1909) developed a commonly accepted scheme for dividing V1 into six layers (I, II, IIIA, IIIB, IVA, IVB, IVCα, IVC*β*, V, and VI).
Hässler	We have used a modification of a nomenclature devised originally by Hässler (1967). The latter allows for more appropriate cross-species comparisons. This nomenclature subdivides cortex into the following layers, with Brodmann's nomenclature in parentheses: I (I), II (II), IIIA (IIIA), IIIBα (IIIB) and IIIB*β* (IVA), IIIC (IVB), IVα (IVCα), IV*β* (IVC*β*), V (V), and VI (VI).
homology	Similarity between parts of different organisms due to evolution from the same part of a common ancestor.
homoplasy	Correspondence between parts or organs as a result of evolutionary convergence.
K, M, P cells	Koniocellular (K), magnocellular (M), and parvocellular (P) cells found in different layers of the lateral geniculate nucleus of primates.
ON-/OFF-center cells	Retinal ganglion and lateral geniculate nucleus cells that respond with increases in response to either the onset or offset of light in the receptive field center.
ontogeny	Developmental progression of an organism from embryo to adult.

4.05.1 Introduction

The primate order to which we belong is quite heterogeneous in size, form, and lifestyle. Primate species range in size from some prosimians that can weigh as little as 100 g (e.g., the mouse lemur, *Microcebus murinus*) to species of great apes, whose males can weigh more than 300 kg (e.g., the gorilla; Figure 1). Such size differences can also be seen in the brain, which varies in weight from 1.73 g in the mouse lemur to 1400 g in humans (Bons *et al*., 1998; Williams, 2002).

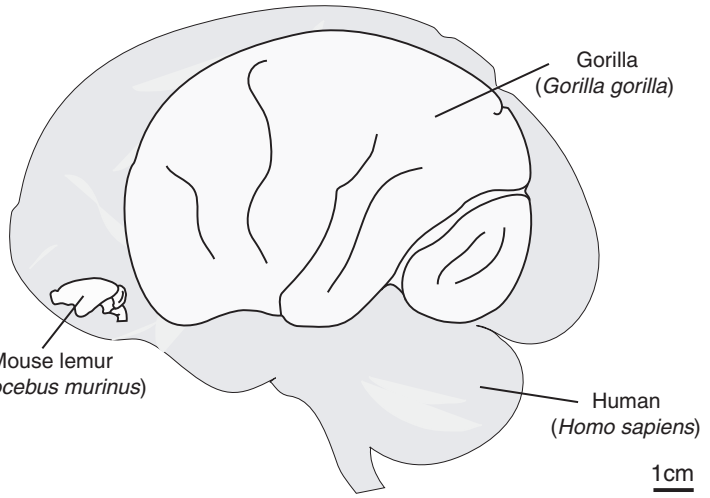
These differences in body/brain size and lifestyle of existing primate species can make it difficult to trace the evolutionary history of brain parts and connections, particularly since big differences in brain size and lifestyle result in both addition and deletion of brain parts, and changes in connections due to scaling issues (Kaas, 2004). Moreover, the clues about brain evolution left by ancestors are limited. These clues rely on incomplete fossil records, and genes whose rate of change cannot be predicted precisely, or (in most cases) be linked to specific brain parts. Finally, relevant visual pathway data have been gathered for relatively small numbers of existing primate species. None of these clues alone, including current powerful genetic approaches, offer sufficient evidence to trace the evolutionary history of specific brain components and connections in primate evolution. The strongest evidence for evolutionary relationships between brain parts and connections of different primates is likely to be the common presence of a feature in several distantly related primates. The difficulty lies in trying to determine

(a)

Relative body and brain sizes

Mouse lemur (*Microcebus murinus*)	Body:	0.06 kg
	Brain:	1.73 g
Gorilla (*Gorilla gorilla*)	Body:	175 kg
	Brain:	535 g
Human (*Homo sapiens*)	Body:	65 kg
	Brain:	1400 g

(b)



(c)

Figure 1 Relative sizes of primates and their brains. The primate order includes mammals that range widely in body and brain size from mouse lemur to gorilla and human. a, Artistic depiction of the relative size differences between a mouse lemur and a gorilla; b, comparison of body and brain weights of mouse lemurs, gorillas, and humans (Bons *et al.*, 1998; Williams, 2002); c, schematic representation of relative brain sizes of these primates. a, Reproduced by permission of David Royal.

the history of these brain parts and connections since similarities may simply reflect a form of parallel evolution (homoplasy) and not necessarily homologous relationships. Also, the fact that connections can be added, deleted, or evolve at different rates in a mosaic fashion magnifies the problem. Nevertheless, some inferences can be made by careful comparisons across existing species and by combining this information with emerging genetic maps of relationships between species.

Our goal in this article is to review relevant evidence from a variety of sources in an effort to reconstruct a reasonable scenario as to how parallel visual pathways might have evolved in primates. Given that visual system studies of living primates are limited to only a few of the many existing primate species, we must rely on work on other mammals, and even nonmammals, to construct a reasonable scenario of the evolution of the visual pathways in primates. Historically, it has been argued that the main parallel visual pathways to cortex in mammals are the retinocolliculopulvinar and retinogeniculo-V1 pathways (see Casagrande and Royal, 2004; Casagrande and Xu, 2004).

For this article, we have chosen to focus on channels passing to and through the lateral geniculate nucleus (LGN), since these pathways may have become differentially specialized in primates and

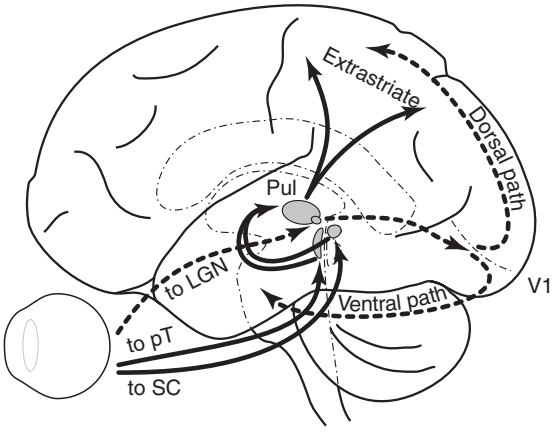


Figure 2 Parallel visual pathways from retina to cortex. In primates, visual information reaches cortex from retina via several pathways. The most studied, and important, is the pathway from the eye to the LGN to V1 (also called striate cortex or area 17), shown with dashed arrows. In V1, new pathways are constructed that enter two hierarchies of visual areas known as the dorsal and ventral paths or streams of processing, also referred to by some authors as the 'where stream' or vision for action stream and the 'what stream', respectively, in reference to their proposed function. Less studied is the pathway from retina (eye) via superior colliculus (SC) and pretectum (pT) to pulvinar (Pul). Pulvinar, in turn, sends widely distributed projections to most extrastriate visual areas to which the dorsal and ventral pathways also project.

are known to be the main pathways for conscious visual perception in primates (Figure 2). We have divided the article into eight sections, including this introduction. In Section 4.05.2, we define what we mean by parallel pathways and provide some other operational definitions that are used in the remaining sections. In Section 4.05.3, we consider whether magnocellular (M) and parvocellular (P) retinogeniculocortical pathways are homologous across primates and whether these pathways exist in nonprimates (e.g., Y and X streams in cats) as some have proposed (Casagrande and Xu, 2004). In Section 4.05.4, we address the controversies over whether the fine fiber system identified by Bishop (1933) in frog and rabbit optic nerve becomes the koniocellular (K) pathway in primates. Given that the K pathway is heterogeneous, we argue that the K pathway is actually made up of a number of pathways of which some are likely to have been present in the common ancestor of primates. A related issue, namely the evolution of chromatic channels and color vision in primates, is addressed separately in Section 4.05.5. Here we defend the position that one type of K pathway likely transmitted cone signals to the LGN even in the ancestors of primates, given that these cone signals have been found in K LGN cells in both New World and Old World primates and in some cat W cells (which share other features with primate K cells). In Section 4.05.6, we consider other properties that remain segregated in the LGN and cortex, such as input from the two eyes and whether it existed in the common ancestor of primates. We support the position that the laminar pattern of ocular segregation in the LGN and the columnar organization of ocular segregation in cortex show the same basic features across primates, suggesting that both were present in the common ancestor of primates. In Section 4.05.7, we examine the issue of whether parallel LGN pathways evolved as starting points for specific hierarchies of visual cortical areas that have been referred to as the dorsal and ventral streams of visual processing in the common ancestor of primates. In Section 4.05.7, we also consider the issue of whether such cortical streams are conserved across mammals or evolved separately in such species as cats. We take the position that the basic subdivisions into dorsal and ventral streams of visual processing at the cortical level can be identified in a diverse range of primates and so are likely to be homologous, but components may have been added, deleted, or modified in different primate lines. In Section 4.05.8, we provide a summary and also outline questions that need to be addressed in order to arrive at more definitive conclusions concerning the evolution of parallel visual pathways. We also outline some practical strategies for answering some of these questions.

4.05.2 Background and Some Definitions

In order to examine the issue of the evolution of parallel visual pathways we need to consider how to define the specifics of the problem. For example, how do we know if a visual pathway is homologous (derived from a common ancestor) or simply analogous (functionally similar but not inherited from a common ancestor)? Since parallel visual pathways are made up of cells at different levels of the neuraxis that differ in terms of neurochemistry, morphology, connections, and function, we need to clarify our level of analysis. For example, can we consider a pathway that carries chromatic signals from two cone types in the retina of a diurnal primate species as homologous to a pathway that appears similar in all other respects to one that carries signals from a single cone type in a monochromatic nocturnal species? We would argue that if this similarity extends to other defining features of the pathway and extends to several distantly related

species the answer should be yes. What would be useful is to understand which particular neural characters at any level in the pathway are conservative. It is likely that answers lie in the ontogeny of these pathways given that early embryological stages are quite conservative across mammals. Unfortunately, since there are almost no studies available comparing the neural development of the visual system of different primate species, we are unlikely to be able to identify such ontological characters, although some clues can be obtained by making comparisons between available primate and nonprimate developmental data. An additional related problem is that it is not clear how modifications at one level of the visual system (e.g., the retina) affect the development of more central target structures and vice versa. For example, Kaskan *et al.* (2005) have argued that major changes in retinal ganglion cell number or shifts in the proportions of rods and cones do not result in major differences in the size of the primary visual cortex (V1) which, instead, appears to scale with overall brain size (see Visual Cortex: Evolution of Maps and Mapping). This result implies that the developmental programs for visual areas in the telencephalon and diencephalon (forebrain) are relatively independent (at least at the early stages) from changes that occur in the original out-pocketing of the forebrain, the retina. If this is the case, then using the retina as the starting point for investigating the evolution of parallel visual pathways may be the wrong approach. Careful examination of V1, however, indicates that there may be differences in relative laminar development across primates that appear to correlate with changes in the eye. Examination of the thalamus, especially the LGN, also indicates that relative laminar development varies in predictable ways with phylogeny and visual niche in primates (Figure 3). Thus, examination of detailed structure (not just gross size) may offer more insights concerning the evolution of brain parts (see Elston *et al.*, 2001).

We argue that, although the programs of neural development that establish peripheral tissues and each level of the neuraxis can differ, they are never evolutionarily divorced from each other if they are connected in the adult. After all, the entire machine needs to run reasonably well for the adult organism to survive and reproduce and this requires that connections be made appropriately. Changes at one level can never be completely divorced from changes at the next level. The latter also raises the issue of epigenetic effects. Clearly, there are a number of epigenetic mechanisms, including neural activity/experience and competition for growth factors, that must be used to match neuronal populations at different levels in large brains since the number of synapses far exceeds the number of genes available for individual specification by a large margin. For example, in humans there are about 15×10^8 synapses per mm^3 of neuropil (DeFelipe *et al.*, 1999) compared with $26–38 \times 10^3$ genes (Venter *et al.*, 2001). Still, these epigenetic mechanisms must have a genetic base and must be selected in order to ensure that brain areas wire correctly (Easter *et al.*, 1985).

Another big question that must be answered before we can even begin thinking about evolution of parallel visual pathways is the question of why these pathways arose in the first place. Parallel pathways likely arise in evolution in response to incompatibilities. A cell cannot have a large dendritic field that integrates information across many receptors and have a small dendritic field capable of discrete fine grain sampling from just one or two receptors. Such incompatibilities could also provide an evolutionary drive for parallel pathway specialization. Parallel pathways presumably also arise from the constraints on the speed of transmission, particularly in relatively large mammalian brains. It seems likely then that true parallel visual pathways originate from ganglion cells that are clearly distinct in a number of ways. As argued eloquently by Rowe and Stone (1980), dividing ganglion cells into different classes needs to be based upon a parametric approach using a variety of criteria, given that it is difficult to prove that any single characteristic defines an entire class. A true class of ganglion cells should also tile the retina without visuotopic holes, otherwise differences may simply reflect natural variation within a cell class. Presumably once the number of ganglion cell types can be established then the number of parallel pathways to the brain/LGN will be limited to that number, assuming that each ganglion cell class projects to its own unique set of cells. In the case of the LGN, the number of different ganglion cells that provide input has still not been established, but, as explained more fully below, one can make comparisons between species based upon examination of some of the established pathways. Similarly, at the level of the LGN and V1, a true visual pathway should show anatomical segregation in terms of connections even if specific functional signatures cannot be traced from level to level. Beyond the first synapse in V1, however, it appears that a separate set of parallel pathways is established that links V1 to extrastriate areas (Casagrande and Kaas, 1994). The degree to which the geniculocortical pathways are actually linked directly to the pathways leading to extrastriate areas is a matter of debate given that most signatures of early pathways disappear at the level of V1

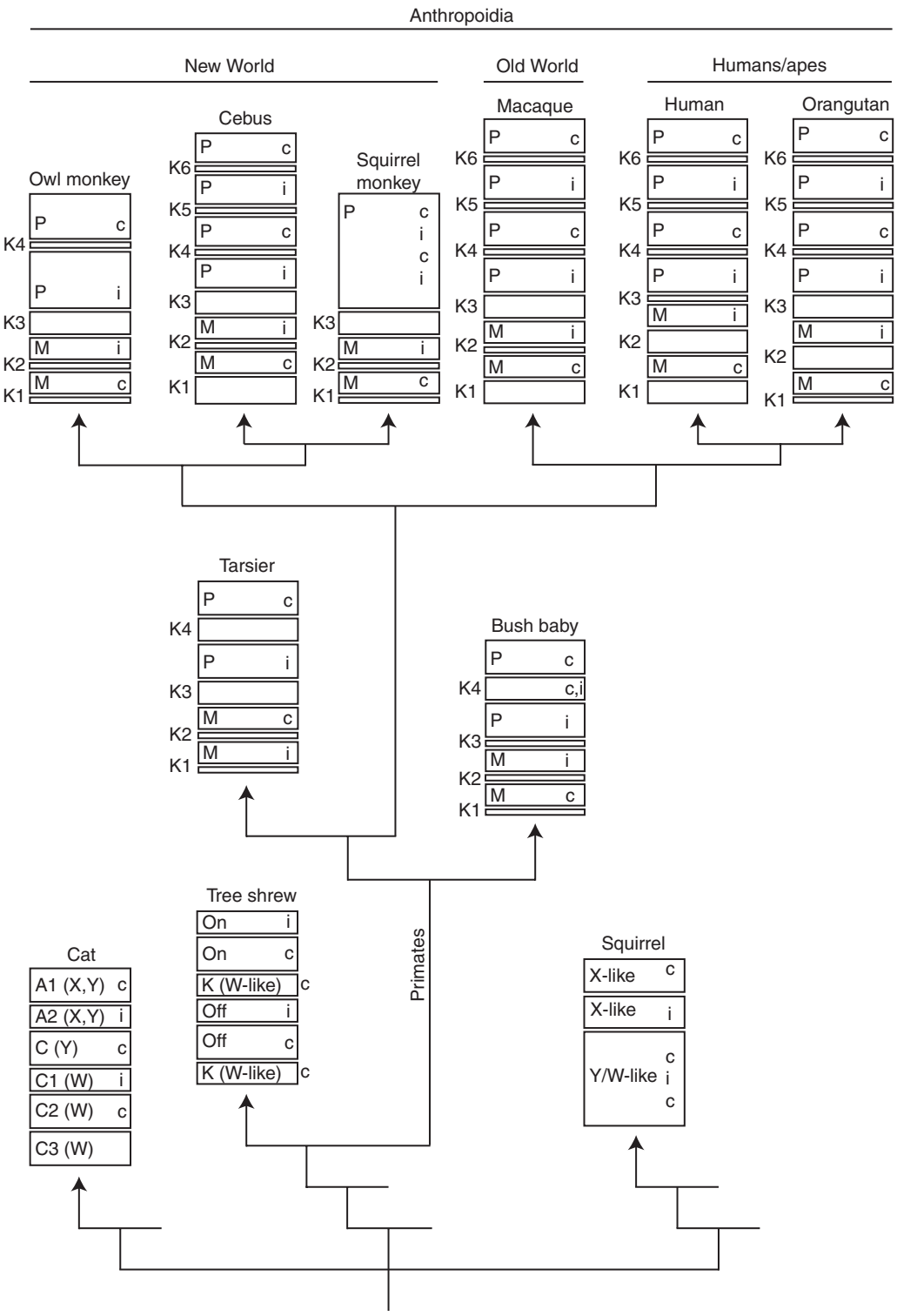


Figure 3 Laminar organization of the LGN in primates, tree shrews, squirrels, and cats. LGN cell layers for each species are indicated in boxes. The phylogenetic relationships between mammalian species are indicated by arrows, with the top branches indicating the relationships between primates. Only a few examples are shown. Note that in all primates the LGN is organized in a similar manner with two P layer and two M layers. In some primates (e.g., macaque monkeys), the P layers can split into four layers in a portion of the nucleus, and in others (squirrel monkeys) P cells exist as a cell mass where layers exist only based upon separate input from each eye. Tree shrews (Scandentia) are the closest living relatives of primates but have a very different LGN laminar organization as do squirrels (Rodentia) and cats (Carnivora). See text for details. c, contralateral retinal input; i, ipsilateral retinal input; K, koniocellular; M, magnocellular; P, parvocellular. Numerals refer to different K layers.

(Merigan and Maunsell, 1993; Casagrande and Xu, 2004). Nevertheless, similarities in the output of V1 to other cortical areas and their connections with each other allow us to ask whether similar hierarchies of visual areas are established across various primate species. As discussed in more detail below, it appears that V1 projects to the same areas in a range of primates (Casagrande and Kaas, 1994) but that, beyond V1, the evidence from connections, lesions, and behavior studies can support the idea that two major hierarchies of visual areas existed in a common ancestor of primates only in the broadest sense.

4.05.3 The Evolution of P and M Pathways

In almost all mammalian species so far examined, retinal and LGN cells can be physiologically classified into those that appear to convey information about higher spatial frequencies and respond in a more sustained manner, those that appear to respond better to higher temporal frequencies in a more transient manner, and those with slowly conducting axons and heterogeneous response properties (Stone, 1983; Lennie, 1993; Casagrande and Xu, 2004). In primate LGN, these classes correspond to P, M, and K neurons, respectively. In this section, we focus on the P and M pathways; the K pathway will be dealt with more fully in Section 4.05.4. Here, we consider the competing hypotheses that the P and M pathways (1) were present early in mammalian evolution and are thus homologous with similar pathways in nonprimates (e.g., X and Y cells in cats), (2) appeared early in primate evolution and their similarities with other mammalian species thus represent examples of parallel evolution, or (3) evolved independently in different primate lineages. Evidence for or against these hypotheses is sought from comparisons of response properties, anatomical organization, and neurochemistry in the retinogeniculocortical pathways of New World and Old World primates, cats, tree shrews, and rodents. Most of the nonprimate data come from cats, as their visual systems have been the most thoroughly studied of all nonprimate mammals.

In all primates, the M pathway originates from large retinal ganglion cells (parasol cells) which project to the M layers of the LGN, whereas the P pathway originates from smaller retinal ganglion cells (midget cells), which project to the P layers of the LGN (Figure 3). M cells in the retina and M LGN have larger receptive fields, lower preferred spatial frequencies, higher preferred temporal frequencies, and higher contrast sensitivities than their P counterparts. A similar dichotomy is found between Y and X cells in the cat retina and LGN (Table 1). Although X and Y cells in cats were first distinguished on the basis of a single criterion, linearity of spatial summation, the X versus Y classification was found to correspond to a host of other characteristics, and it is this extended sense of X and Y that is used here (Norton and Casagrande, 1982). Indeed, when W cells were described in cats, it was found that some were linear and some nonlinear, yet they were clearly a separate population based on the extended criteria that define X and Y cells (Table 1). Although it has been proposed that M and P cells are homologous to Y and X cells, respectively, an alternative hypothesis is that cat X and Y cells correspond to the linear and nonlinear subgroups of M cells, respectively, and that the P pathway is primate-specific (Kaplan and Benardete, 2001). X and Y cells, however, differ in many morphological and physiological characteristics in a similar way to M and P cells, while it is not clear that the linear and nonlinear M cells differ in characteristics other than linearity (see, however, Kaplan, 2004). It should be noted that linearity arises from a special mechanism that is added to the linear center surround mechanism present in all

Table 1 Comparison of primate M and P cells with cat X and Y cells

Attribute	*Primate M cells*	*Cat Y cells*	*Primate P cells*	*Cat X cells*
Cell size	Large	Large	Small	Small
Conduction velocity	Fast	Fast	Slow	Slow
Response dynamics	Transient	Transient	Sustained	Sustained
Spatial resolution	Lower	Lower	Higher	Higher
Temporal resolution	Higher	Higher	Lower	Lower
Contrast sensitivity	Higher	Higher	Lower	Lower
V1 projection	Upper tier of layer 4	Upper tier of layer 4	Lower tier of layer 4	Lower tier of layer 4
Linearity of spatial summation	Most linear, some nonlinear	Nonlinear	Linear	Linear
Chromatic opponency	No	No	Yes (in trichromatic primates)	No

retinal ganglion cells, so linearity of certain cell classes could be gained or lost in evolution without compromising other physiological properties.

Another physiological property that differs between primates and cats is color selectivity in that P cells have chromatic opponency, whereas X cells do not. However, this difference is affected by the fact that cats are dichromats, adapted for a nocturnal existence. As discussed more fully in the following sections, long-wavelength cones were gained (or, more likely, regained) independently in New World and Old World primates. The P cells of some dichromatic (or even monochromatic in the case of galagos and owl monkeys) primates also lack color opponency for similar reasons as cat X cells, yet they have all the other characteristics of P cells in trichromatic primates. Thus, P cells in all primate species should be considered homologous, regardless of color selectivity, because such differences can be explained by changes in single photopigment genes. By the same reasoning, lack of color opponency should not be used as evidence against homology of cat X cells and primate P cells (see Evolution of Color Vision and Visual Pigments in Invertebrates).

Although fewer data are available, distinct physiological classes, possibly corresponding to P and M pathways, have been found in other species. In gray squirrels, P-like cells with longer latencies, sustained firing, and linear spatial summation could be distinguished from M-like cells with short latencies, transient responses, and linear or nonlinear summation (Van Hooser *et al.*, 2003). In tree shrews, although linear and nonlinear cells have been found (Sherman *et al.*, 1975), it appears that the nonlinear cells are more like K or W cells than M cells, and that nonlinear M cells are lacking (Holdefer and Norton, 1995). A clear dichotomy between transient and sustained responses is found in the tree shrew, however (Sherman *et al.*, 1975; Lu and Petry, 2003).

Within the LGN, M and P cells are segregated into different layers. The standard primate laminar pattern consists of four layers: two M layers adjacent to the optic tract, followed internally by two P layers (Casagrande and Norton, 1991; Kaas, 2004). Each of these layers receives input from one hemiretina, with the first M layer receiving crossed (nasal hemiretina) input and the second M layer receiving uncrossed (temporal hemiretina) input. The P layer closest to the second M layer also receives an uncrossed retinal input, while the most internal P layer receives a crossed retinal input. The K layers, discussed in more detail below, lie mainly between or ventral to each of the P and M layers (Casagrande, 1994; Hendry and Reid, 2000; Casagrande and Xu, 2004). In some primates, the two P layers can split into four layers, but this occurs for only a topographically limited portion of the nucleus. For example, in macaque monkeys, four P layers can be identified only within the part of the nucleus representing about 2–3° to 17° of eccentricity (Malpeli *et al.*, 1996). In some humans, P layers split into as many as eight layers in some parts of the nucleus, but in other humans only two P layers exist across the whole extent of the LGN (Hickey and Guillery, 1979). In some primates, portions of the ipsilaterally innervated M layer can split off and form an extra layer next to the optic tract within a portion of the nucleus (Casagrande and Joseph, 1980). The latter is the standard condition for M layers in the tarsier, where it has been suggested that the ipsilaterally and contralaterally innervated M layers are reversed (Rosa *et al.*, 1996). Finally, in many New World primates (e.g., squirrel monkeys), P cells exist as an unlaminated cell mass where layers can only be defined based upon segregated input from the axons from the two eyes (Tigges and O'Steen, 1974; Fitzpatrick *et al.*, 1983). All of these differences, however, can easily be recognized as modifications of the basic primate laminar pattern (Figure 3).

In most nonprimate mammals with well-developed visual systems, three main subdivisions of the LGN can be recognized, progressing internally to externally (i.e., toward the optic tract): (1) a main contralateral layer receiving X- and Y-type input, (2) a main ipsilateral layer receiving X- and Y-type input, and (3) an outermost layer comprising sublayers receiving various combinations of contralateral Y-type input and ipsilateral and contralateral W-type input. The LGN of the cat, for example, consists of paired layers A and A1 receiving mixed X and Y inputs from the contralateral and ipsilateral eyes respectively, a magnocellular C layer receiving contralateral Y cell input, and several small-celled layers receiving either contralateral or ipsilateral W cell input. The LGN of sheep and other ungulates has a similar organization (Karamanlidis and Magras, 1972; Ebinger, 1975; Karamanlidis *et al.*, 1979; Clarke *et al.*, 1988). Additionally, carnivores and ungulates possess a medial interlaminar nucleus (MIN) which receives Y and W input. Layers A and A1 are subdivided into sublayers receiving input from either ON-center or OFF-center retinal ganglion cells in such mustelid carnivores as ferrets and mink (LeVay and McConnell, 1982; Stryker and Zahs, 1983). In squirrels, contralateral layer 1 and ipsilateral layer 2 receive X- and Y-like input (referred to by some as P-like and M-like; see above), while Y-like input is found in layers 1, 2, and especially 3, and W-like input is confined to layer 3 (Kaas *et al.*, 1972; Van Hooser *et al.*, 2003).

The primate LGN thus differs from the standard mammalian plan in having complete, not partial, segregation of different cell classes. As previously pointed out (Boyd and Matsubara, 1996; Matsubara and Boyd, 2002), a simple scenario for transitioning to the primate organization involves the coalescing of ipsilateral Y cells ventrally in layer A into a separate layer. The resulting lamination pattern would have the same contra-M, ipsi-M, ipsi-P, contra-P organization as seen in primates.

Interestingly, the tree shrew, which is considered phylogenetically closer to primates than the groups considered above, has a unique LGN organization which is unlike that in primates or other mammals. The tree shrew has a six-layered LGN with two layers containing W-like cells, and the remaining four layers segregated by both eye input and contrast sign (ON center vs. OFF center). Projections from sustained and transient retinal ganglion cells do not appear to segregate into different LGN layers in the tree shrew. The tree shrew visual system thus appears to have many derived characteristics that arose independently of those in primates (Rager, 1991; Kaas, 2002).

Another criterion that has been used to determine homology in the LGN is neurochemical content. M cells (but not P cells) in the LGN of primates and Y cells (but not X cells) in the LGN of cats are selectively labeled by antibodies against a cell surface antigen, Cat-301 (Hockfield and Mckay, 1983; Hockfield *et al.*, 1983; Hendry *et al.*, 1988), or against nonphosphorylated neurofilaments (Chaudhuri *et al.*, 1996; Bickford *et al.*, 1998). These molecular markers thus support the hypothesis of homologies between LGN cell classes in different mammalian lines.

Finally, the geniculocortical projections of the different classes of relay cells provide evidence for homology between different groups. In all primates, M cells project to the upper portion of layer IV, P cells project to the lower portion of layer IV, and K cells project above layer IV (Casagrande and Norton, 1991). In cats, the laminar segregation between X and Y cells is similar (though likely not as absolute) with X-cell terminations concentrated in lower layer IV and Y cell terminations concentrated in upper layer IV. W cells project outside of layer 4 (see Section 4.05.4 on K pathway for further discussion). In both cats and primates, the simple laminar dichotomy between X and Y cells is likely to be complicated by subclasses of X and Y cells and M and P cells. For example, the Y cells in layer C have larger receptive fields, higher contrast sensitivity, and more pronounced nonlinearities than A-layer Y cells (Frascella and Lehmkuhle, 1984; Yeh *et al.*, 2003). Their terminations are confined to the topmost third of layer IV and, moreover, selectively target cytochrome oxidase (CO) blob columns (Boyd and Matsubara, 1996). It has been argued that a similarly defined subclass of M cells exists in primates (Hawken *et al.*, 1988; Bauer *et al.*, 1999), although the evidence for this is not as conclusive.

The sublaminar organization of geniculocortical organization in other animals is not as well described as for cats and primates, but it can be noted that the layer 3 complex in squirrels, which contains Y-like and W-like cells, projects to the upper part of layer 4 and supragranularly, and these two projections likely come from Y-like and W-like cells, respectively (Weber *et al.*, 1977; Harting and Huerta, 1983). Tree shrews have a very different geniculocortical arrangement, whereby terminations from ON-center and OFF-center cells segregate within different sublamina of layer 4 (Fitzpatrick and Raczkowski, 1990). The W-like LGN layers, however, still terminate outside of layer 4.

The data reviewed here strongly support the hypothesis that the precursors to M and P cells were present in the earliest primates, so M and P cells in all primates are homologous. Moreover, the similarities in organization of the M and P pathways in primates and similar pathways in some other mammals provide some support for the hypothesis that the M versus P dichotomy arose prior to the divergence of primates from other mammals, with the unique differences found in tree shrews representing a derived condition, not primitive characteristics representative of early primates.

4.05.4 Is the K Pathway Evolutionarily Old?

In Section 4.05.3, we focused on the parallel M and P pathways connecting the retina with V1; in this section, we focus on a third parallel pathway, currently referred to as the koniocellular, or K, pathway (for reviews see Casagrande, 1994; Hendry and Reid, 2000; Casagrande and Xu, 2004). As for the M and P pathways, the K pathway consists of a distinct class (or classes, as K cells are heterogeneous) of retinal ganglion cells that project to distinct groups of cells in the LGN, which are in turn connected to distinct layers of V1. The K pathway has a constellation of features that distinguish it from the M and P pathways and that have led some to suggest that the K pathway is phylogenetically older than the M and P pathways. In this section, we review this hypothesis, while at the same time

reviewing the data for homologues of the K pathway in other mammalian species, particularly the W pathway in the cat, the nonprimate for which the greatest amount of data on the visual system is available.

The cat W pathway was relatively well studied years before the primate K pathway was closely examined (Stone, 1983), and indeed even before the extent and importance of the K pathway in primates was widely acknowledged. There are a large number of similarities between K and W pathways. At the level of the retina, both cat W and primate K retinal ganglion cells have small cell bodies, thin but extensive dendrites, and the thinnest most slowly conducting axons in the optic tract (Casagrande and Norton, 1991). There is evidence for a similar class of retinal ganglion cells in other mammals as well, including rats, rabbits, and tree shrews.

The geniculate projections of both K and W retinal ganglion cells are to small-celled layers that are either next to the optic tract or intercalated between the main layers. Neurochemically, these small-celled layers have been identified using antibodies to the calcium-binding protein calbindin. In prosimian bush babies, and both New World and Old World simians including owl monkeys, marmosets, and macaque monkeys, calbindin is found in K layers, but not in M or P layers of the LGN (Johnson and Casagrande, 1995; Hendry and Reid, 2000; White *et al.*, 2001; Xu *et al.*, 2001). Calbindin also labels cells in the tree shrew LGN exclusively in the layers that contain W-like cells, layers 3 and 6 (Diamond *et al.*, 1993). In the cat, although W-cell layers in the LGN contain calbindin, many GABAergic (γ-aminobutryric acid, GABA) interneurons in the LGN also contain calbindin (Demeulemeester *et al.*, 1991), obscuring a possible relationship between the W-cell pathway and calbindin content.

K cells in the LGN differ in their relative laminar development in different primate lines (Hendry and Casagrande, 1996). The K pathway also appears to be physiologically and anatomically more heterogeneous than either the P or M pathways (for review, see Casagrande and Xu, 2004). For example, K cells lying ventral to the M layers in K layer 1 project mainly to layers IIIA and I of primate V1, and can be distinguished physiologically from K cells that lie close to the P layers and send axons to the CO blobs located in layer IIIBα of V1. Some K cells carry S-cone input although most K cells do not, at least in marmosets (White *et al.*, 2001). Some K cells defined by calbindin antibody labeling appear to project exclusively to the middle temporal visual area (MT) in macaque monkeys (Stepniewska *et al.*, 1999; Sincich *et al.*, 2004). This means that subdivisions of the K pathway could have been lost or added in different primate lines (Ding and Casagrande, 1998; Shostak *et al.*, 2002). In cats, W cells have similar projections: to layer 1 and to the CO blobs in layer III of V1 (Boyd and Matsubara, 1996), and to extrastriate cortex (Kawano, 1998). It is not yet clear if these different structures are targeted by different classes of W cells, or by collaterals of the same cells.

The K-cell pathway and the W-cell pathway are also similar in that they have close interconnections with the superior colliculus. Some retinal ganglion cells of the K and W classes project to the colliculus, and the colliculus makes projections to the K- and W-cell layers of primate and cat LGN. In tree shrews as well, there is a projection from the colliculus to LGN layers 3 and 6. Because the colliculus is considered by some to be phylogenically older than the LGN, being homologous with the main target of retinal axons in nonmammalian vertebrates, the optic tectum, it has been suggested that the K/W pathway is phylogenetically older than the M/X and P/Y pathways (Bishop, 1959). Other features of the K/W pathway, such as finer axons with more diffuse projections, have also been suggested to be primitive conditions. Ultimately, the question of pathway evolutionary age is extremely difficult to answer since we have no good biological markers of relative age specific for visual pathways. If anything, the K pathway in primates shows more morphological and physiological variation than the P or M pathways, so could be considered biologically (perhaps evolutionarily) less stable.

In summary, there is strong evidence from anatomy to support the conclusion that K cells in all primates are homologous and that at least some K cells have homologues in other mammals: (1) both K and W cells receive midbrain input from the parabigeminal nucleus and superior colliculus; (2) some W-like and K cells always lie adjacent to the optic tract in a large variety of mammals (Harting *et al.*, 1991); (3) K and W LGN cells send axons that terminate above layer IV in V1; (4) K cells are more likely than other LGN cell classes to project to extrastriate areas outside of V1; (5) K and W cells tend to be slowly conducting and have smaller cell bodies on average; and (6) K LGN cells in all primates and tree shrews (and perhaps W cells in cats) contain calbindin. There is also a variety of physiologically defined similarities between these cell classes, although the overlap in response properties between all relay cell classes in the LGNs of mammals and the influence of lifestyle on spatial and temporal thresholds make it difficult to make useful comparisons.

4.05.5 Color Vision in Primates and the Evolution of P and K Pathways

The ability to see color derives from the ability to compare wavelengths. Such color opponency is constructed at the retina from cones sensitive to short (S; e.g., blue), medium (M; e.g., green), and long (L; e.g., red) wavelengths by creating receptive fields with ON responses to one wavelength and OFF responses to an opposing wavelength or wavelengths. Thus, S cones oppose the M plus L cones to create a blue/yellow color axis, M opposes L to create a green/red axis and all three cones oppose each other to create an achromatic OFF/ON black/white axis. This simple view is complicated by the facts that some primates have only a single cone type and are therefore presumably color blind, most primates are dichromatic possessing two cone types, and some primates (such as humans) are trichomatic (Jacobs, 1996, 1998). Trichromacy, however, appears to have evolved separately in different primate lines (Jacobs, 1996; see The Comparative Biology of Photopigments and Color Vision in Primates). A number of articles have been written about the evolution of color vision in primates as well as the genetics of color vision (Jacobs, 1996, 1998; Nathans, 1999; Tan and Li, 1999; Dacey and Packer, 2003). A commonly held belief is that primates evolved from a nocturnal ancestor. Support for this argument has been recently reviewed (Ross, 2000) and will not be considered in detail here except where relevant to parallel pathway evolution. Relevant to the current article are proposals concerning which parallel pathways carry chromatic signals and what this might tell us about the evolution of parallel pathways in primates. At least four types of ganglion cells carrying cone signals have been identified in macaque monkeys. Of these, three carry signals from S cones, small and large bistratified ganglion cells carrying blue ON signals and large monostratified ganglion cells carrying blue OFF signals. It has been proposed that these blue pathways project to LGN K cells given that some K cells have been identified at the level of the LGN to carry S cone signals in macaque monkeys and marmosets (White *et al.*, 1998). In marmosets, approximately 20% of K cells carry S cone signals based upon studies in which immunocytochemistry for calbindin was used to directly identify K cells at the level of the LGN after single unit recording (White *et al.*, 1998). In addition, it has been argued by many that midget ganglion cells in several primates carry L/M opponent signals to the P LGN layers (see Dacey and Packer, 2003; but see, however, Calkins and Sterling, 1996). At present, it is unclear if some P cells also carry S cone signals, as was originally proposed by Wiesel and Hubel (1966), given that K cells, defined by either calbindin or CamKII immunocytochemistry, can lie below each P and M layer, can be found scattered within these layers, or can even form bridges of cells that pass directly through the P layers (Johnson and Casagrande, 1995; Hendry and Casagrande, 1996; Hendry and Calkins, 1998). Definitive data linking particular ganglion cell classes whose chromatic signature is well defined to particular visual pathways that project through the LGN to cortex is still lacking.

Evidence that does exist suggests the following. In all primates examined it has been demonstrated that K cells in the LGN defined by immunocytochemistry or laminar location send axons above layer IV (IVC of Brodmann) of V1 (Lachica and Casagrande, 1992; Ding and Casagrande, 1998). These K axons terminate in the CO blobs of V1, in cortical layer I and probably also in cortical layer IIIBβ (IVA of Brodmann) (Yazar *et al.*, 2004). With the exception of projections to layer IIIBβ this pattern of axonal projections can be demonstrated in prosimians as well as in New World and Old World simians, apes, and humans. Since, as discussed earlier, some prosimians such as the bush baby and at least one simian, the owl monkey, have only a single cone type and lack S cones entirely (Jacobs, 2002), it could be argued that the K pathway evolved before the evolution of color vision in primates. This would have to be the case if the prosimian bush baby represents the ancestral original nocturnal condition of primates.

An alternative proposal is that ancestral primates were actually dichromatic (Tan and Li, 1999). The evidence to support this view is as follows. First, S cones are considered to be of ancient origin genetically and are present in many mammalian groups, including carnivores, ungulates, and primates (Calkins, 2001). More important is the fact that both prosimian bush babies and simian owl monkeys appear to have the gene for S cones but this gene is not expressed in either species due to defects in the gene (Jacobs, 2002). The presence of the gene strongly suggests that functional S cones existed in their ancestors. Support for this view comes from studies that have examined for the S opsin gene in a relative of the bush baby, the slow loris (*Nycticebus coucang*) (Kawamura and Kubotera, 2004) and found evidence to support the view that this gene was disrupted in the common ancestor of galagiods (e.g., bush babies) and lorisids. Second, S and M cones are both present in at least one nocturnal prosimian, the mouse lemur (*M. murinus*), as well as in several diurnal lemurs and in the tarsier (Dkhissi-Benyahya *et al.*, 2001). Third, S and M

cones have been identified in cat W cells (Wilson *et al.*, 1976). Cat W cells also project to the CO blobs in V1 (area 17) just as K cells do in primates. Cat W cells also share many other characteristics in common with primate K cells as reviewed above and earlier (Casagrande and Norton, 1991). Taken together, these data support the view that the common ancestor of primates may actually have been diurnal with S and M cone signals passing to a population of K cells. Since all LGN cells receive input from cones, this does not inform us about the evolution of color vision relative to the parallel pathways. If this hypothesis is correct, it does predict that, as in cats, S cone signals should be confined to K cells in those prosimians that have functional S cones, a hypothesis that could be tested directly by examining for S cone input to LGN K cells in the mouse lemur. It also predicts that in dichromatic lemurs (perhaps all dichromatic primates) wavelength discrimination would depend upon K cells since P and M cells would only receive from a single M cone.

The issue of whether some primate ancestors were trichromatic is more complicated given the different ways prosimians, Old World primates, and New World primates construct color vision. All Old World primates have two separate opsin genes (M and L) on the X chromosome in addition to the single S autosomal gene. New World simians, and prosimian lorises, and lemurs have only one opsin gene on the X chromosome, but polymorphism of this gene allows females with different versions of the opsin gene on each of their two X chromosomes to achieve trichromacy. Males, with only one X chromosome, can never be trichromats in species that rely on polymorphism. Tan and Li (1999) have argued that the phylogenetic distribution of the M and L opsins across strepsirhine primates (lemurs, lorisids, and tarsiers) supports the idea that the X-linked polymorphism and primate trichromacy arose early in primate evolution. It is interesting that, regardless of whether trichromacy is achieved via polymorphism of a single gene on the X chromosome as in New World simians or on two separate genes on the same chromosome as in Old World simians, it would appear that the M/L (green/red) opponency can be identified electrophysiologically in some P cells in both cases but not so far in K cells (White *et al.*, 1998). This would support the view that M cone input to P cells via midget ganglion cells was the default condition in dichromatic primates with L cone opponency added later. Whether the P pathway further specializes when trichromacy is the norm as in Old World simians, one branch of New World primates, as well as apes and humans, remains unclear (Jacobs, 2002).

One aspect of the chromatic pathway to V1 that appears to show species-specific differences concerns the S cone input to V1 layer IIIBβ (IVA of Brodmann). Callaway and colleagues (Chatterjee and Callaway, 2003) have shown that, in macaque monkeys, cells in layer IIIBβ respond to S cone input in the form of blue ON- and blue OFF-center cells. Since thalamic axons project to layer IIIBβ in many diurnal simians but not in the nocturnal owl monkey, the prosimian bush baby, or in some apes (chimpanzee), or in humans, this pathway appears to be a specialization of some primates and not others (Preuss and Coleman, 2002). These findings indicate that apes and humans may have diverged from a primate ancestor in which the K pathway carrying S cone input did not innervate layer IIIBβ. In macaque monkeys, there is no physiological evidence for a direct S cone input via the thalamus to the CO blobs based upon recording from LGN axons in V1 where cell responses were silenced with the $GABA_A$-receptor agonist muscimol (Chatterjee and Callaway, 2003). Presumably, S cone input reaches cortex via another pathway in apes and humans. Taken together, these observations support the hypothesis that components of the K pathway may either have been lost in the evolution of apes and humans or that their common ancestor showed a parallel pathway organization more like that of present-day prosimians where the thalamus does not project to layer IIIBβ.

4.05.6 Ocular Dominance and Other Properties

At the level of the LGN in primates, retinal ganglion cells within the left and right eyes send input to separate layers. Additionally, ganglion cells with either ON-center or OFF-center responses innervate separate sets of cells at the level of the LGN. These parallel pathway features from retina to LGN appear to generalize across placental mammals. At the level of V1, however, the degree to which these properties remain segregated at the first synapse varies widely among mammals (see Casagrande and Norton, 1991, for review). For example, although close relatives of primates (e.g., tree shrews) show both ocular segregation and segregation of ON- and OFF-center responses to separate cortical layers, primates do not. Instead ON- and OFF-center responses appear combined at the first synapse in all primates examined to date, even

Table 2 Ocular dominance columns in striate cortex

Columns present	*Columns present*	*Columns absent*
Macaque (Hubel and Wiesel, 1969)	Talapoin monkey (Florence and Kaas, 1992)	Rat (Hubel and Wiesel, 1977)
Human (Hitchcock and Hickey, 1980; Horton and Hedley-Whyte, 1984)	Capuchin monkey (Hess and Edwards, 1987; Rosa *et al.*, 1988)	Mouse (Drager, 1974)
Owl monkey (Rowe *et al.*, 1978; Diamond *et al.*, 1985)	White-faced saki (Florence and Kaas, 1992)	Tree shrew (Casagrande and Harting, 1975; Hubel, 1975)
Marmoset (DeBruyn and Casagrande, 1981; Spatz, 1989)	Chimpanzee (Tigges and Tigges, 1979)	Gray squirrel (Weber *et al.*, 1977)
Green vervet (Hendrickson *et al.*, 1978)	Cat (Shatz *et al.*, 1977)	Brushtailed possum (Sanderson *et al.*, 1980)
Red monkey (Hendrickson *et al.*, 1978)	Ferret (Law *et al.*, 1988)	Rabbit (Hollander and Halbig, 1980)
Baboon (Hendrickson *et al.*, 1978)	Mink (McConnell and LeVay, 1986)	Sheep (Pettigrew *et al.*, 1984)
Spider monkey (Florence *et al.*, 1986)	Bush baby (Glendenning *et al.*, 1976; Hubel and Wiesel, 1977; Diamond *et al.*, 1985)	Goat (Pettigrew *et al.*, 1984)

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though ON- and OFF-center cells have been reported by some to be segregated to separate layers in the macaque LGN (Schiller and Colby, 1983). The finding that ferrets, but not cats, show segregation of ON and OFF pathways through the LGN to V1, suggests that parallel ON and OFF pathways that extend to cortex evolved several times in different mammalian lines of descent (Zahs and Stryker, 1988). The advantage of maintaining separability of ON and OFF pathways to cortex in diurnal tree shrews and nocturnal ferrets remains unclear given that these species have very different lifestyles and evolutionary histories.

Similarly, although eye input remains segregated at the first cortical synapse in cortex in tree shrews and many other mammals including some primates, the variability in both the pattern and degree of segregation of ocular inputs suggests that the organization of ocular dominance pathways from LGN to cortex evolved independently in primates and other mammalian species. In tree shrews, left and right eye input to cortex is segregated into sublayers within layer IV of V1 (Casagrande and Harting, 1975), whereas in all primates ocular input segregation (if present) occurs in the form of columns not layers. Among primates, examples of well-developed cortical ocular dominance columns can be found in some members of a number of distantly related groups including prosimian bush babies, New World simian spider monkeys, and all Old World simians and apes thus far examined, including humans (Florence *et al.*, 1986; Florence and Kaas, 1992; Preuss and Coleman, 2002; see Table 2). Even in primates in which ocular dominance columns show high interindividual variability, such as New World squirrel monkeys, or show very weak segregation, as in New World owl monkeys and marmosets, segregation occurs in the form of columns and not in the form of layers as in tree shrews (Florence *et al.*, 1986). These findings suggest that the tendency to segregate ocular information into columnar dominance columns in V1 was present already in the common ancestor of primates but not in the ancestor of tree shrews and primates.

Examination of the different patterns of ocular dominance columns in different primate species, however, indicates that well-developed ocular dominance columns either evolved several times in different lines of descent or regressed in different lines of descent from a well-developed pattern (Florence *et al.*, 1986; Florence and Kaas, 1992). Distinguishing between these different scenarios is difficult given that we do not understand the functional significance of ocular dominance columns since they appear to occur in both small nocturnal primates with no color vision and in large diurnal primates with good color vision, and appear variable across simians (Florence *et al.*, 1986). It may also be the case that such segregation is simply a byproduct of the degree of synchrony between active ganglion cells in the two eyes during a critical phase of development, especially since the expression pattern shows such a high degree of interindividual variation in squirrel monkeys (Horton and Hocking, 1996).

4.05.7 The Evolution of Dorsal and Ventral Cortical Streams

It has been proposed that there are basically two cortical streams for processing visual information

in primates – a ventral stream to the temporal lobe and a dorsal stream to the parietal lobe – the first being involved with object vision and the second with spatial vision or vision for action (Mishkin *et al.*, 1983). The dorsal and ventral streams both start with the intracortical circuitry in V1, which processes the three main classes of LGN inputs described earlier (M, P, and K) to create multiple distinct outputs originating from separate classes of projection neurons in cortical layer III and projecting to several different extrastriate areas. Two hierarchical chains of connections, one to the temporal lobe and one to the parietal lobe (albeit with some connections between areas and compartments belonging to the different streams), can be traced through the multiple (more than 30) extrastriate areas found in primates (DeYoe *et al.*, 1994).

The ventral stream, in order, consists of layer IIIBα blobs and layer IIIBα interblobs in V1, thin stripes and interstripes in the secondary visual cortex (V2), DL/V4, and various inferotemporal areas. The temporal areas at the top of this hierarchy are physically close to and interconnected with perirhinal cortex and hippocampus, structures involved with object recognition and encoding visual memories. The dorsal stream consists of layer IIIC and layer IIIBα interblobs, which give rise, respectively, to a direct and an indirect pathway via V2 to V5/MT, CO thick strips in V2, MT/V5, and surrounding superior temporal areas, and, finally, parietal cortex. These parietal areas are close to, and interconnected with, premotor cortical areas involved with programming eye movements and other visually guided behaviors. In this section, we examine the evolution of these processing streams. We will first consider the early stages of processing through V1 and V2, and then the later stages through specialized extrastriate areas.

To review, in primates, M LGN afferents terminate in upper layer IV, P afferents in lower layer IV, and K afferents in the blobs in layer IIIBα and in layer I. As mentioned in previous sections, these pathways likely have homologues in other mammals, so the building blocks for the two streams will at least be homologous structures. Immediately above the M input layer is found a population of projection neurons that are an important early part of the dorsal pathway, receiving M input and projecting directly to V5/MT. These cells are found in all primates, where they may be pyramidal (prosimians) or stellate (Old World monkeys) or both (New World monkeys). In cats, large pyramidal cells at the base of layer III receive Y-cell input and project to lateral suprasylvian cortex (Matsubara and Boyd, 2002), which is, like V5/MT, an area that processes motion (see below). These projections are probably homologous. In cats, prosimians, and New World primates, these projections are robust, and are concentrated directly below CO blobs. In macaque monkeys, there are far fewer MT-projecting cells in V1 and it has been debated as to whether or not these are concentrated beneath CO blobs (Boyd and Casagrande, 1999; Boyd and Matsubara, 1999; Sincich and Horton, 2003). This could represent a gradual evolutionary reduction of the fast direct pathway to V5/MT in primate evolution, and perhaps of the entire dorsal stream, as increased emphasis is placed on slower indirect pathways passing through upper layer III, which is proportionately thicker and more differentiated in primates (especially simians) then in other mammals. This scenerio suggests a change in visual processing, with more emphasis on analysis of visual form and less emphasis on reaction to movement.

The indirect dorsal stream through V2 originates from neurons in layer IIIBα interblobs that probably receive both M and P input via intralaminar projections from layer IV. The neurons of the ventral stream to V2 are in layer IIIBα blobs and interblobs, and so receive K input in addition to M and P. In species with color vision, the K input may carry color information (see previous sections), an important cue for object recognition but not for visuomotor tasks. Within V2, recent evidence points to the existence of four stripe compartments, two which stain darkly for CO (the CO thick and thin stripes) and receive input from dorsal and ventral stream neurons in V1, and two interstripe compartments that may also receive from both streams but definitely get input from ventral stream neurons in V1 (Xu *et al.*, 2004). In some prosimians, CO stripes are faint or absent in V2, but there still is evidence that projections from blobs and interblobs are segregated into different compartments within V2, so the striped architecture likely is homologous across all primates. There is no clear example yet of a similar striped architecture (with or without accompanying CO staining) in nonprimates. There is some evidence for segregation of blob and interblob projections to extrastriate areas in the cat, but this occurs not in V2, but in area 19. The cortical hierarchy in primates continues through V2 into V5/MT for the dorsal stream, and into V4/DL for the ventral stream. Again, although the prosimian galago does not have distinct CO stripes or functional compartments with low orientation selectivity as in simians (Xu *et al.*, 2005), neurons projecting from V2 to V5/MT and to V4/DL form interdigitated stripes (Krubitzer and Kaas, 1986), showing that the underlying architecture (albeit perhaps less complex) is the same in prosimian V2 as for other primates.

Thus, the earliest levels of the dorsal and ventral streams can be recognized in all primates. Can the same be said of the higher levels? This is an important question because an increase in neocortex size and an increase in the number of sulci and gyri have occurred independently in the evolution of different mammalian lines, and even in the evolution of different primate lines. Both Old World primates and sheep, for example, have large, gyrencephalic brains, but examination of fossil endocasts suggests that their last common ancestor had a small lissencephalic brain (Radinsky, 1967, 1975, 1981). Not surprisingly, sheep and primate neocortex, while superficially similar, show important differences, as, for example, the relative development of the temporal lobe, which is proportionately less prominent in sheep brains than in primate brains, and the olfactory cortex, which in sheep is proportionately enormous by primate standards. Also, the obvious occipital development and landmarks that characterize the primate visual cortex such as the calcarine fissue are not obvious in sheep (nor in other nonprimate mammals) in spite of the fact that other fissures are well developed and the sheep brain is larger and more fissured than many primate brains. In summary, primitive mammals had small brains and likely possessed only a few cortical areas for each sensory modality, perhaps only V1 and V2 for vision (Northcutt and Kaas, 1995). The number of extrastriate visual areas has increased independently in different mammalian lines, so it might be impossible to define homologies across mammalian groups for many extrastriate areas.

Even within the primate lineage, the patterns of sulci and gyri vary between New World and Old World monkeys, apes, and prosimians, and brain size has increased independently in these lines. It is therefore important to determine which of the multitude of visual areas can be unambiguously identified in all primates and are thus likely to be homologous. Homologies among visual areas in different primate lines are recognized on the criteria of size, shape, and position in the cortex with respect to other cortical areas, layout of the visual field map, physiological response properties, patterns of connections with other cortical areas and subcortical structures, and cortical architecture. For example, the V1 can be recognized, not just in primates but also in all mammals, by its position in the occipital lobe, by receiving strong projections from the LGN, by the complete map of the visual field it contains, and by its distinctive histological architecture.

In all primates (and likely all mammals), V2 forms a narrow strip immediately lateral to V1. In addition to its position, it can be recognized by its visual field organization, sharing a representation of the vertical meridian with V1, and by its distinctive mosaic pattern of connections with V1, which are related to the CO architecture (Casagrande and Kaas, 1994). In all primates, an important dorsal stream area, called MT (sometimes referred to as V5) in Old World primates, New World primates and prosimians, occupies a densely myelinated oval-shaped area in the dorsal temporal lobe. This area contains many motion-sensitive neurons, most selective for the direction of stimulus motion. MT/V5 is also identified by its distinctive patterns of projections from V1 and V2, and by its projections to parietal cortex. In all primates, an important ventral stream area, called V4 in Old World primates and DL in New World primates and prosimians, occupies cortex caudal to V5/MT and receives inputs from compartments in V2 not projecting to V5/MT. The homology, however, of this region is less well established, perhaps due to uncertainties in the extent and possible subdivisions of this region of cortex, as it does not have a distinctive architecture, and its visual field map is not as regular as that of MT. Proposed homologies of primate cortical areas higher in the hierarchy are even more tenuous, for similar reasons. It is possible that more homologies will become apparent when the cortical organization of different primates becomes better understood. (This presupposes that regions of cortex outside of primary areas and certain easily identifiable areas such as V5/MT are, in fact, best described as collections of discrete areas with sharply defined borders, and not as larger fields of loosely graded response properties and connections.) With presently available information, then, only areas on the lower levels of the visual-processing hierarchy can be homologized across different primate species, suggesting that areas higher in the hierarchy were added independently in different primate lines. Even so, the dorsal and ventral streams in different primate lineages can be identified without concomitantly identifying homologues for all of the visual areas involved.

Is it possible to identify dorsal and ventral streams in other mammals, given that so few extrastriate areas are likely to be homologous between primates and other mammals? As suggested above, processing in the dorsal and ventral streams prepares visual information for the ultimate use by motor cortex and limbic cortex, respectively, structures that are likely homologous in all mammals. Even if the primitive mammalian visual system consisted of a single area, V1 (although V2 at least was likely also present in the earliest mammals), separate

dorsal and ventral streams could still exist, consisting of separate populations of V1 neurons projecting directly to motor and limbic cortex, respectively. As was suggested to be the case for different primate groups, extra areas could be inserted to form processing hierarchies independently in different mammalian lineages. Inserting areas between V1 and limbic cortex will route the ventral stream through the temporal lobe based on simple proximity to the hippocampus. Similarly, inserting areas between V1 and motor cortex will result in a dorsal stream through parietal cortex.

There is evidence for dorsal and ventral streams in mammalian lineages as different as carnivores and rodents, both of which have multiple extrastriate visual areas that are unlikely to be homologous with any primate areas. The cat has about 15 different extrastriate areas and, as a model species, has the rare advantage that many of these areas have been extensively investigated (Payne, 1993). Evidence for dorsal and ventral streams in cats comes from studies of connections, physiological response properties, and behavioral deficits. Similar to V5/MT in primates, an area in the lateral suprasylvian (LS) sulcus of the cat receives a direct input from V1, projects to parietal and visuomotor areas, and displays motion selectivity. Inactivating this area leads to visual orienting and motion processing deficits (Lomber, 2001), as would be expected from a dorsal stream area. The cat also possesses a temporal visual stream consisting of multiple areas progressing through the temporal lobe to the hippocampus. As would be expected for the ventral stream, inactivation of the temporal lobe areas does not impair visual orienting behavior (Lomber, 2001).

The similarities between V5/MT and LS cortex are strong enough that it has been proposed that these areas are homologous (Payne, 1993). If V5/MT was present in the last common ancestor of cats and primates (more than 65 Mya), one would expect it to also be present in all mammalian lines that share a common ancestor with either cats or primates that is more recent than their last common ancestor (Northcutt and Kaas, 1995). Current mammalian classifications place primates in the superorder Euarchontoglires along with Glires (rodents and rabbits), flying lemurs, and tree shrews. As carnivores, cats are members of the superorder Laurasiatheria, which also includes insectivores, bats, ungulates, and whales (Madsen *et al.*, 2001; Murphy *et al.*, 2001; Waddell *et al.*, 2001; Amrine-Madsen *et al.*, 2003). Thus, if V5/MT and LS are homologous, a similar area should be identifiable in other members of these two superorders; such identifications are currently hampered by lack of data from relevant species.

For Euarchontoglires, at least partial data on extrastriate cortical organization are available from tree shrews and some rodents. Tree shrews have a series of visual areas adjoining V2, one of which, the temporal dorsal area (TD), has been proposed as a possible homologue for MT. Like MT, TD contains a complete representation of the visual field (Sesma *et al.*, 1984), stains more strongly than surrounding cortex for myelin and the Cat-301 antibody (Jain *et al.*, 1994), and receives inputs from V1 (Lyon *et al.*, 1998). However, TD in tree shrews is adjacent to V2, unlike MT, which is separated from V2 by DL/V4, and TD appears to lack connections with visuomotor areas of frontal cortex (Lyon *et al.*, 1998), which is part of the connectional signature of MT in at least some primates (Krubitzer and Kaas, 1990). No data on the detailed response properties in TD are currently available, so it is not yet known if this area contains direction-selective neurons.

The organization of extrastriate visual cortex in rodents is not completely clear, and appears to show substantial species variability (Rosa and Krubitzer, 1999). Germane to the present discussion is that rodents are thought to be monophyletic, and that mice and rats share a more recent common ancestor than either do with squirrels (Reyes *et al.*, 2004). In squirrels, V2 forms the lateral border of V1, with at least two tiers of multiple extrastriate areas lateral to it (Kaas *et al.*, 1972, 1989). In the rat, microelectrode mapping studies suggest that V1 is bordered laterally, not by a single area V2, but by multiple small retinotopically defined extrastriate visual areas named topographically (rostromedial, anterolateral, lateromedial, posterolateral, etc.) and corresponding to regions free of callosal connections (Espinoza *et al.*, 1992; Montero, 1993). Injections of tracers in different retinotopic locations in V1 lead to changes in the location of patches of label within these extrastriate areas that is consistent with the electrophysiological maps (Coogan and Burkhalter, 1993; Montero, 1993), mitigating against the argument that these projections correspond to multiple modules within a traditional retinotopically mapped V2 which, similar to other mammalian groups, extends along the entire lateral border of V1 (Malach, 1989). In mouse, microelectrode mapping shows a single V2 bordering V1 laterally, with at least one other area lateral to that. However, corticocortical projections from mouse V1 had a similar pattern as in the rat (Olavarria and Montero, 1989), suggesting that multiple visual areas adjacent to V1 were common at least to mice and rats. In order to resolve the

differences in cortical organization between different rodent species, it has been assumed that the largest of the areas bordering V1 laterally in rats (the lateromedial area, LM) is homologous to V2 in other species (Rosa and Krubitzer, 1999). According to this hypothesis, either new areas adjoining V1 were added in the mouse/rat lineage, or regressive events caused more lateral visual areas (perhaps homologous to the lateral visual areas in squirrels) to be shifted toward V1, at the expense of V2. A recent optical imaging study of mouse visual cortex (Kalatsky and Stryker, 2003), however, not only found evidence for multiple retinotopically defined extrastriate areas, but also suggested a narrow V2 with only a central visual field representation; detailed optical imaging maps of rat extrastriate cortex have not yet been published. The many patches following a V1 injection, and the tendency of visual fields to be congruent across borders, means that V1 projections to a narrow V2 could be continuous with a patch of labeling in an adjacent area, and thus overlooked in the anatomical mapping studies. The coarse sampling of microelectrode mapping, combined with the large receptive fields, may also have made it possible to have missed a narrow V2. Projections from V1 need to be combined with functional mapping and histological verification of the extent of V1 to determine if there really is a narrow V2 interposed between V1 and the lateral extrastriate areas in rats and mice.

Returning to the original question of functional streams, areas responding preferentially to moving stimuli can be found in both squirrels and mice/rats. In rats, the anterolateral area (AL) appears to have cells selective for movement (Montero and Jian, 1995), while, in mice, AL and another area (LM) bordering V1 laterally give rise to different connectional streams, AL preferentially connecting with dorsal and medial regions of cortex, LM with ventral regions of cortex (Wang and Burkhalter, 2004). In ground squirrels, an area (ML) with large receptive fields and direction-selective cells was found lateral to V2 (Paolini and Sereno, 1998), and thus in the right position to be homologous with MT. Both AL in rats and mice and LM in squirrels receive direct projections from V1, which is another similarity with V5/MT, although neither area appears to have the extensive myelination, an anatomical signature of V5/MT.

On the cat (Laurasiatheria) side, there is even less evidence from which to draw conclusions. It does appear that LS cortex, at least, has homologues in fellow carnivores, the mustelid ferrets (Manger *et al.*, 2002). Another laurasiatherian animal whose extrastriate cortex has been mapped is the megachirpopteran flying fox (*Pteropus*). Although once thought to be more closely related to primates than to michrochipoteran bats, all bats are now thought to comprise a single group within the Laurasiatheria (Van Den Bussche *et al.*, 2002). The occipitotemporal visual area (OT) was proposed as a possible megachiropteran homologue to LS/V5/MT based on its location lateral from V2, and its receptive field organization (Rosa, 1999). Microbats, relying on echolocation for navigation, have an enlarged auditory cortex, and very little extrastriate visual cortex. If this is a primitive condition for bats, it would mitigate against any proposed homologies of megachiropteran visual areas, given that bats are likely monophyletic. It is also possible that extrastriate cortex may have been reduced during microbat evolution.

In conclusion, specialized extrastriate areas belonging to dorsal and ventral cortical streams can be recognized in a wide range of mammals. Only the earliest stages of these streams and the last stages in motor and limbic cortex are likely to be homologous across mammalian lines, however. Even within primates, only a few areas can be unequivocally identified as homologues. Different lineages have added areas to the middle levels of these cortical streams independently. The constraint of proximity of the inserted areas to limbic cortex or motor cortex keeps the temporal stream temporal and the dorsal stream dorsal.

4.05.8 Conclusions, Questions, and Future Strategies

What can we usefully conclude about the evolution of parallel pathways in primates? We need to constantly remind ourselves that without specific definitions of what we are comparing and at what level (genes, molecules, cells, or pathways), we cannot develop definite or testable hypotheses. In this article we have focused on pathways originating with distinct classes of retinal ganglion cells and asked whether homologues of these visual pathways can be found across different primate species or between primates and nonprimates. We hypothesized that examining for similarities across distantly related species is the most important initial step in arguing for homology given the lack of genetic and fossil signatures of visual pathways. Nevertheless, we remain cognizant of the fact that different regions of the nervous system (e.g., retina, thalamus, and cortex) have different patterns of gene expression controlling their cellular composition and distribution. Therefore, we cannot simultaneously address the issue of homology at different levels of comparison (i.e., proteins, cells,

pathways, or brain regions). It is even more difficult to determine if similarities result from homology or homoplasy given that the developmental programs that establish visual cells and pathways are conservative and presumably have a restricted set of viable functional solutions for species to survive using the visible portion of the energy spectrum here on earth. Therefore, a useful future approach would be to compare the ontogeny (both early and late) of distantly related primates (e.g., a prosimian with a New World simian and an Old World simian) and primates and nonprimates (e.g., macaque monkey with rodent) examining for similarities at both the genetic and systems levels. A fuller understanding of commonalities in the ontogeny of different species would aid enormously in examining for homology in visual pathways.

Our examination of P, M, and K pathways leads to the hypothesis that these pathways are homologous across primates in spite of vast differences in the lifestyles and retinal organization in different primate species. It is also likely that what we call the P, M, and K pathways have general counterparts in other mammals since cats certainly appear to have pathways that specialize in spatial versus temporal resolution (i.e., X vs. Y cells) in a similar way to P and M cells in primates; W cells also resemble K cells anatomically and physiologically. Nevertheless, details of these pathways in nonprimates (even close relatives like the tree shrew) differ significantly; so significant changes have occurred independently in the P, M, and K pathways of different lineages.

We have also argued that the K pathway may be made up of more than one pathway so its evolutionary history is more difficult to try to define. Nevertheless, it does appear that cells in this pathway across a range of species can be recognized by the presence of calbindin. Other similarities to W cells in cats and other mammals suggest that a K-like pathway may have originated early in mammalian evolution. This does not necessarily make the primate K-cell pathway phylogenically older or newer than the P and M pathways since the K pathway shows enormous variability in the relative numbers of cells present in different LGN layers (identified neurochemically) across different primate species. What would be useful to know is which ganglion cells actually project to K layers in different primates and in close primate relatives such as tree shrews. For example, do bistratified ganglion cells project uniquely to K layers in tree shrews as would be predicted from work in macaque monkeys? This easily tested question would reinforce the view that some K cells evolved prior to the split between tree shrews and primates. Examining the same issue in cat W cells would extend the evolution of this component of the K pathway to other mammals.

A closely related issue concerns the evolution of chromatic pathways in different primates. Since some K cells receive input from S cones in some New World (marmosets) and Old World (macaque monkeys) primates, and K cells carrying S cone signals project to cortical layer IIIBβ in macaque monkeys, it will be important to understand how S cone signals are transmitted to V1 in primates such as apes and humans that lack an LGN projection to cortical layer IIIBβ. Such information could potentially inform us about the evolutionary split between monkeys and apes. Similarly, it would be informative to know if tarsiers or any diurnal lemurs that have functional S cones send these signals via K cells to cortical layer IIIBβ.

We have argued that, since nocturnal prosimians, such as bush babies, have the S cone gene (even though it is not functional) in addition to functional M cones, and that other prosimians (and tarsiers) also have both M and S cones, it is likely that earliest ancestors of primates were dichromatic like present-day tree shrews. If all nocturnal prosimians, however, show the same defect in the S cone gene, this would argue in favor of a nocturnal bottleneck. Alternatively, if distantly related nocturnal primates, such as galagos and owl monkeys, show that S cone genes were disabled in different ways, this would argue that the lack of functional S cones evolved secondarily when species moved from a diurnal to a nocturnal niche.

We reviewed also the evidence that segregation of ON and OFF pathways and segregation of left and right eye inputs (ocular dominance columns) evolved independently in different lines. ON and OFF pathways are combined at the first level in all primates examined, and the tendency to segregate ocular inputs into columns, although variable across primate species, exists in distantly related primates. These observations support the presence of at least weak ocular dominance segregation into columns in the common ancestor of primates and support the view that the ON and OFF pathways were not segregated to columns or layers in a primate ancestor. Why ocular dominance columns exist in primates remains a mystery. Given the high inter-animal variability of ocular dominance columns in squirrel monkeys, it might be useful to examine both the genetics and visual experience of animals that do with those that do not appear to show clear columns. It would also be useful to examine for ocular segregation in a wider range of primates.

Finally, we examined the most difficult issue, namely the evolution of dorsal and ventral cortical pathways originating in V1. Given that there is

disagreement even about the definitions of cortical areas that receive input from V1, we cannot provide solid conclusions about the homologies of dorsal and ventral streams beyond the statement that there is evidence for sets of projections to similar hierarchies of areas in all primates thus far examined. There is also evidence that the general cortical design for such streams may exist in nonprimate mammals even if specific cortical areas within each hierarchy are not homologous. Clearly, much more evidence concerning the number of visual areas in a range of primates and other mammals will need to be examined before more definitive statements can be made. Perhaps with the advent of high-resolution functional magnetic resonance imaging we will be in a position to more rapidly map visual areas in a variety of species.

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4.06 Organization and Correspondence of the Auditory Cortex of Humans and Nonhuman Primates

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Glossary

architectonic features	Refer to the anatomical features that comprise brain tissue. These features typically vary among distinct areas of the cerebral cortex. Specific architectonic features include the number, arrangement, and types of neurons in a particular area of cortex. Other features include the differential expression of proteins or density of myelinated axons within an area. Comparison of architectonic profiles derived from multiple architectonic features facilitates identification of individual areas, as well as the grouping of areas with similar architectonic profiles into regions.
auditory cortex	Refers specifically to those areas of the cerebral cortex for which the primary sources of thalamic input are the principal nuclei of the medial geniculate complex.
auditory-related cortex	Refers specifically to those areas of the cerebral cortex for which the primary source of auditory input is the auditory cortex, with relatively little, if any, input from the principal nuclei of the medial geniculate complex.
cortical area	A subdivision of cerebral cortex defined by a unique set of anatomical and physiological features. An area may be considered a functional module with unique patterns of connections with other cortical areas.
cortical region	A group of cortical areas that share a defined set of features. In auditory cortex, for example, the core region includes three areas that occupy the first stage of auditory cortical processing, as defined by their patterns of connections with the medial geniculate complex and other areas of cortex.

4.06.1 Introduction

The identification and characterization of areas that contribute to auditory processing in the mammalian cerebral cortex has been the subject of sporadic investigation for over 130 years. The first insights were derived from lesion studies and detailed descriptions of anatomical features during the late 1800s and early 1900s (Beck, 1928, 1929; Broca, 1865; Brodmann, 1905, 1909; Clark,1936; Ferrier, 1875; Poljak, 1932; von Bonin, 1938; von Economo and Horn, 1930; von Economo and Koskinas, 1925; Walker, 1937; Wernicke, 1874). These studies, which often provided detailed parcellations of the superior temporal region, comprise the classical descriptions of human and nonhuman primate auditory cortex and remain influential to this day. In the mid-1900s, electrophysiological studies confirmed the location of auditory-responsive cortex on the superior temporal plane of monkeys and chimpanzees, and produced evidence that a representation of the basilar membrane was preserved in the organization of the primary auditory region (Ades and Felder, 1942; Bailey *et al.*, 1943; Celesia and Puletti, 1969; Gross *et al.*, 1967; Hind *et al.*, 1958; Katsuki *et al.*, 1962; Licklider and Kryter, 1942; Pribram *et al.*, 1954). These landmark studies were accompanied by others in which the connections of the superior temporal region were explored (Nauta, 1957; Pandya *et al.*, 1969; van Buren and Yakovlev, 1959). Interest in the organization of primate auditory cortex increased during the 1970s, marked by studies that related patterns of thalamic and cortical connections to architectonic subdivisions and tonotopic maps (Imig *et al.*, 1977; Merzenich and Brugge, 1973; Mesulam and Pandya, 1973; Pandya and Sanides, 1973; Sanides, 1975). Studies of the auditory cortex of nonhuman

primates and humans since that time have been characterized by stepwise refinements of earlier findings, culminating in a working model of the primate auditory cortex that is the subject of ongoing testing and modification (Kaas and Hackett, 1998, 2000; Kaas *et al.*, 1999). The development of the primate model has paralleled that of other mammalian models, yet the extent to which findings from one species can be generalized to another remains uncertain. The number of auditory areas identified varies between species, and presently only the primary auditory area, A1, is considered to be homologous. Additional homologies are likely, and may eventually be established on the bases of relative location, receptive field organization, and other anatomical and physiological features. The extension of findings from research animals to humans is especially problematic because experimental constraints greatly limit direct comparisons. Nevertheless, the model of auditory cortex established in nonhuman primates shares a number of key features with humans, and therefore provides a useful foundation for comparative study that will improve our understanding of audition in primates (Hackett, 2002).

4.06.2 Gross Anatomical Features of the Superior Temporal Lobe

Allometric measurements of surface area and volume indicate that the superior temporal gyrus (STG) increases in size successively by a factor of nearly three times each between squirrel monkeys, macaque monkeys, chimpanzees, and humans (Rilling and Seligman, 2002). The threefold expansion of the STG is proportional to overall temporal lobe expansion for all of these primates, except humans, where temporal lobe volume is about four times that of the chimpanzee (Figure 1). This appears to reflect greater expansion of temporal fields beyond the STG in humans.

In all primates, the auditory cortex is located on the dorsal surface of the temporal lobe where it occupies a large portion of the STG and lower bank of the lateral sulcus (LS). The relative size, location, and orientation of the auditory fields varies between species, reflecting differences in the morphology of the temporal lobe (Sanides, 1975). The length and depth of the LS increases with brain size, as does the prominence of the circular sulcus and insular region (Figure 1). In prosimians (e.g., lemur, galago), there are no major sulci on the lateral surface of the brain other than the LS; therefore, there is no obvious division between the superior and inferior temporal regions. In some New World monkeys (e.g., marmoset), a shallow superior temporal sulcus (STS) extends for a short distance, partly dividing the temporal lobe into superior and inferior gyri. STS depth in the marmoset ranges from 2.5 mm to little more than a shallow depression (de la Mothe *et al.*, 2006). In other New World monkeys (e.g., squirrel monkey), the STS is deeper and longer, clearly demarcating the superior and inferior gyri. In Old World macaque monkeys and great apes, the STS completely divides the temporal lobe into superior and inferior gyri, except at its rostral pole. Caudally, the LS and STS merge in the temporoparietal junction of these primates. In humans, gyrification of the ventral temporal lobe is more elaborate, but the STG remains bounded by the LS and STS.

4.06.3 Location of the Auditory Cortex in the Superior Temporal Lobe

'Auditory cortex' is defined as the array of cortical areas that receives its principal thalamic input from either the ventral (MGv) or dorsal (MGd) divisions of the medial geniculate complex (MGC) (see Shared Features of the Auditory System of Birds and Mammals, Specialization of the Neocortical Pyramidal Cell during Primate Evolution, Cerebral Cortical Folding Patterns in Primates: Why They Vary and What They Signify). Accordingly, the auditory cortex occupies a large portion of the ventral (lower) bank of the LS and STG in nonhuman primates (Figures 1 and 2). Cortical areas that receive inputs from auditory cortex, but not the MGv or MGd, are referred to as 'auditory related'. These areas are located in the temporal pole, and portions of the STS, intraparietal sulcus, and prefrontal cortex. The precise boundaries of the auditory cortex are not entirely known for any primate species, but are most certain in marmoset, owl, and macaque monkeys. In these primates, the medial boundary lies within the LS, where auditory areas adjoin the insula (rostrally) and parietal operculum (caudally). The caudal boundary is located near the junction of the LS and STS, bordering the temporal parietotemporal (Tpt) area, an auditory-related multisensory area that occupies the caudal terminus of the STG (de la Mothe *et al.*, 2006; Galaburda and Pandya, 1983; Hackett *et al.*, 1998; Leinonen *et al.*, 1980; Pandya and Sanides, 1973). The rostral boundary has not been defined with certainty, but thalamic and cortical connections indicate that auditory cortex does not extend all the way to the temporal pole (Galaburda and

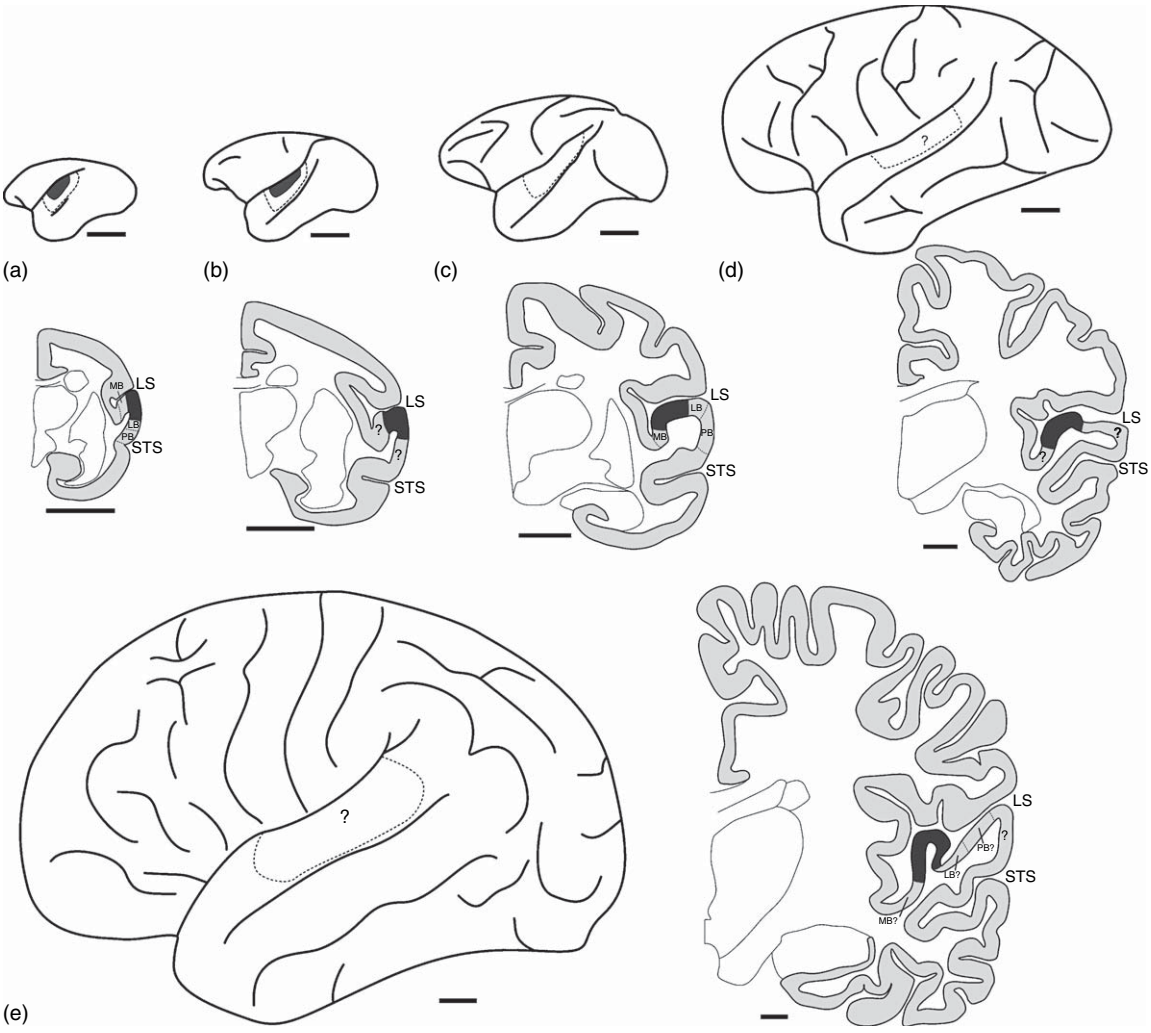

Figure 1 Schematic drawings of the cerebral cortex and location of auditory cortex in several primates. A lateral view of the left hemisphere and a coronal section through auditory cortex is illustrated in each panel. a, Marmoset monkey (*Callithrix jacchus jacchus*); b, squirrel monkey (*Saimiri sciureus*); c, macaque monkey (*Macaca mulatta*); d, chimpanzee (*Pan troglodytes*); e, Human (*Homo sapiens*). Dark shading, core region; MB, medial belt region; LB, lateral belt region; PB, parabelt region; ?, region not defined. Scale bars: coronal sections, 5mm; lateral views, 10mm.

Pandya, 1983; Hackett *et al.*, 1998; Kosmal *et al.*, 1997; Pandya and Sanides, 1973). The ventral (lateral) boundary has not been defined, but the connections of the STG and STS suggest that auditory cortex covers most of the STG, but does not extend far onto the dorsal (upper) bank of the STS (de la Mothe *et al.*, 2006; Hackett *et al.*, 1998).

Classic and modern studies of the human temporal lobe have localized the auditory cortex to a region encompassing the posterior STG, transverse temporal gyri (TTG) of Heschl (HG), and the planum temporale (Hackett, 2002). This corresponds generally to areas 41, 42, 52, and 22 of Brodmann (1909). Compared to monkeys, however, the precise location and extent of auditory cortex in great apes and humans is much less certain, because the thalamic or cortical connections of individual areas cannot be determined experimentally. Therefore, areas must be identified from other anatomical or functional characteristics. This is problematic for several reasons. First, auditory areas cannot be distinguished from auditory-related or nonauditory fields purely on the basis of architectonic features (e.g., cytoarchitecture, myeloarchitecture, chemoarchitecture), as there is no distinctive set of traits that defines a cortical area as auditory. Second, functional imaging and noninvasive electrophysiological techniques currently lack either the spatial resolution or functional specificity to distinguish one area of auditory cortex from another. In general, auditory stimulation activates numerous auditory and auditory-related cortical areas within and beyond the superior temporal cortex (Hall *et al.*, 2003; Scott and Johnsrude, 2003). The sources of this

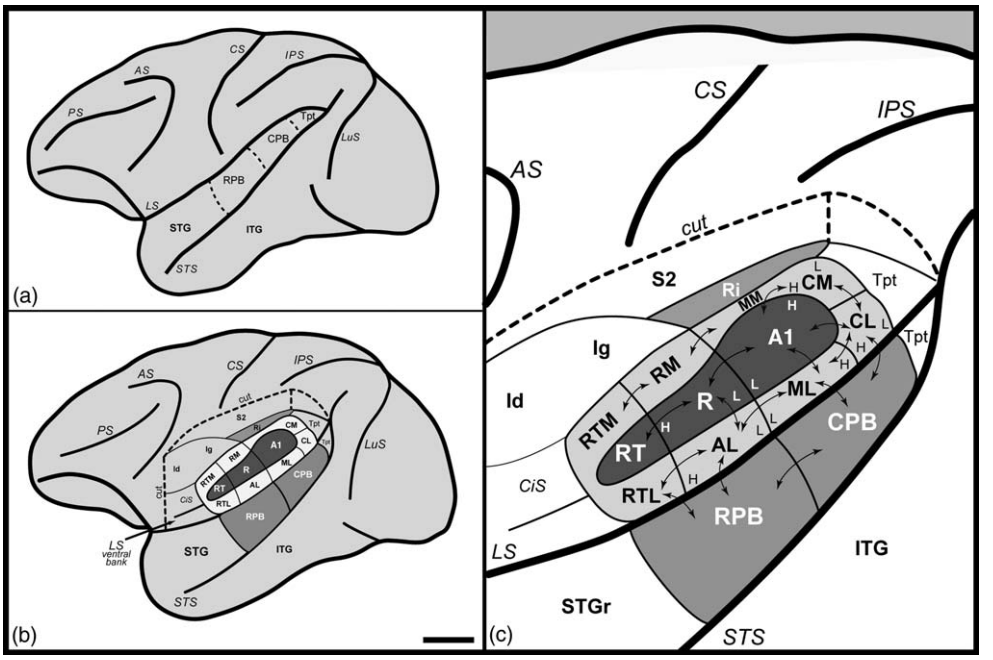

Figure 2 Schematic diagram of primate auditory cortex model illustrated for the macaque monkey. a, Lateral view of the left hemisphere showing locations of the rostral (RPB) and caudal (CPB) parabelt areas on the surface of the superior temporal gyrus (STG); b, the lateral sulcus of the left hemisphere was graphically opened (cut) to reveal the locations of auditory cortical areas on the lower bank of the lateral sulcus (LS); c, expanded view of auditory cortex, showing major connections between areas (arrows). The circular sulcus (CiS) has been flattened to show the rostromedial (RM) and rostrotemporal medial (RTM) areas that occupy its lateral wall. The upper bank of the LS was partly opened to show the locations of areas adjoining auditory cortex: the retroinsular area (Ri) in the fundus, second somatosensory area (S2) on the upper bank, granular insula (Ig), and dysgranular insula (Id). The three areas that comprise the core region of auditory cortex (dark shading) are located on the lower bank (A1, auditory area 1; R, rostral; RT, rostrotemporal). The core is surrounded by eight areas that belong to the belt region (light shading) (CM, caudomedial; CL, caudolateral; MM, middle medial; ML, middle lateral; RM, rostromedial; AL, anterolateral; RTM, rostrotemporal medial; RTL, rostrotemporal lateral). The core and belt regions are mostly contained within the LS. On the surface of the STG are the two areas that make up the parabelt region (medium shading) (RPB and CPB). The rostral part of the STG (STGr) extends to the temporal pole. The temporal parietotemporal (Tpt) area occupies the caudal end of the STG and extends onto the supratemporal plane within the LS. Tonotopic gradients within areas are indicated by H (high frequency) and L (low frequency). Other sulci and gyri shown include the arcuate sulcus (AS), central sulcus (CS), intraparietal sulcus (IPS), superior temporal sulcus (STS), lunate sulcus (LuS), and inferior temporal gyrus (ITG). Scale bar: 10 mm.

activity are largely indistinguishable, because the 'physiological signatures' of the auditory areas involved are not known. These uncertainties highlight the need for studies directed at the identification and characterization of auditory areas in the human brain. To some extent, this can be achieved through comparative studies involving nonhuman primates, for which the organization of the auditory cortex is more certain. As reviewed below, there is both direct and indirect evidence that major features of monkey auditory cortex organization are conserved in humans.

4.06.4 Organization of the Auditory Cortex of Monkeys

Models of auditory cortex organization in mammalian species other than humans are the subject of ongoing testing and refinement. Currently, most of this work is being accomplished in bats, cats, ferrets, mice, rats, New World monkeys, and Old World monkeys and apes. A common theme across models is that a central primary, or 'core region', containing one or more areas, is adjoined by a variable number of secondary areas comprising a 'belt region' (Figure 2). The core and belt regions are strongly interconnected, but the main source of thalamic inputs to the core areas is the MGv, whereas the MGd is the principal source of inputs to the belt areas. Species differ with respect to the number of areas present, their relative position and arrangement, connections (input/output), and tonotopic organization. For example, the number of areas identified ranges from 5 to 6 in rodents, 6 to 9 for cats and ferrets, 10 to 12 in monkeys, and over 30 in humans. Thus, there is a tendency to identify more auditory areas in larger brains. In nonhuman primates, the core–belt scheme has been extended to

include a third region, known as the 'parabelt region' (de la Mothe *et al.*, 2006; Hackett *et al.*, 1998; Morel *et al.*, 1993; Morel and Kaas, 1992). The parabelt region receives thalamic inputs from the MGd, but is distinguished from the belt region in that it does not receive direct inputs from the core (Figure 2c) (Hackett *et al.*, 1998). Information reaching the parabelt region from the core is mediated by connections with the belt region. Accordingly, a processing hierarchy (core–belt–parabelt) is formed across these three regions (Kaas and Hackett, 1998).

Each of the three major regions of the monkey auditory cortex contains two or more areas, or subdivisions (Figure 2). In our working model, there are three subdivisions of the core (Al, R, and RT), seven areas within the belt (CM, RM, RTM, CL, ML, AL, and RTL), and two divisions of the parabelt (RPB and CPB). Each subdivision receives inputs from lower and higher stages of processing, and subdivisions within a region appear to process inputs from multiple sources in parallel. Within the core, for example, each subdivision receives parallel inputs from the MGv (not illustrated), and has reciprocal connections with more than one belt area. In the belt region, all subdivisions receive inputs from the MGd and have reciprocal connections with one or more subdivisions of the core and parabelt. Thus, the connections between regions and areas within regions indicate that both serial and parallel processing is accomplished within the auditory cortical network (Kaas and Hackett, 1998; Kaas *et al.*, 1999; Rauschecker, 1998; Rauschecker *et al.*, 1997).

The establishment of individual subdivisions depends on the identification of unique subsets of anatomical and physiological features. Confidence in the delineation of an area is increased when its profile is based on multiple anatomical and physiological criteria; thus, some areas are defined better than others. The greatest differences are found between areas located in different regions (e.g., core vs. belt). By contrast, areas within a region share several key features, and since adjacent areas tend to share more features than nonadjacent areas, they are the most difficult to delineate. Within the core, for example, areas AI and R have similar architecture and connections. At present, the most reliable distinction between them is receptive field topography. The frequency organization of the cochlea is represented in both A1 and R, but the tonotopic gradients run in opposite directions from a common low-frequency border (see Figure 2) (Aitkin *et al.*, 1986; Brugge, 1982; Cheung *et al.*, 2001; Imig *et al.*, 1977; Kosaki *et al.*, 1997; Luethke *et al.*, 1989; Merzenich and Brugge, 1973; Morel *et al.*, 1993; Morel and Kaas, 1992; Rauschecker *et al.*, 1995, 1997; Recanzone *et al.*, 2000; Tian *et al.*, 2001). A reversal in the tonotopic gradient also distinguishes areas R and RT within the core (Bendor and Wang, 2005; Morel *et al.*, 1993; Morel and Kaas, 1992). Microelectrode recordings have also revealed tonotopic gradients in the belt region, supporting the existence of subdivisions that were previously distinguished on the basis of anatomical features alone. In these experiments, pure tones and narrowband noise stimuli were used to demonstrate complementary tonotopic gradients among the lateral belt areas (AL, ML, CL) (Kosaki *et al.*, 1997; Rauschecker *et al.*, 1995). In areas AL and ML, the gradients match the adjacent core areas (A1, R), while the tonotopic gradients in CM and CL mirror that of AI (Kajikawa *et al.*, 2005; Recanzone, 2000). Thus, an independent representation of the cochlea, or absence thereof, is an important criterion in the establishment of auditory cortical areas, especially when anatomical features are similar.

4.06.5 Organization of the Auditory Cortex of Great Apes and Humans

Compared to monkeys, much less is known about the organization of the auditory cortex in the great apes and humans. Due to the absence of information about connections and near-field electrophysiology, extending findings from monkeys to these primates is primarily limited to descriptions of architectonic features and noninvasive neurophysiology. Detailed parcelations have been produced by multiple investigators spanning about 100 years (Hackett, 2002). Most of these were derived from the analyses of cytoarchitecture and/or myeloarchitecture (Beck, 1928, 1929; Brodmann, 1909; Campbell, 1905; Flechsig, 1920; Galaburda and Sanides, 1980; Hopf, 1954; Morosan *et al.*, 2001; Pandya and Sanides, 1973; Poljak, 1932; Rademacher *et al.*, 1993; Seldon, 1981a, 1981b, 1982; Vogt and Vogt, 1919; von Economo and Horn, 1930; von Economo and Koskinas, 1925). The most recent studies also used the distribution of various markers (e.g., acetylcholinesterase, cytochrome oxidase, parvalbumin, receptor autoradiography) to identify or characterize auditory-related cortical fields (Clarke and Rivier, 1998; Hackett *et al.*, 2001; Hutsler and Gazzaniga, 1996; Morosan *et al.*, 2005; Nakahara *et al.*, 2000; Ong and Garey, 1991; Rivier and Clarke, 1997; Sweet *et al.*, 2005; Wallace *et al.*, 2002). Despite substantial variations in conclusions

and nomenclature across studies, a common finding has been the identification of a central core region with primary, or primary-like, architectonic features surrounded by belts of several nonprimary fields (Figure 3). The correspondence between the core region of monkeys, apes, and humans is rather certain, whereas the homology of areas located beyond the core is not.

In monkeys, the core region is elongated along the anterior–posterior axis of the temporal lobe. In apes and humans, the core is mainly confined to the posteromedial two-thirds of the TTG, which is oriented from posteromedial to anterolateral across the superior temporal plane (Figure 3; Hackett, 2002; Hackett *et al.*, 2001). This region most closely corresponds to area 41 of Brodmann (1908, 1909), as well as to comparable territory identified by other investigators (Table 1). The distinctive cytoarchitecture of the core is commonly referred to as 'granulous' or 'koniocellular', named for the dense concentration of small cells in layers II and IV. Other cytoarchitectonic features include a conspicuous absence of large pyramidal cells in layer III, and a relatively sparse population of pyramidal cells in layer V. Myelin density is higher in the core, compared to most of the surrounding fields. The myelination pattern of the core is characterized by a matrix of small to large caliber fibers of such high density that the inner and outer striae of Baillarger in layers IV and Vb are difficult to resolve (Hackett *et al.*, 2001; Hopf, 1954; Pandya and Sanides, 1973; Sanides, 1972). Other studies have added that the expression of the acetylcholinesterase, cytochrome oxidase, and parvalbumin is greater in layers IIIc and IV of the core than in the belt or parabelt regions (Hackett *et al.*, 1998, 2001; Jones *et al.*, 1995; Kosaki *et al.*, 1997; Morel *et al.*, 1993; Morel and Kaas, 1992; Nakahara *et al.*, 2000; Rivier and Clarke, 1997; Sweet *et al.*, 2005; Wallace *et al.*, 2002). The consistency of this architectonic profile is such that the core can be easily identified in monkeys, apes, and humans. Thus, there is significant conservation of these features among these primates.

Despite the architectonic similarities, there is greater variability in the gross morphological features of the superior temporal cortex of apes and humans, compared to monkeys. In chimpanzees and humans, the number of TTG varies between individuals and sometimes between hemispheres (Hackett *et al.*, 2001; Leonard *et al.*, 1998; Rademacher *et al.*, 1993). The position of the core region varies relative to sulcal and gyral landmarks; therefore, precise localization depends on the detailed architectonic analyses of individual specimens. In humans, the most common configurations are a single or paired TTG, also referred to as a posterior duplication or bifid HG. In humans with a single HG, the core occupies most of the gyrus and usually does not extend beyond its anterior and posterior sulcal boundaries. When the HG is divided by an intermediate transverse sulcus (i.e., bifid HG), the core occupies portions of both gyri and spans the intermediate sulcus. Most chimpanzees have a single HG, but some lack a definitive HG. In these cases, the core is situated deep in the LS and elongated along the medial edge of the superior temporal

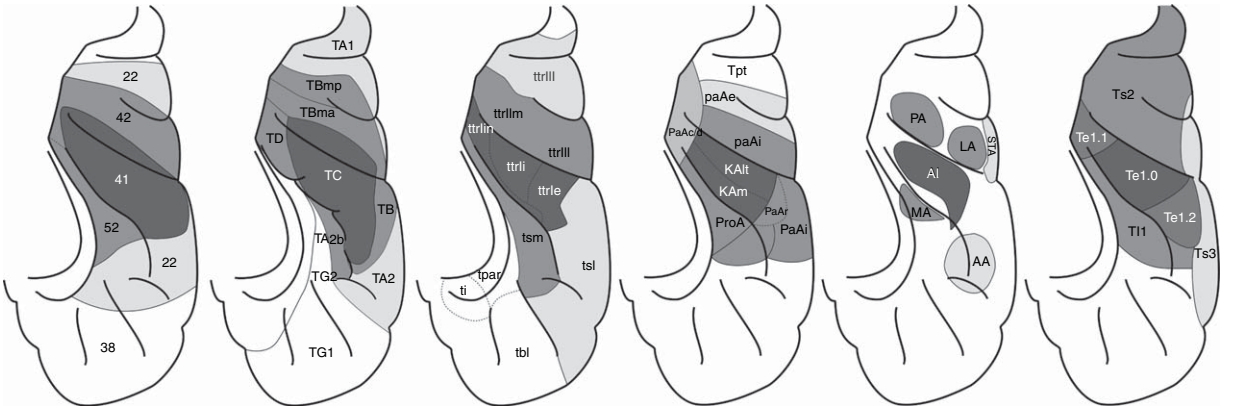

Figure 3 Parcelations of the human superior temporal cortex by different investigators. For each panel, the locations of major auditory cortical regions are drawn on a standardized schematic of the superior temporal plane. The STG is not visible. Dark shading, core region; medium shading, belt region; light shading, parabelt and possibly other regions. Posterior is up, lateral is right. Modified from Hackett, T. 2002. The comparative anatomy of the primate auditory cortex. In: Primate Audition: Behavior and Neurobiology (*ed.* A. Ghazanfar). CRC Press with permission from Taylor & Francis Group LLC.

Table 1 Proposed homologous auditory cortical regions in great apes and humans, with reference to the model of auditory cortex established in monkeys. For each study cited, the species is listed, along with the corresponding region or areas, and the anatomical methods used to identify the region. Note that the size and location of areas varies widely between species and corresponding regions are approximate

Study	*Species*	*Core*	*Medial belt*	*Lateral belt*	*Parabelt*	*Methods*
Bailey *et al.* (1950)	Chimpanzee	TC	TC	TB	TA	C
Bailey and von Bonin (1951)	Human	TC, koniosus supratemporalis	TB, 42	TB, 42	TA, 22	C
Beck (1928, 1929)	Human, chimpanzee	Ttrli/e	Tsm	TtrII	ND	M
Braak (1978)	Human	Temporalis granulosa	Temporalis progranulosa	Temporalis paragranulosa	Temoralis magnopyramidalis	L, C
Brodmann (1908, 1909)	Human	41	52	42	22	C
Campbell (1905)	Human, chimpanzee, orangutan	Auditosensory	ND	ND	Auditopsychic	C, M
Von Economo and Koskinas (1925)	Human	TC, supratemporalis granulosa	TB, TD	TB	TA_1, TA_2?	C
Von Economo and Horn (1930)	Human	TC, supratemporalis granulosa	TD, (TA_2a)	TBma	TA_1, TA_2?	C
Flechsig (1876)	Human	7	14?	14?	14	M
Flechsig (1920)	Human	10	18?	19?	19?	M
Galaburda and Sanides (1980)	Human	Kam, kalt	ProA, PaAc/d	PaAi	PaAe	C, M
Hackett *et al.* (2001)	Human, chimpanzee, macaque	Core	Medial belt	Lateral belt	ND	C, M, A
Hopf (1954)	Human	Ttr1	Tsep	Ttr2	Tpart, Tmag	M
Mauss (1911)	Orangutan, gibbon	40				M
Morosan *et al.* (2001, 2005)	Human	Te1.0	TI1	Te2	Te3	C; RA
Nakahara *et al.* (2000)	Human	Zone 1	Zone 2	Zone 2	ND	PV, C
Ong and Garey (1990)	Human	41	ND	42	22	C, G, M
Rademacher *et al.* (1993)	Human	41	ND	ND	ND	C
Rivier and Clarke (1997)	Human	AI	MA	LA, PA	PA, STA	A, CO, N
Smith (1907)	Human	No. 27	21, postcentral insular	26, temporalis superior	26, temporalis superior	G
Sweet *et al.* (2005)	Human, macaque	Core	ND	Lateral belt	Internal and external parabelt	C, A, PV
Vogt and Vogt (1919)	Human, macaque	41, temporalis transversa interna	ND	42, temporalis transversa externa	22aB	M, C
Wallace *et al.* (2002)	Human	AI, LP?	MA, AA?	PA, LA, ALA	STA	C, A, M, CO, PV, N

A, acetylcholinesterase; C, cytoarchitecture; CO, cytochrome oxidase; G, Golgi; L, lipofuscin (pigment architecture); M, myeloarchitecture; NADPH, NADPH-diaphorase; PV, parvalbumin; RA, receptor autoradiography.

plane. In chimpanzees with a prominent HG, the orientation and appearance of the core is more similar to that found in humans (Hackett *et al.*, 2001). The core in these cases is confined to the HG. These findings highlight the variable relationship of the core region to the surface landmarks. The anatomical variability is more problematic for functional studies in which delineation of the core and belt regions is important for interpretation of experimental results. Functional imaging and electrophysiological approaches have been broadly used to study activity in auditory cortex (for reviews, see Hall *et al.*, 2003; Scott and Johnsrude, 2003). However, since auditory stimulation tends to activate core and belt regions, it has been difficult to precisely dissociate their respective contributions, especially given the anatomical variability between subjects (Seifritz *et al.*, 2002; Lehmann *et al.*, 2006).

Flanking the core region on the anteromedial and posterolateral sides of the TTG are two distinct regions that are likely to comprise part of the auditory cortex of humans and apes. The anterior region, interposed between the core and circular sulcus of the insula, is a region with distinctive architecture that most closely corresponds to the medial belt region of monkeys. Although variably named by different investigators (e.g., area 52 of Brodmann) (Figure 3), the various architectonic descriptions of this region are remarkably similar. The planum temporale (PT) occupies the superior temporal plane posterolateral to the TTG. Aside from the TTG, the expansion of the PT is perhaps the clearest differences in the gross morphology of the superior temporal lobe among primates. Compared to monkeys, the PT and STG appear to be greatly expanded in apes and humans. Accurately accounting for this expansion poses a significant challenge, because comparative studies are lacking. With respect to the PT, most descriptions have noted significant architectonic heterogeneity, consistent with the presence of at least two subdivisions in apes and humans (Figure 3). Brodmann (1908, 1909) identified area 42 and part of area 22 on the human PT, and while details vary between investigators, subsequent reports have generally not departed greatly from this interpretation (Figure 3, Table 1).

The rotation of the core in apes and humans suggests that the PT may contain parts of the core and parabelt regions of monkeys (Figure 3). Anatomical support for this hypothesis includes evidence that belt areas corresponding to CM and CL cap the core region posteromedially in both chimpanzees and humans (Hackett *et al.*, 2001). Most recently, areas possibly corresponding to the lateral belt and parabelt of monkeys were identified in the human PT adjacent to the core on the TTG (Sweet *et al.*, 2005). These assignments were made on the basis of position, relative to the core region, and architectonic similarities between species. While further comparative studies are needed to validate and extend these findings, it follows that a significant portion of the STG (area 22) would have no clear homologue among monkeys, apes, and humans. That is, if the PT contains most of the lateral belt and parabelt regions identified in monkeys, then most of the STG of apes and humans is comprised of cortex with no clear homologue in monkeys. Since there have been no comparative studies of the STG among these primates, correspondence remains an open question.

4.06.6 Conclusions and Directions for Future Research

Anatomical and functional studies suggest that certain elements of the monkey model of auditory cortex are directly applicable to apes and humans. The homology of the core region is the most well established at present, and homologies among the medial and lateral belt regions appear likely. Undoubtedly, further comparative studies will be needed to identify other similarities and differences in the organization of auditory cortex across taxonomic groups. For architectonic studies, progress will depend on the establishment of anatomical profiles to distinguish cortical fields. Functional studies may help to validate the anatomical predictions through the discovery of common physiological features, such as tonotopic organization. While it is not expected that the anatomical and functional features will be identical among areas identified as homologous, it is likely that many corresponding areas will be revealed, with the possibility that additional areas have been added in apes and humans. The differences in auditory cortex organization are expected to contribute to well-known differences in the perception and production of speech and music among primates, and may also reveal clues about the evolution of these abilities.

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4.07 The Evolution of the Sweetness Receptor in Primates

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Glossary

acesulfame K	A saccharin-type sweetener with a sweetness potency about 200 times that of sucrose (see sweetness potency entry in the glossary).
alitame (ALT)	A dipeptide-type sweetener with a potency of 2000.
Asp-	Side chain of a specific aspartate residue; recognition site of the sweet taste receptor.
aspartame (APM)	First sweet-tasting dipeptide discovered, with a potency of about 200.
bernadame	Guanidine-type sweetener with a potency of about 200 000.
Glu-	Side chain of a specific glutamate residue; recognition site of the sweet taste receptor.
G-proteins	Family of intracellular proteins that bind receptor complexes and mediate a cell surface receptor (for instance, sweet taste receptor) to intracellular responses.
interaction sites	Parts of a sweet molecule interacting with the recognition sites of the sweetness receptor.
interaction points	Each interaction site is made up of two interaction points or interaction subsites (with the exception of the D-site, where there is only one interaction point).
lugduname	Guanidine-type sweetener, the most potent sweetener ever described, with a potency of about 225 000.
Lys-	Side chain of a specific lysine residue; recognition site of the sweet taste receptor.
monellin	Protein isolated from the fruits of an African plant *Dioscoreophyllum cumminsii*, with a potency of about 2250 (about 100 000 times on a molar basis).
MPA theory	Multipoint attachment theory: a model to explain the interactions of sweet molecules with sweet taste receptors in humans.
neotame (NTM)	A dipeptide-type sweetener with a potency of about 10 000.
recognition sites	Specific side chains of some amino acids forming the sweet taste receptor and assumed to be able to interact with sweet molecules.
Ser-	Side chain of a specific serine residue; recognition site of the sweet taste receptor.
sucrononate	Guanidine-type sweetener with a potency of about 200 000.
sweetness potency	Sweetness potencies are given for humans on a weight basis relative to a 2% sucrose solution.
thaumatin	Protein isolated from the berries of an African plant *Thaumatococcus daniellii* with a potency of 5500 (100 000 on a molar basis).
umami	Taste typically represented by monosodium glutamate (umami is a fifth taste quality in addition to sweet, sour, salty, and bitter, and is derived from the Japanese word meaning deliciousness).
Val-	Side chain of a specific valine residue; recognition site of the sweet taste receptor.

4.07.1 The Importance of the Sense of Taste

The intake of energy is vital for life for animals and humans and is a key determinant in the evolution of animals from primitive to more advanced levels. For most animals, visual and/or olfaction senses are initially essential in finding nutritional energy. Later the sense of taste remains a powerful controlling system of food intake. The importance of the gustatory system is observed in regulation of food intake, recognition and distinction of food substances, and

protection of the body from toxic substances. In many species, the sense of taste is also involved in other functions, such as increase in sexual excitation (i.e., for many insects), or in allowing and regulating the co-habitation of different species of animals and plants (exudates play a regulatory role in interspecific interactions), or, as in humans, in increasing pleasure (see The Evolution of Sensory and Motory Systems in Primates, The Development and Evolutionary Expansion of the Cerebral Cortex in Primates, Organization and Correspondence of the Auditory Cortex of Humans and Nonhuman Primates, The Vomeronasal Organ and Its Evolutionary Loss in Catarrhine Primates, Evolution of Taste).

4.07.2 Classification of the Order Primates

Primates have stimulated more interest than any other group, as humans are ranked in this mammalian order. Primates are represented by following main groups: suborder Prosimmii, with the infraorders Lemuriformes, Lorisiformes, and Tarsiiformes; suborder Anthropoidea, with the infraorders Platyrrhini (the New World simians with the Callitrichidae and Cebidae) and Catarrhini (the Old World simians with the superfamilies Cercopithecoidea (one family cereopithecidae) and the Hominoidea with three families Hylobatidae (lesser apes), Pongidae (great apes), and Hominidae (humans)). A simplified classification is given in Figure 1.

The history of the order Primates began in late Cretaceous times, about 65 Mya. There have been several lively current disputes anticipating primate origins as early as 80 Mya, but up to now there has been no clear evidence for this suggestion. Future fossil discoveries will probably clarify this divergence in the timescale.

4.07.3 Early Greek Theories of Taste Sensations

Until about 10 000 years BC, humans were little more than successful predators living according to the law of the jungle and surviving as they were well adapted to it. Direct evidence about primate diets is exceedingly rare in prehistoric records. But archeologists have dug up tools and food residues, making it possible to sketch an outline of the diet of prehistoric humans. Indications exist that, in relation to sweet substances, about 50 000 years BC humans were cultivating the date palm in southern Mesopotamia. The date palm probably supplied one of the first sweet compounds (81% sucrose dry weight) to humans. Furthermore, the Stone Age paintings (over 20 000 years old) in the caves of Arana in southern Spain depict an individual collecting honey from a bee's nest. Few facts are available in relation to sweet substances in human diet before 3000 BC, the approximate date of the first written records. With the emergence of the early Greek philosophers are found the first written records on taste sensation

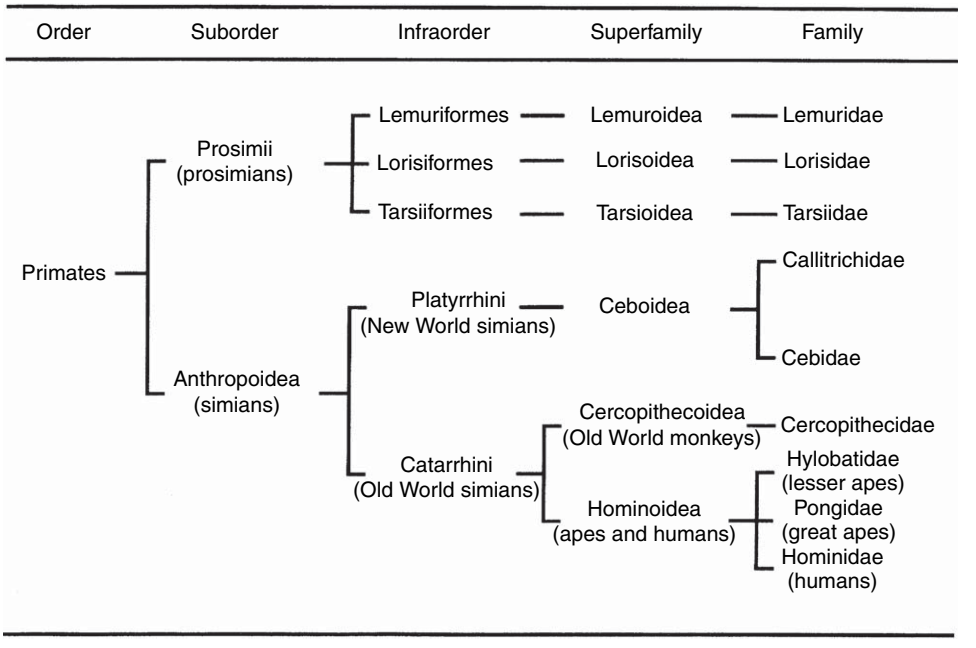

Figure 1 A simplified classification of the order Primates.

(Burnet, 1975): the concept of canals ("Perception is due to the meeting of an element in us with the same element outside") was prevalent. However, Alkmaion (*c.* 500 BC) first connected pores with sensation ("It is with the tongue, however, that we distinguish the tastes") and he suggested the concept that the sensation reaches the brain through these pores.

Influenced by the Pythagorean (532–497 BC) ideals concerning opposites (even–uneven, right–left, hot–cold, black–white, finite–infinite, wet–dry, male–female, good–bad, love–hate), Alkmaion mentions the opposites of sweet–bitter. At this time, the Pythagorean teaching on the battle of the opposites and their fusion into harmony continued to influence biology. In the fifth and fourth centuries BC, probably instigated by Empedokles (*c.* 495–435 BC), the doctrine of the four bodily humors (blood, phlegm, choler, and melancholy) was created in connection with humoral pathology. This further evolved to produce the doctrine of the four basic or primary qualities (probably credited to Zenon, *c.* 490–430 BC). Later, Aristotle (384–322 BC) expresses himself diplomatically: "The types of taste are – as are colours – simple opposites; sweet–bitter, following these two oily and salty; as intermediates pungent, astringent, sour, prickly." Aristotle proposes the existence of two opposites, instead of four, but recognizes the existence of intermediate qualities. However, it is difficult to arrange the Aristotelian terms, oily and salty, as well as the intermediates – pungent, astringent, sour, prickly – on a straight line. This shows how difficult it is to describe the characteristics of taste in an accurate semantic way.

Nowadays, we generally refer to the four primary qualities of taste as sweet, sour, salty, bitter, and in modern times, umami, and it is possible to depict this in a three-dimensional spherical model (Figure 2) (Haefeli and Glaser, 1985). More details of the development of models on taste qualities are given by Glaser (1999).

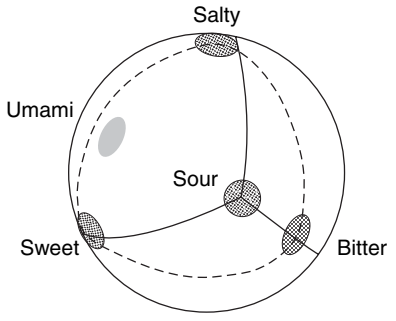

Figure 2 Areas of the spherical model represent diversity within the qualities of taste. This is in contrast to Henning's tetrahedron, where only one point represents a different taste quality. Reproduced from Glaser, D. 1999. The evolution of taste perception. In: Low-Calorie Sweeteners: Present and Future (*ed.* A. Corti), vol. 85, pp. 18–38. Karger, with permission from Karger, Basel.

4.07.4 Sweet Receptor Theories

As mentioned above, Alkmaion discussed pores in connection with taste, while Empedokles wrote that "Perception, then, is due to the meeting of an element in us with the same element outside." This takes place when the pores of the sensory organ are neither too large nor too small for the effluences constantly emitted by all things (Burnet, 1975).

Democritus (460–370 BC) was one of the first originators of the atomic theory of nature: he explained that various taste qualities resulted from the shapes of the atoms (now molecules) in different taste compounds. For example, he attributed sweetness to medium-sized spherical shapes entering special pores, bitterness to small spherical shapes attached with hooks, saltiness to big, aspherical shapes, and sourness to angular shapes.

It was only much later that the concept proposed by Democritus was substantiated by the German chemist Emil Fischer in the following sentence: "Visually speaking, I would say that the enzyme and glucoside must fit together as lock and key, in order to chemically act upon one another." The validity of this concept continues to stand today.

Among basic tastes, the sweet taste has attracted wide attention with regard to its importance to the taste of food. How a molecule binds to the receptor on the microvilli of a taste bud and initiates a sensory effect will be discussed here for sweetness in different steps.

An important step was that of Shallenberger and Acree's (1967) bipartite hydrogen-bonding principle, or the AH–B system, in which "the initial chemistry of the sweet taste response must be analogous to concerted and antiparallel intermolecular hydrogen bonding with a chemically and sterically commensurate receptor." Both A and B are negatively charged atoms in suitable geometrical proximity. A is a hydrogen-bond donor, while B is an acceptor. This bipartite binding was thought to be all that is required to elicit the sensation of sweetness.

Through a second step, a tripartite binding site completed the two hydrogen bonds. Kier (1972) proposed the existence of a third binding site, a hydrophobic site capable of cooperating with the AH–B system.

Later van der Heijden *et al.* (1979) tried to locate the distances of the AH–B moiety from this third binding site. The distance between this new site and A was estimated to be approximately 5.5 nm, and the distance to B approximately 3.5 nm. This third binding site was designated as X by Kier (1972), y by

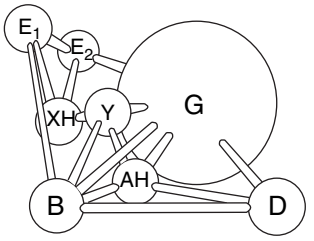

Figure 3 Spatial arrangement of the eight interaction sites of sweetening compounds of the initial MPA model, with four high-affinity sites: B, an anionic group; AH, hydrogen-bond donor group; G, hydrophobic group; and D, hydrogen-bond acceptor group. Four secondary sites are designated as Y, E_1, and E_2 sites, hydrogen-bond acceptor ligands, and XH is a hydrogen-bond donor group. Reproduced from Glaser, D. 1999. The evolution of taste perception. In: Low-Calorie Sweeteners: Present and Future (*ed.* A. Corti), vol. 85, pp. 18–38. Karger, with permission from Karger, Basel.

Shallenberger and Lindley (1977), δ by van der Heijden *et al.* (1979) and later G by Tinti and Nofre (1991).

A fourth step was suggested by Tinti *et al.* (1982), studying structure–activity relationships in the suosan series. These researchers found that an NO_2 or a CN group attached to an aromatic ring (such as a phenyl group) was an essential feature for a high-potency sweetener. They designated this hydrogen-bond acceptor group (such as NO_2/CN) as the D-site. Following this finding, structurally different sweeteners were reviewed by these authors, who proposed a multipoint attachment (MPA) theory of sweetness reception (Figure 3) (Tinti and Nofre, 1991).

4.07.5 Extensive Research of Primate Taste Responses to Sweeteners Started in 1966

In 1966, I started testing nonhuman primates with sweet compounds at the primate facilities of the Anthropological Institute of the University in Zürich, Switzerland. At first, the main point of interest was the animals' sensitivity to sweet compounds in comparison with that of humans. Later, interest focused on the diversity of taste responses and the mechanistic relationships between a sweet substance and the sweet taste receptor.

The first interesting result in the order of Primates was found with thaumatin, a protein isolated from the berries of the African plant *Thaumatococcus daniellii* Benth., which humans find tastes intensely sweet (on a molecular basis, about 100 000 times sweeter than sucrose). Neither the Prosimii – including *Tarsius* – nor the South American primates tested (Figure 1) showed any behavioral and/or electrophysiological responses to this protein. Only the Cercopithecidae, the Hylobatidae, and the Pongidae responded to this compound in the same way as humans and preferred it (Glaser *et al.*, 1978). Later it was found (Glaser *et al.*, 1992) that aspartame (APM), the first discovered sweet-tasting dipeptide with a potency of about 200, induces a behavior similar to that of thaumatin, i.e., there is a clear dichotomy within the order Primates at the same intersecting line. A third compound, the new high-potency non-nutritive sweetener neotame (NTM) (Nofre and Tinti, 2000), which has a sweetness potency about 10 000 times that of sucrose, confirms the intersecting line found with thaumatin and APM.

4.07.6 The Multipoint Attachment Theory

Since its first presentation, the MPA model (Tinti and Nofre, 1991) has been modified and improved to integrate more structure–activity relationship results in sweetening agents and new observations on the sweet taste reception of primate species.

The new model comprises 10 fundamental interaction sites that are counterparts of 10 opposite recognition sites contained in the sweetness receptor.

Because the sweetness receptor has a proteinaceous structure – it is probably a member of the family of seven-pass transmembrane receptors coupled to a G-protein – it is assumed according to the MPA theory that the recognition sites are represented by the side chain of certain amino acids comprising the sweet taste receptor. The sweetening agents are thus able to interact with some of these recognition sites arranged around the central cavity inside the transmembrane receptor protein.

These recognition sites are assumed to be made up of an aspartate or glutamate residue (Asp-1 or Glu-1), a lysine residue (Lys-2), another aspartate or glutamate residue (Asp-3 or Glu-3), five threonine residues (Thr-4, Thr-5, Thr-6, Thr-7, Thr-9), a valine residue (Val-10) behind Thr-4 and under Thr-5, and a serine residue (Ser-8), comprising only one recognition site and designated as the D-site (Figure 4).

Nine of the recognition sites are dual-interaction subsites (or interaction points); thus the number of attachment points between a sweet molecule and the receptor may be high (up to 19 points) (Glaser *et al.*, 2000). Note, however, that a molecule may elicit a sweet taste while interacting through a notably lower number of these interaction points.

Considering in detail Primates (Figure 5), we find only G_1- and G_4-recognition subsites in the entire

infraorder Platyrrhini (all the New World monkeys). In species of the suborder Prosimii (Lemuridae and Lorisidae) the G_1-, G3-, and G4-recognition subsites are found, and it is only in the Catarrhini (Cercopithecidae, Hylobatidae, Pongidae, and Hominidae) that all four G-recognition subsites are found on the receptor surface. Moreover, if we look at the E-points, we find only the E_1- and E_4-recognition subsites in the Callitrichidae (a family of New World monkeys). In the Cebidae (the other family of New World monkeys), we find the E_1-, E_2-, and E_4-recognition subsites. The suborder Prosimii possesses the E_1-, E_3-, and E_4-recognition subsites and again only the Catarrhini possesses all four E-recognition subsites. The mutations observed in the primate sweetness receptors concern only the G_2-, G_3- and E_2-, E_3-steric recognition subsites.

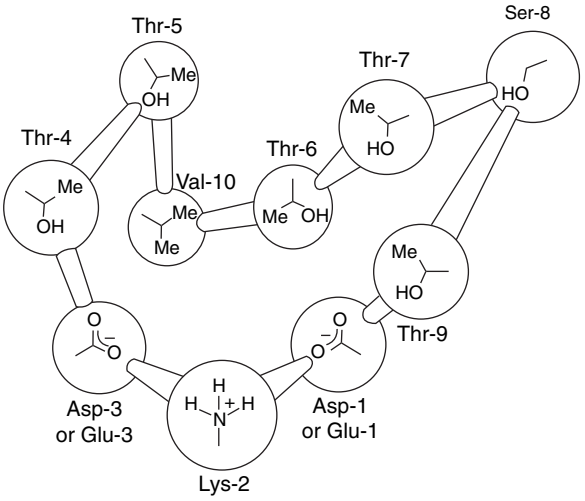

Figure 4 The MPA model of the human sweetness receptor. The spheres of the model represent the approximate spatial positions of the different functional groups that may be involved in the interactions of the human receptor with various natural or artificial sweeteners. Reproduced from Glaser, D., Wanner, M., Tinti, J.-M., and Nofre, C. 2000. Gustatory responses of pigs to various natural and artificial compounds known to be sweet in man. *Food chem.* 68, 375–385, Elsevier.

Initially designed for human sweetness reception, the MPA model proved useful to understand better sweet taste reception by considering the presence or absence of a given recognition site for a specific primate species.

Figure 6 shows the interaction points of the first-known sweet-tasting dipeptide APM (which is only sweet in all Catarrhini) in comparison with another dipeptide-like sweetener, alitame (ALT), which is about 2000 times sweeter than sucrose in humans and which, in contrast to APM, elicits a sweet taste in all primates investigated (Glaser *et al.*, 1996). APM interacts with the receptor through the G_1-, G_2-, and G_4-steric recognition subsites. The G_3-steric interaction subsite is therefore postulated to be the special

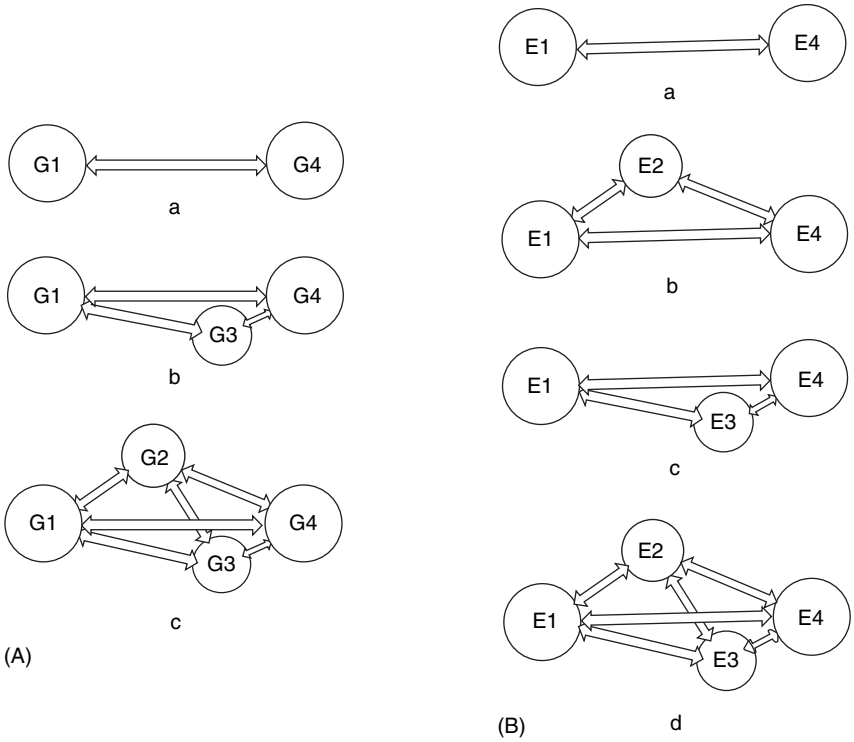

Figure 5 A, The steric G-recognition sites in primates: a, Platyrrhini; b, Prosimii; c, Catarrhini. B, The steric E-recognition sites in Primates: a, Callitrichidae; b, Cebidae; c, Prosimii; and d, Catarrhini. Reproduced from Glaser, D. 1999. The evolution of taste perception. In: Low-Calorie Sweeteners: Present and Future (*ed.* A. Corti), vol. 85, pp. 18–38. Karger, with permission from Karger, Basel.

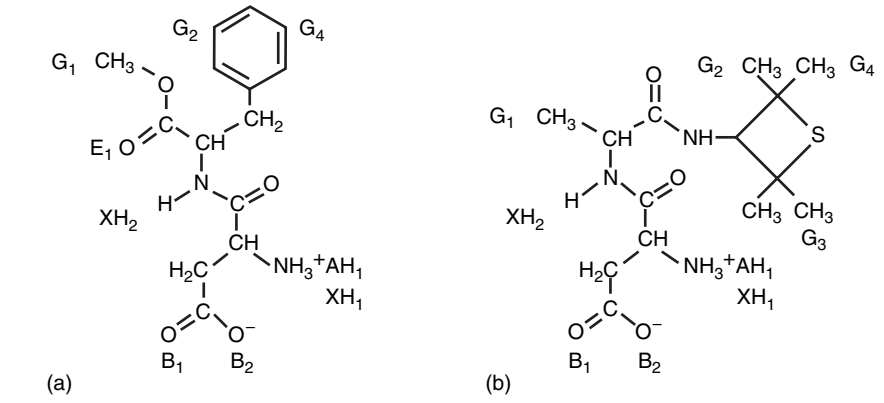

Figure 6 a, Aspartame, a B_1, B_2, AH_1, XH_1, XH_2, E_1, G_1, G_2, G_4-type sweetener and b, alitame, a B_1, B_2, AH_1, XH_1, XH_2, G_1, G_2, G_3, G_4-type sweetener. Reproduced from Glaser, D., Tinti, J.-M., and Nofre, C. 1996. Gustatory response of nonhuman primates to dipeptide derivatives or analogues, sweet in man. *Food Chem.* 56, 313–321, Elsevier.

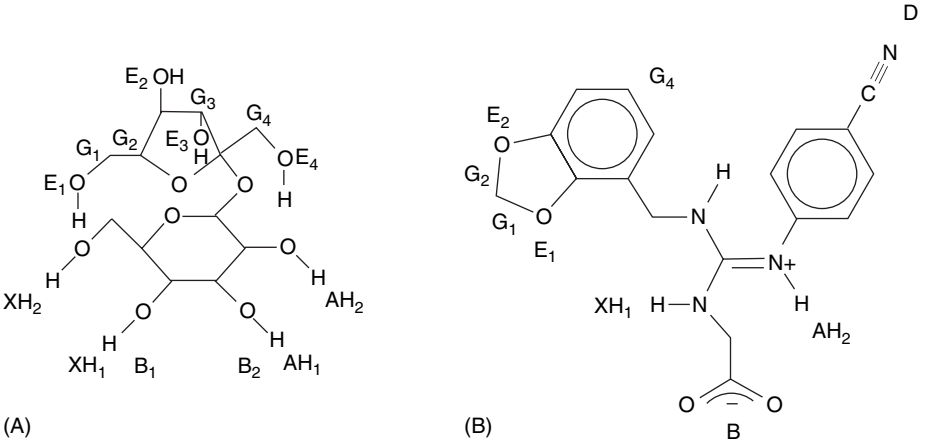

Figure 7 Interaction subsites for (a), naturally occurring sucrose and, in comparison, (b), lugduname, a guanidine derivative, the most potent artificial sweetener known to date, which has a potency 225 000 times that of a 2% sucrose solution. Reproduced from Glaser, D. 1999. The evolution of taste perception. In: Low-Calorie Sweeteners: Present and Future (*ed.* A. Corti), vol. 85, pp. 18–38. Karger, with permission from Karger, Basel.

feature allowing ALT to be sweet in all the primate species tested. The interaction subsites for naturally occurring sucrose and lugduname are illustrated in Figure 7.

4.07.7 Why Are Such a Large Number of Attachment Points Necessary?

We must focus our attention not only on the naturally occurring sweeteners that have been discovered in more than 30 different classes of plants, but also on the enormous number of artificial sweet compounds found in the course of extensive research. All of these sweetening agents belong to more than 25 chemical structural classes (Table 1) and are all coupled to a high biological diversity of animal responses. One way of understanding this diversity is to speculate that there are many receptors (such as, for olfaction), and each family of sweeteners interacts with a different type of receptor. A possible second way is to contemplate that a unique basic receptor exists.

In this second hypothesis, the receptor contains multiple recognition sites, and each sweetener is able to interact with a specific number of these recognition sites. Differences in sweet taste recognition between primate species can thus be easier explained as resulting from evolutional changes (i.e., adapting to environmental conditions) of a common sweet taste receptor ancestor, changes occurring through the mutation or deletion of an existing recognition site or in the apparition of a new one. The many gustatory experiments performed with primates have supported this second approach.

To give just one example, consider the observations with Lemuridae (Prosimii from Madacascar). *Lemur catta* prefers the amino acids glycine and D-tryptophan while *Hapalemur griseus occidentalis* prefers glycine and shows no taste reaction to D-tryptophan. These

Table 1 Sweeteners can be found among diverse chemical structural classes

1. Amino acids (D-tryptophan)
2. Dipeptides (ALT, APM, ampame)
3. Proteins (thaumatin, monellin)
4. Monosaccharides (fructose)
5. Oligosaccharides (sucrose)
6. Polyols (xylitol)
7. Saccharins (saccharin)
8. Urea derivatives (dulcin, suosan)
9. Guanidines (sucrononate, lugduname)
10. Sulfamates (Na-cyclamate)
11. Acesulfames (acesulfame K)
12. Flavonoids (hesperitin)
13. Dihydrochalcones (NHDHC)
14. Nitroanilines (P 4000)
15. Oximes (perillartine)
16. Diterpenes (stevioside)
17. Triterpenes (glycyrrhizic acid)
18. Sesquiterpenes (hernandulcin)
19. Steroid saponins (osladin)
20. Isocoumarins or diphenyls (phyllodulcin)
21. Halogenated hydrocarbons (chloroform)
22. Metal salts (beryllium chloride)
23. Trihalogenated benzamides
24. Malonic diesters
25. Thioureas
26. Anilides

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gustatory differences between primate species can be understood using the MPA theory (Nofre *et al.*, 1996). All our gustatory data support the assumption that a single sweet taste receptor exists in a given primate species – including humans – a receptor that was however subjected to mutations and variations across the millions of years of primate evolution.

4.07.8 Conclusions

Thanks to our knowledge of the MPA model, we can provide a key that allows us to increase our understanding of biodiversity of taste responses and to trace the phylogeny of the sweet receptors in the order Primates. In the course of development from the most ancestral group toward the Cercopithecoidea and Hominoidea, the capability to taste thaumatin, APM, and NTM must have evolved after the South American Platyrrhinai had spilt off. At what point this change occurred is difficult to say, but it may be assumed that this feature is connected with the occurrence of the first catarrhine primates, i.e., 35–38 Mya, with the first confirmed findings of the Catarrhini (*Oligopithecus*) during the Oligocene (Figure 8).

There are indications, however, that this ability evolved specifically in the Catarrhini. All primate species that we tested, whose origin can be traced

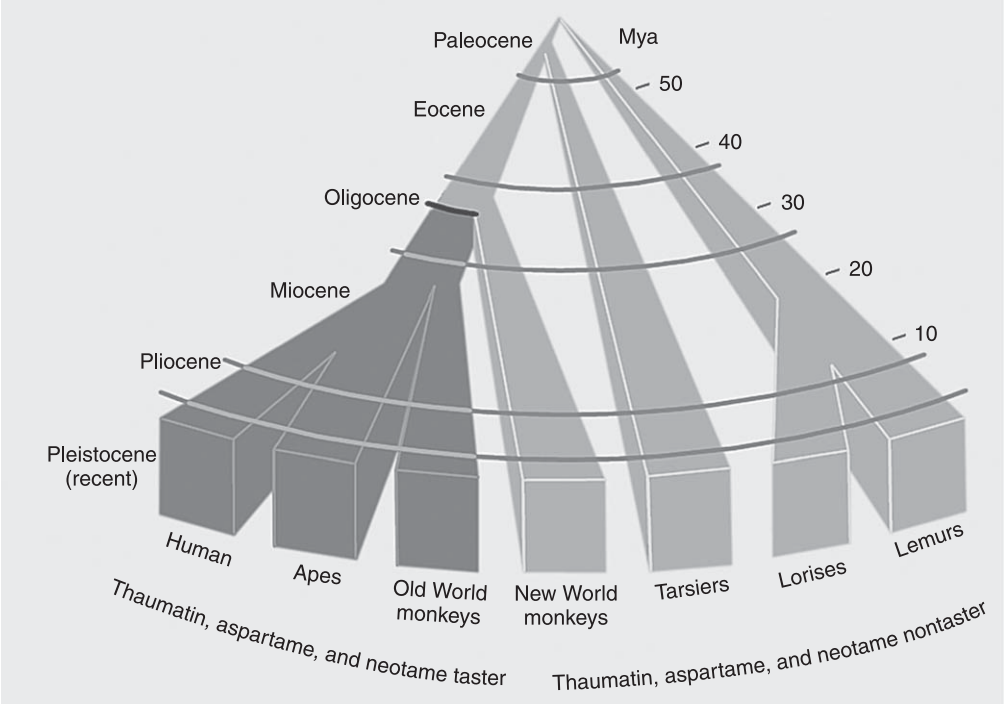

Figure 8 The radiation within the order Primates of tasters and nontasters of thaumatin, aspartame, and neotame. Reproduced from Glaser, D. 2002. Specialization and phyletic trends of sweetness reception in animals. *Pure Appl. Chem.* 74, 1153–1158, with permission from IUPAC.

back to the Cretaceous, Eocene, or Paleocene period, show no response to thaumatin, APM, or NTM (Glaser *et al.*, 2000; Glaser, 2002). All our new and previous data (Nofre *et al.*, 1996, 2002; Tinti *et al.*, 2000) demonstrate that the Catarrhini – the Old World simians – including humans are a compact group and that their sweetness receptor evolved >35–38 Mya in the Oligocene (Glaser *et al.*, 1978, 1992). The Catarrhini possesses the most advanced type of sweetness receptor compared to that of the Callitrichidae, which is considered to be a more primitive sweet taste receptor.

Generally, animals' feeding habits and behavior have been classified into various categories such as carnivorous, omnivorous, and phytophagus, but many species are not highly restricted in their natural diets. Seasonal and/or daily variations sometimes considerably shift feeding habits. Additional efforts in studying comparative gustatory physiology could be helpful to clarify the evolution of further mammalian sweet receptors in connection with feeding habits and other behaviors in the animal kingdom.

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4.08 The Loss of Olfactory Receptor Genes in Human Evolution

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Glossary

apes Great apes comprise chimpanzees, bonobos, gorillas, and orangutans.

cluster A gene cluster is a genomic region containing similar genes and resulting mostly from tandem duplications.

duplication Creation of two copies of a DNA segment in the genome. A tandem duplication results in the duplication of DNA segments (or genes) in close proximity to each other in a given region of a chromosome.

frameshift mutation A mutation that causes a change in the reading frame of a protein-coding region, leading in general to protein inactivation.

G-protein-coupled receptors (GPCR) Seven transmembrane domain receptors that need both ligand binding and coupling to intracellular G-proteins for their activation.

macrosmates and microsmates Animals that either rely (macro-) or do not rely (micro-) on the sense of smell and therefore display high or low olfactory ability.

nonsense mutation A mutation that causes the introduction of a stop codon leading to a premature termination of the encoded protein. This type of mutation results most of the time in protein inactivation.

nonsynonymous mutation (or substitution) A nucleotide change in a codon that changes the encoded amino acid, as opposed to a synonymous mutation that does not change the nature of the encoded amino acid.

Old World monkeys Monkeys from Africa and Asia. New World monkeys are native to America.

open reading frame (ORF) DNA sequence translatable into protein. For known proteins such as olfactory receptors, the initiation Met and the termination stop codon that limit the ORFs are also searched to define a potentially functional gene.

orthologues Genes that are conserved between species and that lie generally in syntenic regions. The sequence similarity is the result of the speciation process.

paralogues In a single organism, paralogues are homologous genes originating from the duplication of an ancestral gene.

positive selection Selection for an advantageous gene.

pseudogene Nonfunctional DNA segment that shares sequence homology with a functional gene. Different processes may lead to the generation of pseudogenes. They generally contain disrupted ORFs.

purifying selection (or negative selection) Removal of an allele or a gene from a population through natural selection.

synteny Synteny conservation arises when DNA regions of different species contain a series of homologous genes (orthologues) in the same order.

4.08.1 Introduction

Olfaction, or the sense of smell, is an ancient sensory system that together with taste enables an organism to detect chemicals in the external environment. Olfaction is present in most species such as insects, worms, fish, amphibians, birds, and mammals. It is essential for survival by permitting the location of food, mates, and predators, although in humans, olfaction is often viewed as an esthetic sense capable of triggering emotion and memory. In mammals, the sense of smell is mediated by two main sensory organs that are located in the nasal cavity: the main olfactory epithelium (MOE), which binds odorants, and the vomeronasal organ (VNO), which is responsible for pheromone detection. This review will focus on genetic aspects of the conscious perception of odors mediated by the MOE. In terrestrial vertebrates, odorants are volatile chemicals of various classes (aliphatic, cyclic, aromatic, etc.). The olfactory perception starts when odorants bind to olfactory receptors (ORs) that are expressed on cilia of the dendrites of olfactory sensory neurons (OSNs), which emerge in the MOE. This interaction induces the activation of a transduction pathway that involves a G-protein-dependent elevation of cyclic 3′, 5′ adenosine monophosphate (cAMP) opening ion channels that in turn results in the transmission of action potentials to specialized glomeruli localized in the olfactory bulb. Sensory inputs are then transmitted to the olfactory cortex where the information is integrated to result in the sensation of smell. A number of reviews have been published on the principles of olfaction (Moulton, 1967; Keverne, 1999; Krieger and Breer, 1999; Laurent, 1999; Mombaerts, 1999, 2001a, 2004a; Mori *et al.*, 1999; Firestein, 2001; Gaillard *et al.*, 2004a).

During the past 6 years, different studies on the evolution of the OR genes in mammals have pointed out the reduction of the functional fraction of the OR gene repertoire during primate evolution leading to a restricted set of functional OR genes in humans (see Evolution of Vertebrate Olfactory Subsystems). These observations led to the hypothesis that the size of the functional OR repertoire reflects the olfactory needs and hence the olfactory capacity/ability of an organism.

This review is an overview on the subject by comparing data obtained by the analysis of the OR subgenome of different primate species with respect to that of mouse and dog.

4.08.2 Olfactory Receptors

4.08.2.1 Receptors

OR genes were first identified in rat (Buck and Axel, 1991) 16 years ago and subsequently in numerous other species. The OR multigene family (see below) encodes proteins 300–350 amino acids in length that belong to the G-protein-coupled receptor (GPCR) superfamily. They share structural features (Figure 1) common to the GPCRs such as seven hydrophobic transmembrane domains (7TM or heptahelical structure) and other characteristics described elsewhere (Liu *et al.*, 2003). Nevertheless, ORs possess specific characteristics such as a long extracellular loop 2 and consensus motifs (for example, MAYDRYVAIC at the end of TM3 and the beginning of intracellular loop 2). Some motifs are specific to mammalian ORs and others are signatures for the mouse subgroup and class I and class II ORs (Liu *et al.*, 2003), which are the two main OR classes defined by sequence comparison. Class I comprises fishlike ORs thought to bind water-soluble odorants, whereas class II contains mammal-like ORs that bind volatile odorants. Transmembrane domains 3, 4, and 5 are hypervariable and are part of the odorant-binding pocket by analogy with other 7TM proteins (Fuchs *et al.*, 2001). Most of the vertebrates and fish possess similar ORs, whereas insects (*Drosophila melanogaster*) and worms (*Caenorhabditis elegans*) possess very specific OR

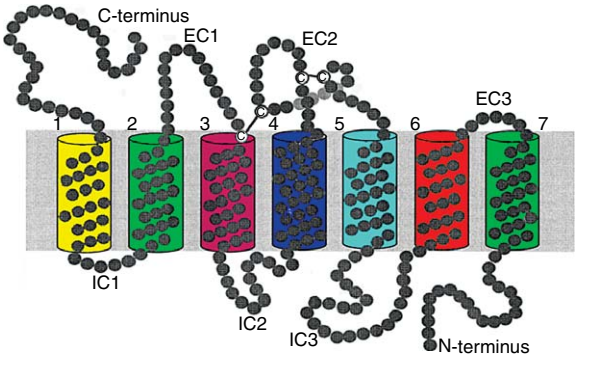

Figure 1 Schematic diagram of an olfactory receptor. The seven transmembrane domains (1–7) are represented embedded in the membrane. Extracellular (EC) and intracellular (IC) loops are indicated as well as the three disulfide bridges.

repertoires that do not share significant sequence homology with any other animal class (Gaillard *et al.*, 2004a).

Classically, on the basis of their sequences, ORs are grouped into protein families (>40% amino acid identity) and subfamilies (>60%).

4.08.2.2 Genes

Although structural features, mode of expression, and gene organization are common to ORs of most species, we will refer to terrestrial mammalian ORs in the next part of this review.

Since the first cloning of OR genes in rat (Buck and Axel, 1991), a plethora of genes have been identified using PCR-based (polymerase chain reaction) approaches with degenerate primers derived from the conserved regions of OR genes. More recently, the complete sequencing of the genome of different species has provided a complete picture of the OR gene repertoire to be drawn. Mammals possess approximately 1000 OR genes that are responsible for the expression of the corresponding receptors. ORs display enough diversity to ensure the detection of thousands of odorants using a combinatorial code (Malnic *et al.*, 1999; Mori *et al.*, 1999; Firestein, 2001; Young and Trask, 2002) to translate complex odorant stimuli into a subtle odor perception. OR genes are expressed mainly in the olfactory epithelium but also in spermatozoa where they appear to be key elements for sperm chemotaxis and the fertilization process (Parmentier *et al.*, 1992; Vanderhaeghen *et al.*, 1993; Spehr *et al.*, 2003). In olfactory neurons, OR genes are expressed in a monoallelic fashion since each OSN expresses only one single OR allele (maternal or paternal) (Chess *et al.*, 1992, 1994; Chess, 1998a, 1998b). This particular expression requires a negative feedback mechanism since the expressed receptor inhibits the expression of the other ORs (Serizawa *et al.*, 2003; Lewcock and Reed, 2004; Li *et al.*, 2004; Mombaerts, 2004b).

OR genes are composed of an intronless coding region of approximately 1kb preceded by a variable number of noncoding 5′ exons (1–4, encompassing up to >15kb) and terminated by a short 3′ end (~1.5kb) containing a polyadenylation signal (Sosinsky *et al.*, 2000). It has been shown that ORs could be alternatively spliced (among the different 5′ nontranslated exons), giving rise to different transcripts encoding the same receptor that might reflect a tissue-specific expression, but no obvious regulatory motifs have been identified (Asai *et al.*, 1996; Sosinsky *et al.*, 2000; Lane *et al.*, 2001).

4.08.3 OR Gene Organization

4.08.3.1 OR Gene Clusters

After the identification of the first OR genes, a number of genomic analyses have been performed to elucidate their organization in the genome by physical mapping. More recently, the complete genome sequencing of diverse species has allowed different groups to perform the precise analysis of the distribution of OR genes in whole genomes.

OR genes are organized in clusters, i.e., genomic regions containing OR genes duplicated in tandem. The first well-studied cluster was located on human chromosome *17p13.3* (Ben-Arie *et al.*, 1993; Glusman *et al.*, 1996, 2000; Sosinsky *et al.*, 2000; Fuchs *et al.*, 2001; Lapidot *et al.*, 2001; Menashe *et al.*, 2002). Other regions were also investigated thereafter (Trask *et al.*, 1998a, 1998b; Brand-Arpon *et al.*, 1999; Lane *et al.*, 2001, 2002; Mefford *et al.*, 2001; Younger *et al.*, 2001; Amadou *et al.*, 2003). OR clusters may contain from 2 to 138 genes (for review, see Gaillard *et al.*, 2004a) with, in general, no non-OR genes interspersed. The human *17p13.3* cluster spans approximately 440kb and contains 17 OR genes (Sosinsky *et al.*, 2000). The average size of human OR clusters is approximately 300kb in a range 100kb to 1Mb with the largest greater than 3Mb. Some of them may contain more than 100 OR genes (Glusman *et al.*, 2001; Zozulya *et al.*, 2001). The OR repertoire is the result of multiple rounds of gene duplication to generate the first ancestral clusters that were subsequently duplicated in multiple locations in the genome (Glusman *et al.*, 2001). Duplications of entire and large genomic segments between chromosomes have been termed segmental duplication (Ohno, 1970; Ohno *et al.*, 1968; Eichler, 2001; Friedman and Hughes, 2001a, 2001b; Bailey *et al.*, 2002a, 2002b; Samonte and Eichler, 2002; van Geel *et al.*, 2002) in contrast to the duplication of a single gene or a whole genome (the tetraploidization process). As a consequence of the dynamics of OR cluster formation, genes lying in the same locus or cluster are closely related (pertaining to the same family), but this does not exclude the possibility that different families may be represented in a single locus. Given that approximately 1000 OR genes (3–5% of all genes) are devoted to olfaction, the OR repertoire is the largest gene family in the genome of mammals and occupies as much as 30Mb, i.e., 1% of the genome.

4.08.3.2 Distribution of the OR Gene Repertoire in Humans

The first picture of the OR gene distribution in the human genome was established before the human

genome was sequenced (Rouquier *et al.*, 1998b). The authors utilized a combination of fluorescence *in situ* hybridization (FISH) analysis, using consensus OR probes to identify the different OR loci on metaphase chromosomes, and the PCR cloning of a number of OR genes, using as templates individual chromosomes sorted by flow cytometry and degenerate OR primers (Ben-Arie *et al.*, 1993) derived from the most conserved OR regions (TM2 and TM7). This analysis showed that:

1. OR genes are distributed in more than 50 loci on virtually all chromosomes except chromosomes 20 and Y.
2. OR genes are not evenly distributed but are preferentially located in subtelomeric regions, which are known as sites for genetic recombination/transposition, and to a lesser extent into pericentromeric regions.
3. Sequence analyses indicate that the OR family expanded by inter- and intrachromosomal duplications.
4. Approximately 70% of the genes are nonfunctional pseudogenes, i.e., whose coding region (open reading frame or ORF) is disrupted by deleterious mutations such as frameshift mutations (insertions or deletions that change the right reading frame in any part of the protein) or nonsense mutations (introducing stop codons leading to truncated proteins).

This latter observation was unexpected and led the authors to hypothesize that there is a parallel between a reduced sense of smell (humans are considered as microsmatic with respect to macrosmatic dogs or rodents) and a reduction in the functional fraction of the OR repertoire. In the same study, it was demonstrated that for some OR pseudogenes, the same mutations were conserved between humans and chimpanzees, meaning that the pseudogenization process occurred before the two species diverged.

Other work confirmed and detailed these data, particularly on the telomeric nature of the OR loci and their role as founders of the present repertoire (Trask *et al.*, 1998a, 1998b; Linardopoulou *et al.*, 2001; Mefford *et al.*, 2001; Mefford and Trask, 2002). At the same time, after sequencing the human genome was nearly complete, two major studies (Glusman *et al.*, 2001; Zozulya *et al.*, 2001) used database mining and sequence analysis to precisely establish a complete genomic map of the OR gene repertoire and its evolutionary origin as well as the sequence of all the OR genes. ORs are distributed in 17 families and dispersed in more than 50 chromosomal locations. OR pseudogenes represent approximately 63% of the gene repertoire, resulting in a functional set of approximately 350 genes. A surprising characteristic of the human repertoire is the presence of a cluster of fishlike class I ORs on chromosome 11. This cluster contains approximately 102 class I ORs, and 52% of them are pseudogenes compared to the more than 75% for the class II, meaning that these genes probably have a functional significance and are not evolutionary relics as was previously supposed. This cluster is actually the ancestral cluster of the whole OR gene repertoire. It first duplicated locally to generate a tetrapod-specific class II OR cluster that in turn was duplicated to the long arm (q arm) tip of chromosome 1 (Figure 2). This chromosome 1 cluster was then duplicated in multiple locations in the genome to generate the complete OR repertoire (Giorgi *et al.*, 2003; Glusman *et al.*, 2001; Zozulya *et al.*, 2001; Gaillard *et al.*, 2004a). Chromosome 11 is therefore the ancestral chromosome of the OR gene repertoire and it contains nine clusters and accounts for approximately 42% of the total number of OR genes. Two databases have compiled these results (for review see Mombaerts, 2001b): the Human Olfactory Receptor Data Exploratorium (HORDE) and the Olfactory Receptor DataBase (ORDB). Other analyses were then published which gave slightly different results (Niimura and Nei, 2003; Malnic *et al.*, 2004). In these publications, the authors count 339–388 intact OR genes and 297–414 pseudogenes, for a total of 636–802 genes located in approximately 51 loci, indicating that 47–52% are pseudogenes. Nevertheless, analyses of the HORDE database tend to consider 1100 human OR genes or more with a pseudogene count comprised in the 63–67% range (Safran *et al.*, 2003; Olender *et al.*, 2004). However, the pseudogene count could be higher since only OR genes with a disrupted ORF were scored, suggesting that nonsynonymous mutations (nucleotide mutations, in general on the first and second base of a codon, that produce amino acid changes that do not disrupt the ORF) could lead to nonfunctional ORs. For example, it has been shown that the replacement of the Arg residue of the DRY motif led to nonfunctional ORs (Gaillard *et al.*, 2004b) and that this change was found in 11% of human ORs (Young *et al.*, 2002), suggesting that the functional human OR repertoire could be at least 11% lower than the theoretical count, leading to approximately 300 functional human genes. As we will see further, among terrestrial mammals, humans possess the highest pseudogene content. For unknown reasons, segmental duplication events have even expanded the human OR 7E gene family

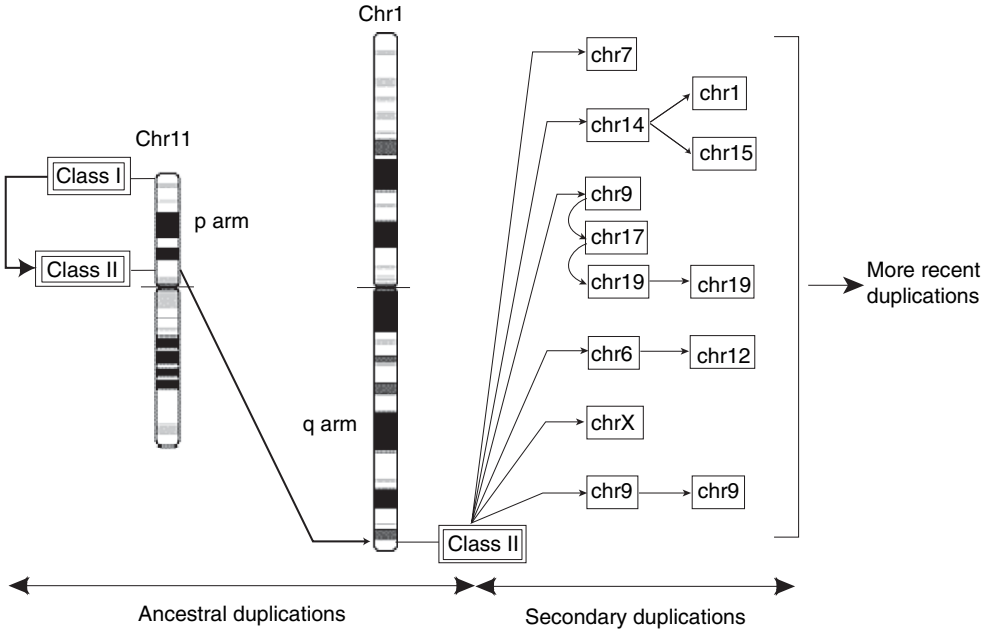

Figure 2 Generation of the OR gene repertoire in the human genome. The ancestral clusters are double boxed. On chromosome 11, the initial duplication of the class I cluster that generated the first class II cluster is indicated. Class II repertoire originated from the duplication of the chromosome 11 class II cluster on chromosome 1, followed by a series of other duplications on other chromosomes. Adapted from Glusman, G., Yanai, I., Rubin, I., and Lancet, D. 2001. The complete human olfactory subgenome. *Genome Res.* 11, 685–702.

that contains approximately 88 genes with a pseudogene count of more than 92% in more than 35 genomic regions (Newman and Trask, 2003). The pseudogenization process accelerated in the human lineage, since it has been shown that humans accumulated OR pseudogenes about fourfold faster than any other species tested (Gilad *et al.*, 2003b).

4.08.3.3 The OR Gene Repertoire in Mouse and Dog

The OR gene repertoire was also investigated thereafter in two macrosmate species (good smellers): the mouse and the dog. These observations are particularly interesting when compared to those of humans, who are considered poor smellers.

4.08.3.3.1 Mouse The mouse offers several advantages for studying ORs:

1. It is a laboratory animal widely used for *in vivo* studies.
2. It is a macrosmatic mammal.
3. Its genetics is known and many mutant mouse lines are available.
4. Its genome is entirely sequenced.

Different studies have shown that the mouse MOE was compartmentalized in four different zones in which different sets of ORs were randomly expressed (Ressler *et al.*, 1993, 1994; Vassar *et al.*, 1993, 1994; Strotmann *et al.*, 1994). The first study of the mouse OR gene repertoire (Sullivan *et al.*, 1996) used 21 OR genes expressed in the four zones of the MOE to study OR gene distribution by genetic analysis. The authors found that OR genes are clustered in multiple loci that lie in paralogous regions generated by duplications. This model predicted what was later shown by database mining and sequence analysis. However, if database searching revealed that mouse ORs are organized in the genome similarly to humans, some important differences emerged (Young *et al.*, 2002; Zhang and Firestein, 2002). Indeed, the mouse genome contains approximately 1500 OR sequences (compared to ~960 in humans) and only 20% are pseudogenes, giving a functional repertoire in mice approximately three times larger than that of humans. Mice possess more than 850–1200 intact ORF-containing genes that map to only 46 genomic locations with very few to subtelomeric or centromeric bands. Also, the mouse gene repertoire seems more compact (less divergence between genes) than in humans, suggesting a rapid evolution/degeneration of the human

repertoire. Most of the characteristics found in humans were also found in mouse, that is:

1. OR genes are distributed on all chromosomes except 12 and Y in a number of subfamilies (228).
2. Roughly 140 class I OR genes are present on chromosome 7 in a region that is syntenic to human chromosome 11.
3. Most clusters are found at syntenic human locations, indicating that they predate primate–mouse divergence (Young *et al.*, 2002).

Another recent study (Godfrey *et al.*, 2004) presented similar data, i.e., approximately 1200 genes among which 24% are pseudogenes. As previously shown, the authors point out that most of the subfamilies are encoded by genes at a single chromosomal locus. As a result of the repertoire evolution/specialization, 22 subfamilies (29 ORs) are found only in humans, whereas 84 (177 ORs) are present only in mouse, and 150 are common to both species.

4.08.3.3.2 Dog Dogs also present several advantages for studying molecular genetics and olfaction. They were domesticated recently (~10000 years ago) and the numerous strains that present readily different morphotypes, sizes, and behaviors are the result of selective breeding, leading to the selection of particular genotypes that are valuable models for the study of genetic diseases (Ostrander *et al.*, 2000; Ostrander and Kruglyak, 2000; Ostrander and Comstock, 2004). On the other hand, all dogs are considered as macrosmatic animals even if they display a great heterogeneity in behavioral traits. The olfactory sensitivity of dogs is empirically known to be much higher than that of humans (Marshall *et al.*, 1981; Smith *et al.*, 2001). For example, trained animals are able to find hidden objects (drugs, explosives, food) or buried people after natural disasters such as avalanches or earthquakes (Ashton and Eayrs, 1970; Komar, 1999).

Different reasons have been proposed to explain the differences between the olfactory performance of dogs and humans. First, dogs have a MOE surface up to 20 times larger than that of humans (Moulton, 1967; Moulton *et al.* 1970; Issel-Tarver and Rine, 1997). This implies that the population of olfactory neurons is larger and might express a higher number of ORs, allowing the animal to develop a better olfactory sensitivity. Second, the brain structures devoted to olfaction, particularly the olfactory bulb, are substantially larger than in microsmatic primates (Stephan *et al.*, 1988). Third, as in the case of mouse, one might expect a high number of functional OR genes.

The dog OR repertoire was first studied by genomics and hybridization studies to assess whether dog OR genes were similar to those of human and mouse, also organized in clusters lying in syntenic regions to mouse, and conserved during evolution (Issel-Tarver and Rine, 1996, 1997; Carver *et al.*, 1998). The complete analyses came recently from the first release of the dog genome sequence (Quignon *et al.*, 2003; Olender *et al.*, 2004). The dog OR repertoire contains approximately 1200 OR genes that are distributed on 24 chromosomes ($n=39$) in 37 loci, and the fraction of pseudogenes is estimated to be 12–18%, similar to the range found in mouse. It seems that this percentage corresponds to the lower value in the gene repertoire of good smellers. Fishlike class I ORs occupy approximately 19% of the repertoire compared to approximately 12% in humans and mouse. The same 17 families are found, and 34 subfamilies are specific to dogs.

In summary, the OR repertoires of humans, mice, and dogs are composed of homologous receptor genes that are organized similarly in the genome. The main information is that there is a striking difference between macrosmates and microsmates, i.e., a higher number of OR genes with a low pseudogene content in macrosmates so that the functional repertoire of macrosmates is more than three times larger than that of microsmates. This difference correlates with the olfactory performance of these species.

4.08.4 Evolution of the OR Gene Repertoire

Starting from the observations cited above, it was interesting to determine whether the loss of functional genes (above the threshold of 20% pseudogenes) was specific to humans and if it was possible to trace the pseudogenization process during evolution. This study naturally focused on primates, since apes (higher primates) were not thought to rely on their sense of smell as mice or dogs do. Preliminary observations showed that some deleterious mutations were conserved from macaque (Old World monkey) to humans, suggesting that particular OR pseudogenes predated the divergence of these species (Rouquier *et al.*, 1998b). At the same time, the analysis of an OR cluster located on human chromosome 3 indicated that orthologous loci were found in syntenic regions of apes (chimpanzee, gorilla, and orangutan), suggesting a similar genomic organization (Trask *et al.*, 1998a, 1998b; Brand-Arpon *et al.*, 1999;

see The Evolution of Neuron Types and Cortical Histology in Apes and Humans). The study of a particular OR gene located on human chromosome 11 revealed a conservation among apes and a localization in syntenic regions. A prominent feature is that it was recently inactivated in humans (pseudogene) by a single deleterious stop mutation (Rouquier *et al.*, 1998a). This mutation was found in all the human populations tested, suggesting that it appeared during the divergence of humans from other apes 4–5 Mya. This observation indicated that the human OR repertoire is still evolving. Later, functional studies showed that the human gene probably incurred additional nonsynonymous mutations (Gaillard *et al.*, 2002, 2004a). The complete analysis in apes of the evolution of the OR gene cluster localized on chromosome *17p13.3* in humans has made it possible to analyze the dynamics of the evolutionary process (Sharon *et al.*, 1998, 1999). A comparison between OR genes in the cluster (paralogues) and between orthologous pairs of the different species indicated that in addition to duplications, gene conversion events are responsible for the evolution of the OR repertoire in the different species. Furthermore, this cluster contains 40% pseudogenes in humans and ape species, with some pseudogenes common to all species. From these observations, the decline of the OR repertoire seems to have occurred approximately 10 Mya before apes diverged. A survey of the OR gene repertoire of different primate species has permitted subsequent evaluation of the pseudogene content in most primate species (Rouquier *et al.*, 2000). This study indicated an increase of the pseudogenization process in humans (pseudogenes represent 70% of the OR gene repertoire), with respect to apes (~45%), Old World monkeys (~27%), and New World monkeys (2%), whereas prosimian lemurs also have a high pseudogene content (~37%) (see the phylogenetic tree in Figure 3). Although this sampling was biased by the PCR primers used for pulling out the OR genes, the general information indicated that there is a progressive loss of functional OR genes from Old World monkeys to humans. Humans present an acceleration in the pseudogenization level with more than 60% pseudogenes. In contrast, New World monkeys seem almost devoid of pseudogenes. This limit between New World monkeys and higher primates parallels that observed for pheromone detection, i.e., New World monkeys are the latest primates to possess an intact and functional VNO, the site of expression of pheromone receptors (see Giorgi and Rouquier, 2002 and references therein), whereas higher primates do not possess this organ. New World monkeys seem to rely heavily on olfaction by using both a VNO-mediated pheromone detection and an olfactory system similar to that of mice or dogs. A more accurate analysis (Gilad *et al.*, 2004) has been done recently on 19 primate species. The results are summarized in Figure 3. Actually, New World monkeys and lemurs have the lowest pseudogene content (~15–20%), similar to that of mice, whereas there is a striking acceleration in OR pseudogenization from Old World monkeys to humans. Surprisingly, the authors report that among New World monkeys, the howler monkey presents a pseudogene count similar to Old World monkeys (Figure 3). Remarkably, the howler monkey is the only New World monkey to possess full trichromatic vision, as do Old World monkey and apes. The authors suggest that the decline of the olfactory repertoire occurred concomitant with the acquisition of trichromatic vision. This observation is very interesting in terms of selective pressure and olfactory needs with respect to vision, and is in accordance with the studies cited above, but one cannot rule out that this could be coincidental. Another study from the same group compared the evolutionary forces that shaped the olfactory repertoires of humans and chimpanzees (Gilad *et al.*, 2003a). In both species, OR pseudogenes evolve neutrally, whereas intact OR genes are submitted to purifying selection in chimpanzees and to positive selection in humans. The authors suggest that selective pressure acted differently because of differences in lifestyle that led to different sensory needs. However, a recent article using the first release of the chimpanzee genome refuted the previous findings on the selective pressures, i.e., there may be a purifying selection over half the repertoire in humans and chimpanzees but no general positive selection (Gimelbrant *et al.*, 2004). Nonetheless, the very recent comparison of the OR repertoire of human and chimpanzee has allowed a refinement of these data (Gilad *et al.*, 2004). First, the OR pseudogene fraction in the human and chimpanzee is 56% and 50%, respectively, or 51% and 41% if the large 7E subfamily that contains only pseudogenes is excluded from the analysis. The number of OR genes in chimpanzee is approximately 26% higher than in human. Second, several subfamilies are either specific to chimpanzee or to human, and some genes are under positive selection in either species, and would therefore be likely candidates for adaptations, as a result of species-specific sensory requirement.

4.08.5 Conclusions

Olfaction requires the binding of odorants to specific receptors (ORs). ORs are encoded by the largest

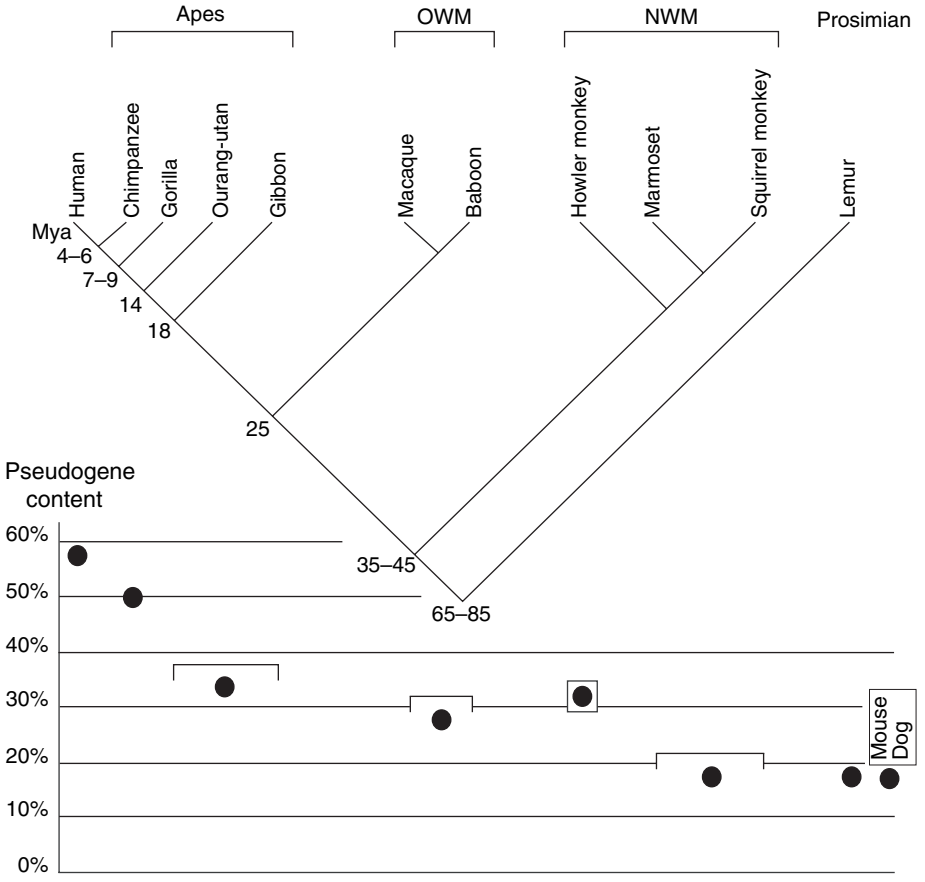

Figure 3 Schematic phylogeny tree (top panel) of the main primate branches used in the study of OR repertoire evolution. The times of divergence are indicated in Mya. OWM, Old World monkeys; NWM, New World monkeys. Lower panel, pseudogene content of the OR repertoire of the species indicated above. The dot for howler monkey is squared. Mouse and dog are indicated for comparison. Adapted from Gilad, Y., Wiebe, V., Przeworski, M., Lancet, D., and Paabo, S. 2004. Loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates. *PLoS Biol.* 2, E5.

multigene family (~1000 members) in the genome of mammals and occupies as much as 1% of the genome. However, there is a rapid decline in the functional fraction of the repertoire in primates, with humans having more than 55% nonfunctional pseudogenes. The pseudogenization process occurred mostly during primate evolution when Old World monkeys and higher primates diverged from New World monkeys. In parallel, genome analyses of macrosmatic mice and dogs revealed a higher number of OR genes with a pseudogene fraction of 20% or less leading to approximately three times more functional ORs than in humans. Although the functional repertoire is reduced in higher primates, most of the OR subfamilies are represented, suggesting that they probably sense the same odor spectrum as mice or dogs but with a reduced sensitivity. Obviously, the decline of the OR repertoire has been correlated to the olfactory ability of the different species. Higher primates are considered as poor smellers compared to dogs, and there is a strong parallel with their OR gene repertoire, even if genes are not the only cause of their reduced olfactory capacities. Indeed, selective pressure has played a major role. For example, when humans developed vision, bipedal posture (verticalization), and language, olfaction was relegated as a secondary need for survival (Shepherd, 2004). However, a number of psychophysical studies comparing the olfactory performance of different species such as primates, dogs, or rodents assume that humans display similar olfactory ability to the other species and sometimes even better, suggesting that humans (and higher primates) do not have a reduced sense of smell (Laska *et al.*, 2000, 2004). However, most of these studies use the sniffing of individual odorants and the data between species are probably very difficult to compare and control. Even if individual measures are likely correct, their interpretation when comparing species may be not an exact reflection of their respective olfactory performance (Giorgi and Rouquier, 2000). It is indeed

difficult to imagine humans detecting hidden explosives or people buried under two meters of snow by their sense of smell, even after intensive training.

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4.09 The Vomeronasal Organ and Its Evolutionary Loss in Catarrhine Primates

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Glossary

accessory olfactory bulb	A miniature, somewhat disorganized, but discreet, part of the forebrain, situated in a posterodorsal position within the olfactory bulb itself. It is the first site in the central nervous system to receive the vomeronasal neuroepithelial projections.
anthropoid	Taxa of the hyporder Anthropoidea, comprising New World monkeys, Old World monkeys, apes, and humans.
catarrhines	Taxa of the infraorder Catarrhini, comprising Old World monkeys, apes, and humans.
cercopithecoid	Old World monkeys.
haplorhine	Primates of the suborder Haplorhini, which include catarrhines, platyrrhines, and tarsiers.
hominoid	Apes and humans.
platyrrhine	Taxa of the infraorder Platyrrhini, comprising the New World monkeys.
receptor-free epithelium	Vomeronasal organ epithelium that is a columnar (frequently pseudostratified and/or ciliated) epithelium, but no receptor neurons.
strepsirrhine	Among primates, taxa of the suborder Strepsirrhini, comprising lemurs and lorises.
vomeronasal neuroepithelium	Vomeronasal organ epithelium that contains receptor neurons.
vomeronasal organ	Bilateral, peripheral receptor organs for the accessory olfactory (vomeronasal) system, a neural system linked to perception of mammalian pheromones.

4.09.1 The Vomeronasal Organ

4.09.1.1 Introduction

The vomeronasal organ (VNO) is a tetrapod innovation that functions in pheromonal and other chemosensory functions (see Evans, 2003; Halpern and Martínez-Marcos, 2003). Two functional categories of VNO are found in mammals: (1) chemosensory, with elements summed up as a VNO complex, and (2) a nonchemosensory homologue as in chimpanzee or humans (Smith *et al.*, 2001a, 2002). A generalized mammalian VNO complex comprises: (1) an epithelium arranged around a lumen; (2) a lamina propria containing blood vessels, glands, nerve fascicles (VNN), paravomeronasal ganglia; and (3) a cartilaginous or osseous capsule (Figures 1a, 1b, 1i, and 2a–2d). The tubular border comprises a ventromedial microvillar receptor neuroepithelium (VNNE) and a dorsolateral, ciliated receptor-free epithelium (RFE; Figure 1b). Posteriorly, the vomeronasal lumen ends blindly, but anteriorly it either leads to the nasopalatine duct, or opens directly into the nasal vestibule. The nerve fascicles ascend

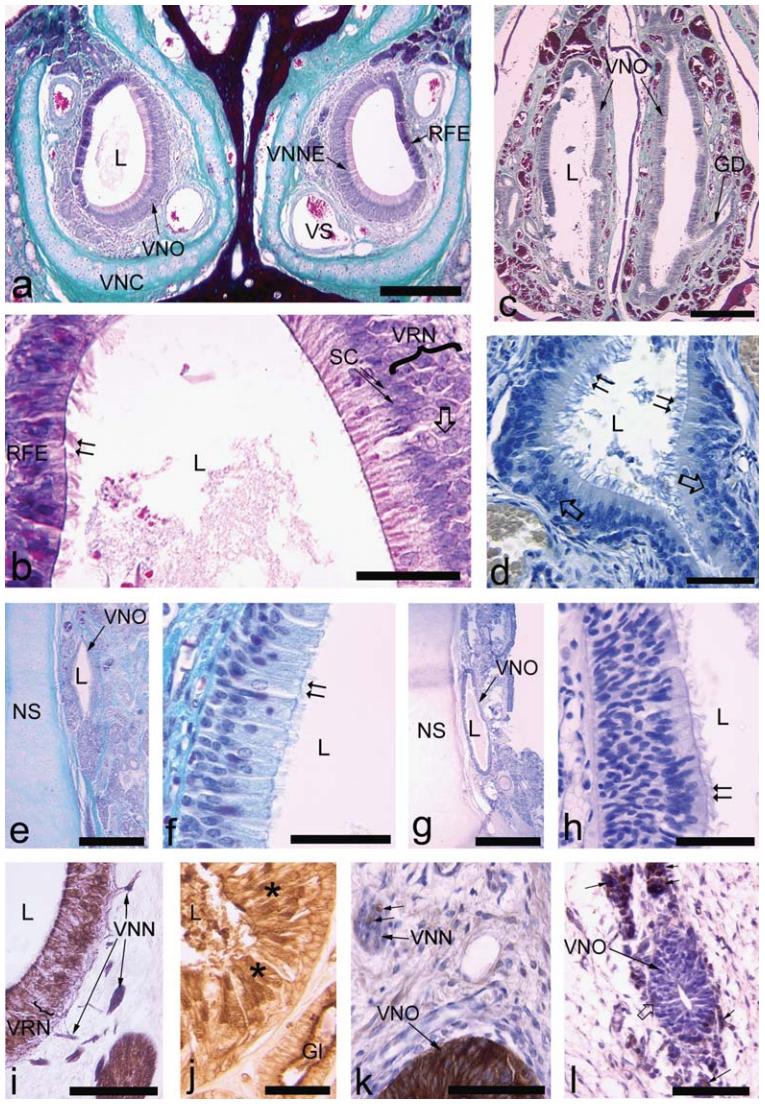

Figure 1 The vomeronasal complex of strepsirrhines such as *Cheirogaleus medius*, a, includes VNO, complete with VNNE (medially) and RFE (laterally), venous sinuses (VS), VNN, glands, and a surrounding capsule (vomeronasal cartilage, VNC). b, The RFE of this species is ciliated (double arrows) while the VNNE possesses an apical row of supporting cell nuclei (SC) and multiple rows of vomeronasal receptor neurons (VRN), which possess round, pale nuclei (open arrow). In adult platyrrhines (c, *Saguinus oedipus*), the medial/lateral differentiation of the epithelium is not evident, and at high magnifications (d) VRN (open arrows) and microvilli (double arrows) can be detected on all sides. In chimpanzees (*Pan troglodytes*, e) and humans (g), VNO homologues are found in an atypical position, displaced superiorly from the base of the nasal septum (NS) and devoid of a cartilaginous capsule. The VNO epithelium in chimpanzees (f) and humans (h) is pseudostratified, columnar, ciliated (double arrows). Reactivity of VNOs with lectins such as UEA-1 (i–l) differs among primates. VRN and VNN are highly reactive in strepsirrhines (i, neonatal *Eulemur macaco*), whereas in humans the mucins of cells resembling goblet cells (*) are strongly reactive (j, 2-year-old child). Presumptive neuron bodies in the VNO and within VNN are UEA-1-reactive in perinatal tamarins (k, *Saguinus geoffroyi*) and fetal humans (l, 9.5-week-old fetus). GD, gland duct; L, lumen. Scale bars: a, 250 μm; b, 50 μm; c, 500 μm; d, 70 μm; e, 500 μm; f, 50 μm; g, 1 mm; h, 50 μm; i, 100 μm; j, 50 μm; k, 70 μm; l, 70 μm.

in the nasal septal mucosa and reach the accessory olfactory bulb (AOB) through the cribriform plate. The AOB is found dorsally on the main olfactory bulb itself (Bhatnagar and Meisami, 1998).

The conclusion that the VNO mediates sociosexual behavior in mammals is supported by track-tracing studies in rodents showing vomeronasal connections to the amygdala and hippocampus and experimental removal of the VNO or VNN lesions (see Halpern and Martínez-Marcos, 2003). It is experimentally demonstrable that similar functions exist for the VNO of strepsirrhine primates (Aujard, 1997), although evidence for such behavioral links in New World monkeys is equivocal thus far. Among mammals, the VNO has been tied to the *Flehmen* behavior (see Evans, 2003, for review); among primates, this has only been observed in *Lemur catta* (Bailey, 1978). Other possible functions have not

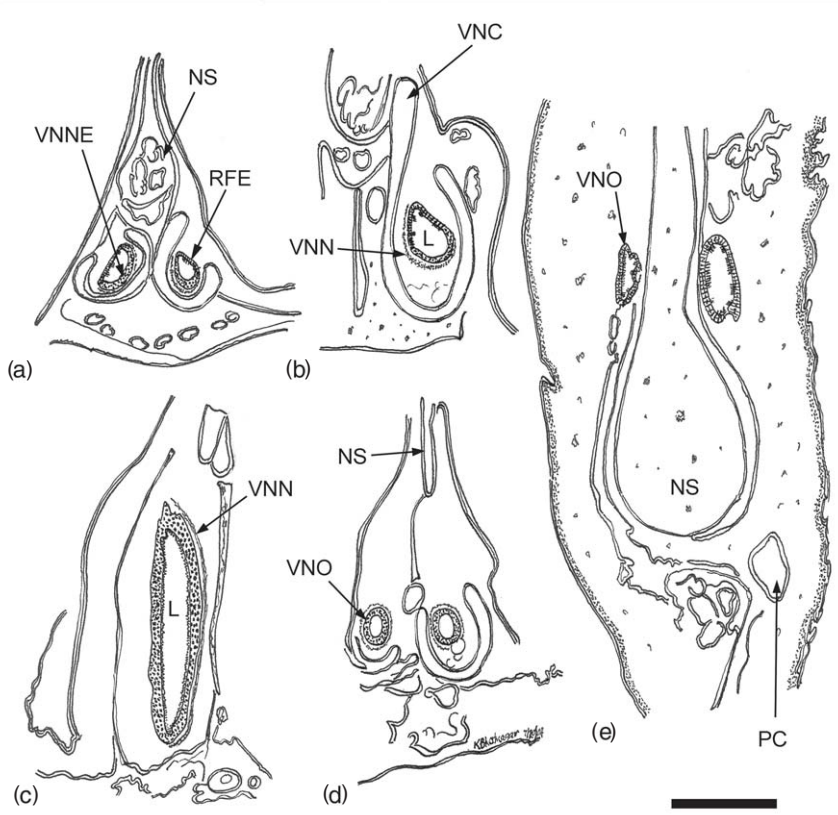

Figure 2 Schematic representations, via camera lucida tracings, of the vomeronasal complex in various primates. Strepsirrhines all have a complete vomeronasal complex (a, *Cheirogaleus medius*; b, *Otolemur crassicaudatus* (left side only)), including VNOs that possess a RFE, VNNE, VNN, and a surrounding vomeronasal cartilage (VNC). Platyrrhines (c, *Saguinus oedipus* (right side only); d, *Callithrix jacchus*) have VNOs with VNNE on all sides and possess VNN and a capsule. Catarrhines either lack a VNO entirely or possess a vestigial tubular VNO that lacks VNNE or VNN (e, *Pan troglodytes*). In such cases, the VNO is spatially separated from the paraseptal cartilages (PC), reduced homologues of the VNC. NS, nasal septum; L, lumen of VNO. Scale bar: 1 mm.

been carefully considered as yet (see Halpern and Martínez-Marcos, 2003). In humans and chimpanzees, the nonchemosensory homologue of the VNO likely serves as a secretory channel, funneling gland secretions to the nasal cavity (Smith *et al.*, 2002).

4.09.1.2 VNO Variability in Mammals

A functional VNO complex is seen in marsupials, rodents, phyllostomid bats, primates except the catarrhines, and all other mammals except the whales. Among placental mammals, taxa of entire suborders likely possess a functional VNO (e.g., species of Rodentia and Carnivora) i.e., with axonal connections to the forebrain (see Halpern and Martínez-Marcos, 2003). The order Chiroptera, in contrast, is characterized by extreme inter- and intrafamilial variations in the VNO complex (Bhatnagar and Meisami, 1998). In primates, such extreme variations have not been reported within families, but are likely to exist at subordinal taxonomic levels (see below).

4.09.2 The Primate VNO

4.09.2.1 Strepsirrhine VNO Complex

Although some of the 23 genera within this order remain uninvestigated, positive evidence of a functional accessory olfactory system exists for every family in this suborder (Schilling, 1970; Stephan *et al.*, 1982; Smith *et al.*, 2001a). Variations of the VNO within Strepsirrhini may only involve epithelial differences. The thickest VNNE is observed in the VNO of cheirogaleids such as *Microcebus murinus* (Schilling, 1970), *Phaner furcifer* (see Evans, 2003), and *Cheirogaleus medius* (T. D. Smith, unpublished observations; Figures 1a and 1b). *Cheirogaleus medius* exhibits a VNNE comprising multiple rows of receptor neurons reminiscent of the VNNE of

rodents. The RFE comprises pseudostratified, columnar, ciliated epithelium. Departures from this morphology have so far included mainly VNNE thickness and lack of cilia in some strepsirrhines (T. D. Smith, unpublished observations). In any event, it can be assumed that all strepsirrhines are endowed with a functional VNO complex.

4.09.2.2 Haplorhine VNO Complex

4.09.2.2.1 Platyrrhines and tarsiers An AOB has been reported in 13 of the 16 platyrrhine genera (Stephan *et al.*, 1982), and tarsiers. Switzer *et al.* (1980) noted an accessory olfactory formation (AOF/AOB) in several strepsirrhines and New World haplorhines, and the through course of the dorsal part of the lateral olfactory tract in relation to the AOF. Switzer *et al.* (1980) have considered the through condition as derived and the under condition of the AOF as primitive (see Evolution of the Neural Circuitry Underlying Laughter and Crying, Evolution of Vertebrate Olfactory Subsystems).

In all platyrrhines studied to date, VNOs have a VNNE (e.g., Smith *et al.*, 2001b, 2003a), although in adults the overall epithelial distribution in the VNO differs from that of strepsirrhines. Adult tarsiers have similar VNO traits. In contrast, our studies on prenatal and perinatal primates have revealed a similar partitioning of sensory and nonsensory divisions of the VNO epithelium among strepsirrhines, some platyrrhines, and tarsiers (Smith *et al.*, 2003a, 2003b; Dennis *et al.*, 2004). Thus, some haplorhines have a transient progression from the primitive VNO morphology to an atypical morphology common to adult platyrrhines and tarsiers (i.e., the VNO lumen is bordered by VNNE on all sides). We hypothesized that the adult morphology of tarsiers and platyrrhines probably reflects that of a haplorhine common ancestor (Smith *et al.*, 2003b). This altered VNO morphology could represent a synapomorphy of Haplorhini (see hypothetical node 'a' on the cladogram shown in Figure 3).

4.09.2.2.2 Catarrhine VNO complex Of the 65 genera of extant primates, 27 (40%) belong to catarrhines, most of which are confined to Africa, Asia, Southeast Asia, and associated islands. Whereas not many catarrhines have been examined by serial sectioning of the nasal septum for VNO presence/absence, 41 extant primate genera were studied for the presence/absence of an AOB (Switzer *et al.*, 1980; Stephan *et al.*, 1982; Table 1); of these, 13 genera (all catarrhines) lacked an AOB. The implication is clear: no extant catarrhines possess a functional accessory olfactory system. Interestingly, the lack of an AOB does not preclude the presence of a nonfunctional VNO (see below). However, two hominoids that possess VNOs are known to lack an AOB (Stephan *et al.*, 1982; Bhatnagar *et al.*, 1987) or VNN in the lamina propria (Bhatnagar and Smith, 2001; Smith *et al.*, 2002). Given this and other data on the VNO itself (see below), the adult hominoid VNO is an atavistic, nonchemosensory vestige.

Until recently, the VNO was reported to be postnatally absent in all apes (Jordan, 1972; Loo, 1973). Then, Smith *et al.* (2001a, 2002) reported homologous structures in postnatal chimpanzees. This has not been investigated in the other hominoids, although it was vaguely suggested that VNO homologues exist perinatally in other hominoids as well (cf. Evans, 2003). Regarding the presumptive hominoid VNO (Figures 1e–1h and 2e), which we hypothesize to be similar in morphology for all hominoids at some postnatal stage, there are positional and microstructural deviations from all other primates. First, the VNO is positionally displaced to a superior (dorsal) position relative to what is seen in any other primate VNO (Figures 1f, 1h, and 2c). The homology of this structure with the VNO of other mammals becomes apparent by comparing late embryos (Smith and Bhatnagar, 2000). Instead of a chemosensory neuroepithelium, a ciliated, pseudostratified columnar epithelium is found (Figures 1f and 1h) (Bhatnagar and Smith, 2001; Smith *et al.*, 2001a, 2001b, 2002).

4.09.2.3 Overview: Morphological Variants of the VNO in Primates

Smith *et al.* (2001b, 2003a) have listed five morphological variants of the primate VNO, including:

1. well-developed sensory epithelium (see description of strepsirrhine VNOs, above);
2. interrupted sensory epithelium (Figures 1c and 1d);
3. sensory epithelium only (Figure 2d);
4. displaced VNOs (Figures 1e–1h); and
5. VNO absence.

Although not all species have been examined, extant catarrhines have been found to exhibit either displaced vestigial VNOs (in at least some hominoids) or none at all (in all Old World monkeys).

4.09.2.4 VNO Functionality in Primates

4.09.2.4.1 Functional categories of VNO

4.09.2.4.1.(i) Chemosensory VNO As long as components of a VNO complex are present (see Figures 1 and 2, Table 1), we have defined it anatomically as a functional (chemosensory) VNO. Even

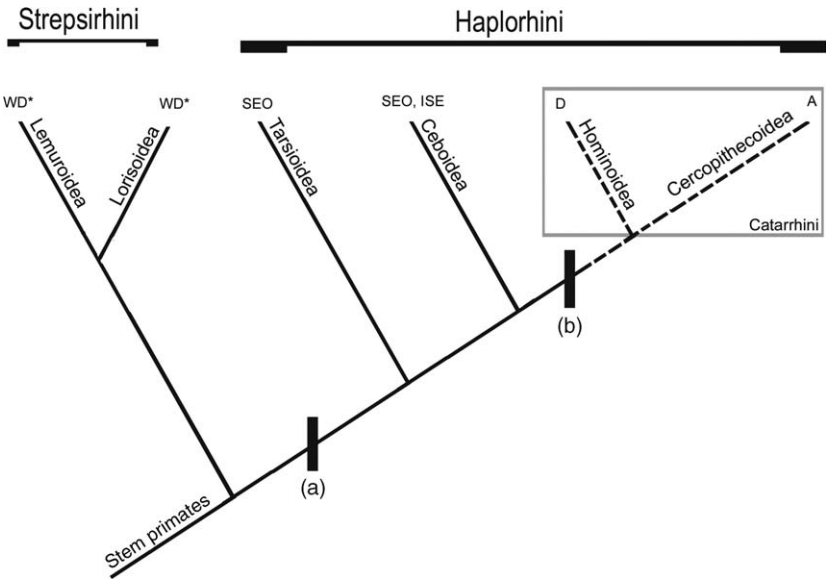

Figure 3 Cladogram based on strepsirrhine-haplorhine subordinal taxonomy (see Fleagle, 1999) showing the distribution of VNO morphologies among extant primates. All strepsirrhines possess well-developed (WD) VNOs with a neuropeithelium and RFE, as in most mammals. Extant tarsioids and ceboids possess VNO epithelium that is either sensory alone (SEO) or sensory epithelium interrupted by patches of RFE (ISE, interrupted sensory epithelium). In the extant Catarrhini (box), at least two hominoids possess a superiorly displaced, nonchemosensory VNO homologue (D), whereas all adult cercopithecoids appear to lack a VNO entirely (A). Node (a) indicates a proposed synapomorphy of Haplorhini (presumably including stem catarrhines), the absence of a distinct RFE. Node (b) indicates a proposed synapomorphy of Catarrhini, the loss of a VNNE. * Experimental evidence for VNO function is firm only for strepsirrhines.

though isolated chemoreceptor cells are distributed sparsely throughout the nasal cavity, the category of chemosensory VNO is distinguished by the presence of a VNNE with connections to the AOB. This stands in contrast to the sparse cells with neuronal characteristics described in the human VNO (Witt *et al.*, 2002). It must be emphasized that functionality is frequently assumed for platyrrhines; there is no anatomical evidence to refute this. However, experimental evidence that the VNO functions similarly in platyrrhines as in strepsirrhines is presently lacking (Table 1).

4.09.2.4.1.(ii) Nonchemosensory VNO In the adults of a few catarrhine VNOs examined (e.g., human, chimpanzee), only a ciliated duct has been observed. This duct clearly drains glandular secretions to the nasal chamber, but lacks a VNNE (Smith *et al.*, 2002). Similar situations are anticipated in the other catarrhines that have not been studied carefully, at least in other hominoids.

4.09.2.4.2 Evidence for VNO functionality among extant primates The basic evidence for postnatal VNO functionality is the presence/absence of the VNO itself or the AOB. Although cercopithecoids lack both, the persistence of the VNO homologue in humans, for instance, has fueled much controversy (see Bhatnagar and Smith, 2001; Wysocki and Preti, 2004). Yet several lines of evidence (Table 1) indicate two generalizations: (1) all strepsirrhines possess a chemosensory VNO that functions, at least in part, in pheromone reception; (2) no extant catarrhines possess chemosensory VNOs as adults. Platyrrhines possess all neural structures essential for VNO chemoreception (Figures 1c, 1d, 2c, and 2d), yet immunohistochemical and experimental evidence only promote more questions regarding the true nature of VNO function in these monkeys (Table 1).

This discussion is limited to postnatal functionality. The role of the prenatal primate VNO as a pool of migratory neurons has been explored little (see Evans, 2003), and such cells are poorly known in nonhuman primates. However, it is clear that cells reactive to neuronal markers (Smith *et al.*, 2004) or lectins such as *Ulex-europeanus-1* (Evans and Griogorieva, 1994; T. D. Smith and K. P. Bhatnagar, unpublished data; Figure 1i–1l) are found in the VNO lamina propria and vomeronasal and terminal nerves of prenatal and infant primates.

4.09.2.5 Phylogenetic Implications

4.09.2.5.1 Fossil evidence The VNO complex is a soft tissue structure and therefore extracting fossil evidence is not easy. Interesting speculation has centered on Eocene primates and inferences about their rhinarial (soft tissue of external nose) and upper lip

Table 1 Classification of living primates and functional status of the vomeronasal system

Taxonomic group[a] *(common names; genera)*	*AOB*[b,c]	*Other evidence for VNS functionality*
Suborder Strepsirrhini		
Infraorder Lemuriformes		
Superfamily Lemuroidea		
Family Indriidae (wooly lemurs, sifakas, indris; *Indri, Propithecus, Avahi*)	P	
Family Megalapididae (sportive lemurs; *Lepilemur*)	P	
Family Daubentonidae (aye-aye; *Daubentonia*)	P	
Family Lemuridae (lemurs and bamboo lemurs; *Lemur, Eulemur, Varecia, Hapalemur*)	P	IHC[d], Fl[e], G[f]
Superfamily Cheirogaleoidea		
Family Cheriogaleidae (dwarf and mouse lemurs; e.g., *Cheirogaleus*,[g] *Microcebus, Phaner*)	P	Exp[h]
Infraorder Lorisiformes		
Family Galagonidae (bush babies; e.g., *Galago*, *Otolemur*)	P	IHC[d,i]
Family Loridae (lorises, pottos; e.g., *Loris, Nycticebus*)	P	
Suborder Haplorhini		
Hyporder Tarsiiformes		
Infraorder Platyrrhini		
Superfamily Ceboidea		
Family Cebidae[j] (e.g., marmosets, tamarins, owl monkeys, spider monkeys; e.g., *Callithrix, Saguinus, Ateles, Alouatta*)	P	IHC (except *Saguinus*[b])[d]; Exp equivocal for *Callithrix jacchus*[k]; G[l,m]
Infraorder Catarrhini		
Superfamily Cercopithecoidea		
Family Cercopithecidae[j] (e.g., macaques, colobus monkeys; e.g., *Macaca, Papio, Colobus, Nasalis*)	A	G[n]
Superfamily Hominoidea		
Family Hylobatidae (gibbons and siamangs; *Hylobates*)	A	G[n]
Family Pongidae (orangutans; *Pongo*)	A	G[n]
Family Hominidae (e.g., chimpanzees, humans; *Pan, Homo*)	A	G[n]

[a] Taxonomic divisions mainly follow Fleagle (1999).
[b] Bhatnagar *et al.*, 1987;
[c] Stephan *et al.*, 1982;
[d] Dennis *et al.*, 2004;
[e] Bailey, 1978;
[f] Liman and Innan, 2003;
[g] data on perinatal animals;
[h] Aujard, 1997;
[i] Smith *et al.*, 2004;
[j] Not every genus examined;
[k] Barrett *et al.*, 1993;
[l] Webb *et al.*, 2004;
[m] Zhang and Webb, 2003.
[n] this line of evidence indicates reduction or loss of function in adults.
AOB, accessory olfactory bulb; A, absent; P, present; Exp, VNO ablation experiment showed behavioral deficits; Fl, *Flehmen* behavior observed; G, genetic evidence for functional signal transduction pathway; IHC, VNNE positive for neuronal markers.

morphology (Beard, 1988), although this has no direct bearing on VNO loss in Catarrhini. Recently, Rossie (2005) described a potential correlate of VNO function that is discernible based on osteology. Primates possessing a chemosensory VNO have a system of turbinals (from anterior to posterior, marginoturbinal–atrioturbinal–maxilloturbinal) that span from the margin of the bony nasal aperture to the nasal fossa proper. In this continuous series, muscles that attach to the most anterior turbinal (marginoturbinal) can affect airflow throughout the entire ventral (inferior) meatus, and therefore can affect access of nonvolatile chemostimuli, which preferentially travel in the ventral meatus. The nasal aperture of the nasopalatine duct, which provides a nasal access route to the VNO, is also located in the inferior meatus. Rossie (2005) noted that extant catarrhines have lost the atrioturbinal and thus, this path of chemostimulus access no longer exists. However, the continuous system of turbinals is present in some oligocene catarrhines (e.g., *Aegyptopithecus*), which may provide an important clue to the timing of VNO loss in cercopithecoids (Rossie, 2005).

4.09.2.5.2 Genetic evidence Among primates, genetic evidence supports the anatomical findings for the lack of a functional vomeronasal system in all catarrhines (Liman and Innan, 2003; Zhang and Webb, 2003). Most recently, such studies have focused on genes relating to function of the *TRPC2* ion channel, considered critical for accessory olfactory system functionality. Liman and Innan (2003) described evidence that an accumulation of mutated *TRPC2* genes has occurred since the divergence of catarrhines and platyrrhines, a finding consistent with the anatomical evidence (Dennis *et al.*, 2004; Table 1). Zhang and Webb (2003) asserted that *TRPC2* ion channels, and V1R pheromone receptors, were impaired about 23 Mya in primate evolution, before hominoids and Old World monkeys diverged. These findings have been interpreted as evidence supporting the hypothesis that sociosexual cues are detected via trichromatic color vision in Old World monkeys, and pheromonal communication is thus largely superfluous (and replaced) for this purpose. However, it is likely that some pheromonal communication occurs in catarrhine primates (Wysocki and Preti, 2004), even in the absence of a functioning vomeronasal system. Thus, functions usually attributed to this system are likely mediated by the main olfactory system in humans and other catarrhines.

4.09.3 Conclusions and Future Directions

Multiple lines of evidence are consistent with the hypothesis that the catarrhine VNO became nonfunctional (in pheromonal communication) prior to the divergence of the cercopithecoid and hominoid lineages (Smith *et al.*, 2001a, 2001b; Zhang and Webb, 2003; Rossie, 2005). At present, we speculate that the cercopithecoid-hominoid common ancestor possessed a VNO vestige similar to that seen in extant chimpanzees and humans (Smith *et al.*, 2001a, 2002). However, further investigation of the other extant hominoids (gibbons, orangutans, gorillas) is needed. If absent in these taxa (as previously claimed by Jordan, 1972; Loo, 1973), it would indicate that a postnatal VNO was lost in parallel in Old World monkeys and some hominoids.

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4.10 The Evolution of the Cerebellum in Anthropoid Primates

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Glossary

afferents	Incoming connections.
cerebellar vermis	The narrow, middle zone between the two hemispheres of the cerebellum.
dentate nucleus	The most lateral and largest of the cerebellar deep nuclei.
efferents	Outgoing connections.
hominid	Any living or extinct habitually bipedal hominoid, including members of the genus *Homo* and *Australopithecus*.
hominoid	Any living or extinct ape or human.
neocortex	A uniquely mammalian type of cerebral cortex involved in perception, thought, and reasoning, and consisting of six cytoarchitectonic layers.
premotor cortex	An area of cortex anterior to motor cortex, corresponding to Brodmann's area 6, that is involved in motor planning.

4.10.1 Anatomy of the Cerebellum

Much like the cerebrum, the cerebellum (Figure 1) consists of cortical gray matter overlying white matter within which lie subcortical nuclei, known as the cerebellar deep nuclei (Figure 2). However, in contrast to the six-layered cerebral cortex (see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates), the cerebellar cortex has only three layers. Within it, Purkinje cells are responsible for integrating excitatory and inhibitory inputs and providing output to the cerebellar deep nuclei, which in turn send projections out of the cerebellum (Figure 3). Thus, most cerebellar efferents originate in the deep cerebellar nuclei. In contrast to the cerebral cortex, cerebellar cortex is cytoarchitectonically homogenous and intercortical connectivity is minimal.

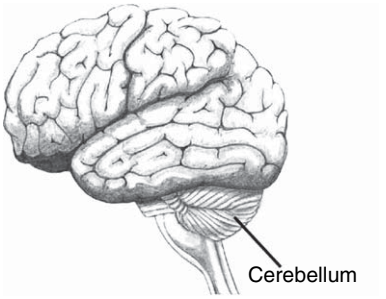

Figure 1 Location of cerebellum in lateral view of human brain. Reproduced from Memory Loss and the Brain. Copyright © Ann L. Myers/Memory Loss and the Brain.

The cerebellum is composed of four different anatomical regions: the flocculonodular lobe (or vestibulocerebellum), the vermis, the intermediate hemispheres, and the lateral hemispheres. The flocculonodular lobe is on the ventral surface of the cerebellum, abutting the brainstem. The vermis occupies the midline of the cerebellum and is separated from the cerebellar hemispheres by longitudinal furrows on either side. The intermediate portion of the hemispheres is just lateral to the vermis and just medial to the lateral portion of the hemispheres (Figure 3).

4.10.2 Cerebellar Connectivity and Function

4.10.2.1 The Flocculonodular Lobe

The flocculonodular lobe receives input from the primary vestibular afferents and projects back to the vestibular nuclei. This portion of the cerebellum

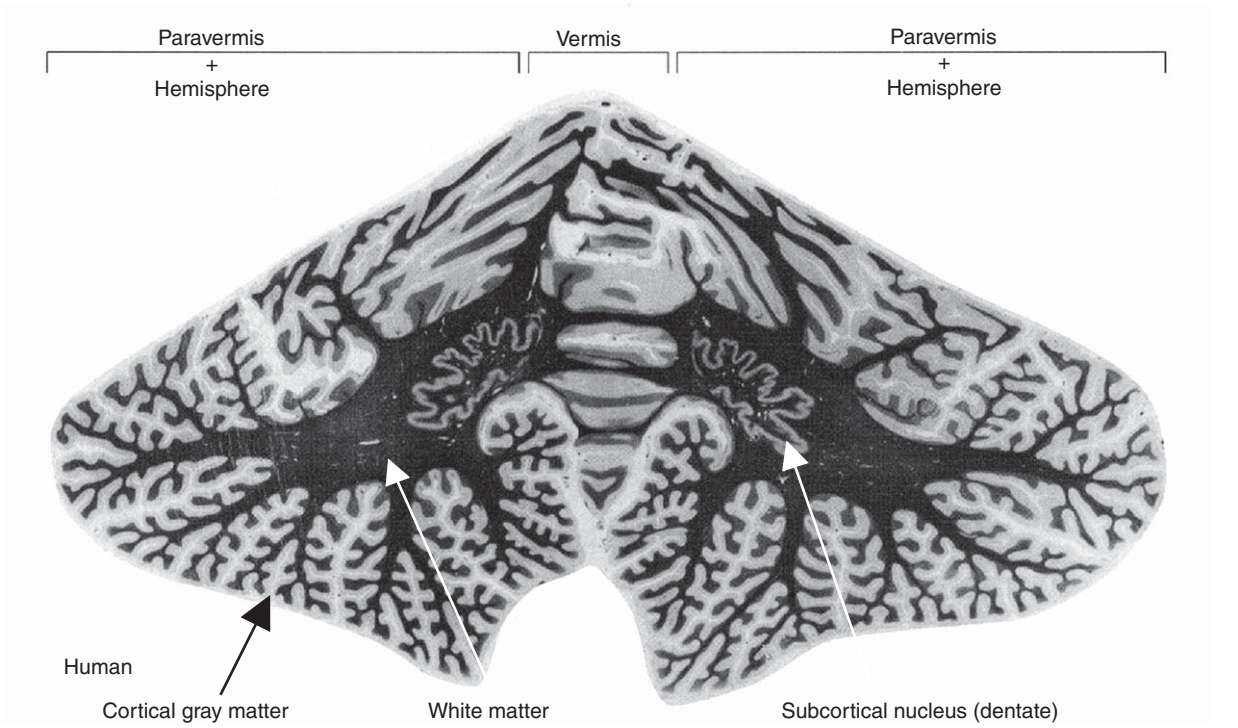

Figure 2 Cerebellar anatomy. Reproduced in full from Altman, J. and Bayer, S. A. 1997. Development of the Cerebellar System in Relation to Its Evolution, Structure and Functions. CRC Press.

governs eye movements and body equilibrium during stance and gait.

4.10.2.2 The Vermis

The primary sources of input to the cerebellar cortex of the vermis are the spinocerebellar tracts, which carry somatosensory information from axial and proximal body parts. This information is relayed from the cortex of the vermis to the most medial of the deep cerebellar nuclei: the fastigial nucleus. All of the deep cerebellar nuclei have both ascending (to midbrain, thalamus, and cortex) and descending (to brainstem and spinal cord) efferents. Fastigial efferents are predominantly descending, projecting to brainstem nuclei that control proximal muscles of the body and limbs (see Figure 3).

4.10.2.3 The Intermediate Hemispheres

The spinocerebellar tracts are also the primary source of input to the cortex of the intermediate hemispheres. The intermediate hemispheres relay information to the interposed cerebellar nuclei (globose and emboliform), which send both ascending and descending efferents to nuclei involved in coordination of distal limb muscles. The ascending projections reach the magnocellular portion of the red nucleus, which gives rise to the rubrospinal tract. Two somatotopic maps of the body are found in the cortex of the vermis and intermediate hemispheres (see Figure 3).

4.10.2.4 The Lateral Hemispheres

In contrast to the vermis and intermediate hemispheres, the lateral hemispheres receive little somatosensory input from the periphery. Instead, the main source of afferents to the cortex of the lateral hemispheres is the cerebral cortex. In macaques, these corticopontocerebellar fibers originate in motor, premotor, posterior parietal, cingulate, and prefrontal cortex and project to the lateral cerebellar hemispheres by way of the pontine gray matter (Brodal, 1978; Glickstein *et al.*, 1985; Schmahmann and Pandya, 1997). Output from the lateral hemispheres is directed to the most lateral of the deep cerebellar nuclei, the dentate nucleus. Most of the dentate's efferents ascend to either the parvicellular portion of the red nucleus or the ventrolateral thalamus, which in turn projects to motor, premotor, prefrontal, and posterior parietal cortices (Kelly and Strick, 2003), thereby forming a loop that links lateral cerebellar and cerebral cortex. Based on tract-tracing studies, Strick and colleagues have introduced the notion of closed cerebellar loops in which inputs from cerebral cortical areas are spatially segregated in both cerebellar cortex and the dentate nucleus. For example, projections from motor (e.g., BA4) and nonmotor (e.g., BA46) cortex

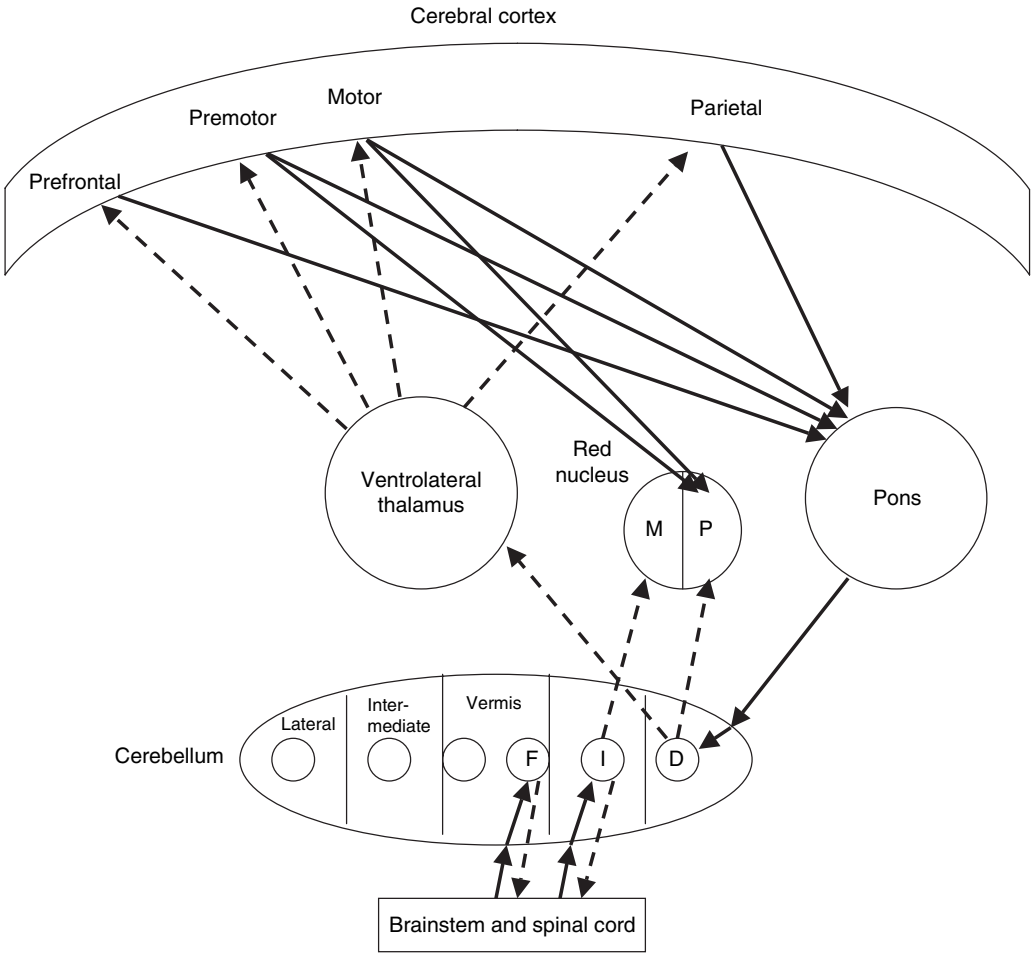

Figure 3 Schematic of cerebrocerebellar system showing prominent cerebellar afferents (solid lines) and efferents (dashed lines). M, magnocellular portion of red nucleus; P, parvicellular portion of red nucleus; F, fastigial nucleus; I, interposed nucleus; D, dentate nucleus.

have distinct, nonoverlapping representations in the cerebellum, both in the cortex (where they enter) and in the dentate nucleus (where they exit). Specifically, motor cortex projects to lobules IV–VI and nonmotor area 46 projects to crus II. In the dentate, the motor domain is dorsal and the nonmotor domain is ventral (Dum and Strick, 2003). The existence of these closed cerebellar loops is consistent with the suggestion that corticocortical projections are absent within the cerebellar cortex (Braitenberg *et al.*, 1997), in stark contrast to the cerebral cortex (see Figure 3).

Consistent with these anatomical data, evidence from neurological patients and functional neuroimaging experiments suggests that the lateral cerebellum of humans has both motor and nonmotor functions. Motor-related functions of the cerebellum include fine motor coordination, motor planning, and motor learning (Ghez and Thach, 2000). The cerebellum has also been implicated in a bewildering array of nonmotor functions, including sensory discrimination, spatial cognition, visuospatial problem solving, attention and attention switching, procedural learning, verbal working memory, verb generation, verbal fluency, lexical retrieval, syntax, semantic and phonological word retrieval, syntactic processing, and abstract reasoning (Dow, 1988; Leiner *et al.*, 1989; Courchesne *et al.*, 1994; Allen *et al.*, 1997; Desmond and Fiez, 1998; Schmahmann, 1998; Ghez and Thach, 2000; Rapoport *et al.*, 2000; Marien *et al.*, 2001). It seems unlikely that all of these processes can be subsumed under a single overarching function that the cerebellum performs. More likely, as originally suggested by Snider (1950), the cerebellum may be "the great modulator of neurologic function." That is, it improves the skilled performance of any cerebral area to which it is linked by two-way neural connections (Leiner *et al.*, 1989). Connections with motor areas would increase the speed and skill of movement and connections with cognitive areas would improve the speed and skill of cognition.

4.10.3 Comparative Cerebellar Anatomy

Evidence with respect to the evolution of the primate cerebellum (see Primate Brain Evolution in Phylogenetic Context) comes mainly from cross-species, comparative studies of living primate brains. Some of these studies are based on postmortem specimens (Stephan *et al.*, 1981), whereas others are based on MRI scans obtained from living primates (Rilling and Insel, 1999). Each data set has both advantages and disadvantages. The primary advantage of the postmortem sample is that it permits analysis of microscopic cytoarchitecture, which cannot be observed in MRI scans. On the other hand, the *in vivo* MRI scans (Figure 4) were obtained from healthy (not old or sick) animals and do not suffer from shrinkage artifacts caused by the postmortem tissue fixation process. Using these data sets, various authors have compared both the size and the histology of the cerebellum and its component structures across the primate order. Below is a summary of what these comparisons have revealed.

To begin with, Figure 5a illustrates variation in absolute cerebellum volume across a sample of 11 anthropoid primate species. At least some of this variation is likely attributable to variation in body weight, since most structures are larger in larger animals. Furthermore, the cerebellum is in close contact with the body surface, receiving somatosensory information from the periphery and modulating descending motor systems that control movement, so that we might expect cerebellar size to track body size. As can be seen in Figure 5a in which species are listed in ascending order of body weight, there is indeed a relationship between body weight and cerebellar volume. In fact, for the sample of 44 anthropoid primates in the Yerkes MRI data set, (log) body weight explained 87% of the variance in (log) cerebellar size ($r = 0.93$, $p < 0.001$; Figure 5b). Figure 5a demonstrates that the human cerebellum is larger than expected for a primate of our body weight. This is confirmed by Figure 5b in which cerebellar volume is plotted against body weight and a least-squares regression line is fit through the data. Much of the unexplained variance can be attributed to the human data points, which lie well above the prediction based on the regression line.

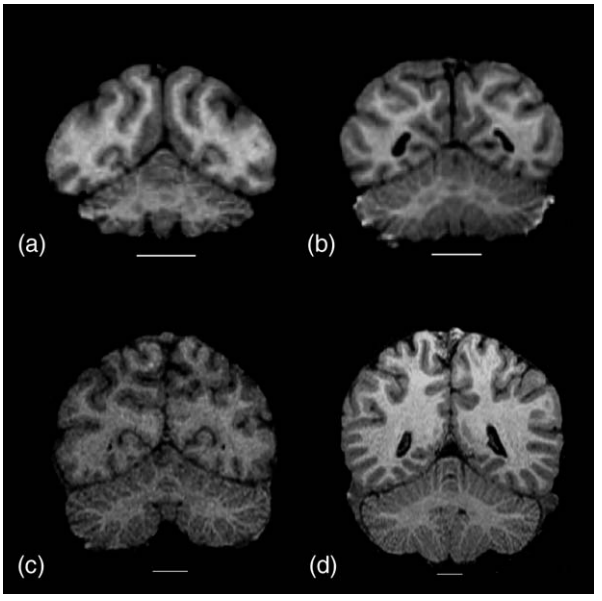

Figure 4 MRIs of primate cerebella. Coronal MRI through the cerebellum of: a, rhesus macaque; b, gibbon; c, chimpanzee; and d, human. Reproduced from Rilling, J. K. and Insel, T. R. 1998. Evolution of the cerebellum in primates: Differences in relative volume among monkeys, apes and humans. *Brain Behav. Evol.* 52, 308–314, with permission from Karger.

Is the cerebellum unusual in this respect or are other human brain structures also disproportionately large relative to body weight? In fact, the cerebellum is second only to the neocortex, albeit a distant second, in terms of its size relative to body size (Stephan *et al.*, 1988). The fact that the neocortex and cerebellum are the two structures that enlarged most relative to body size in humans, coupled with the existence of extensive connections between the two, suggests the possibility that the neocortex and cerebellum have evolved in tandem as a coordinated system (Barton and Harvey, 2000). Using the method of independent contrasts to eliminate the effect of common inheritance, Barton and Harvey showed that this is indeed the case for primates. Cerebellar contrasts are significantly correlated with neocortex contrasts, and this relationship is stronger than that of cerebellar contrasts and other major brain divisions, such as the medulla, mesencephalon, and diencephalon. In a subsequent analysis, Whiting and Barton (2003) demonstrated correlated evolution in other components of this cerebrocerebellar system among primates. For example, after partialing out evolutionary change in the size of the rest of the brain, they found that evolutionary changes in the size of the pons were positively correlated with changes in the size of both neocortex and cerebellar cortex. In other words, the corticopontocerebellar system that provides input to the cerebellum appears to evolve in concert. There was no significant correlation between pons and the deep cerebellar nuclei, in accord with the lack of direct connectivity between these two structures. On the other hand, there was a strong positive correlation between relative contrasts in deep cerebellar nuclei and relative contrasts in the thalamus, to

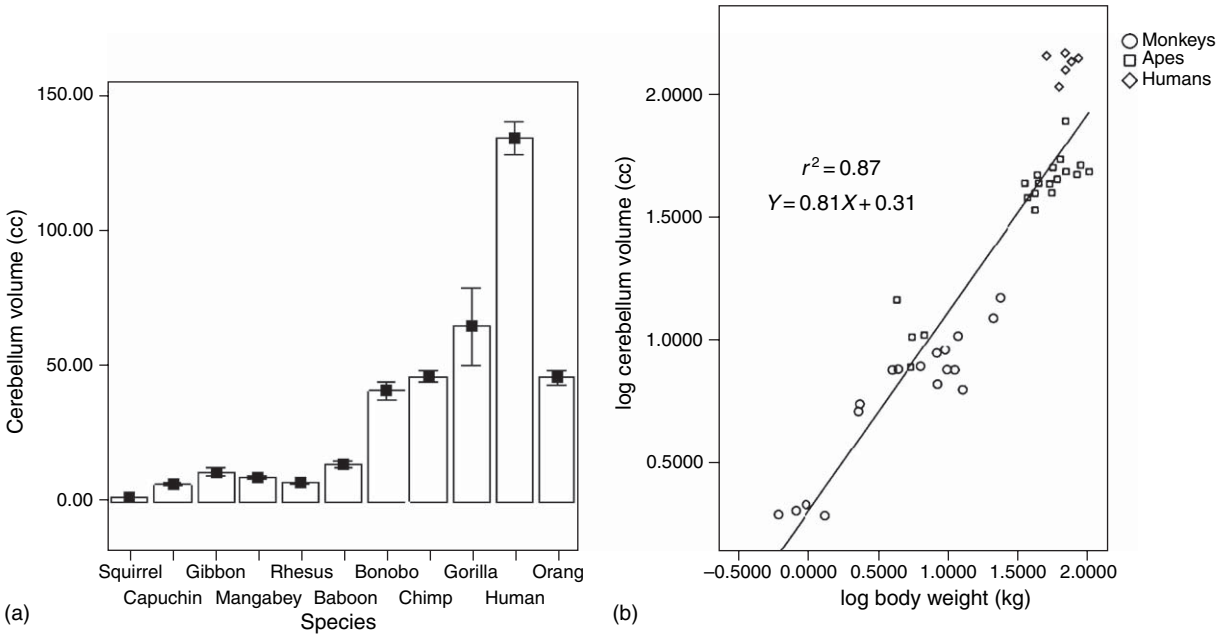

Figure 5 Comparison of cerebellum volume in anthropoid primates. a, Mean absolute cerebellum volume from MRI scans in 11 anthropoid primate species, in ascending order of body weight. Note that the gorilla body weight is low for the MRI data set because it is based on one small female and one subadult male. Error bars are ±1 SE. b, Regression of log cerebellum volume against log body weight, with least squares regression line fit through the entire sample.

which the nuclei send much of their output en route to the neocortex (see Figure 3).

4.10.4 The Ape Cerebellum Is Not an Allometrically Enlarged Monkey Cerebellum

Despite this tendency for the cerebrocerebellar system to evolve as a coordinated whole, it is clear that the relationship between the two structures was altered with the evolution of hominoids (Rilling and Insel, 1998; Semendeferi and Damasio, 2000). When cerebellum volume is regressed on cerebral cortex volume for anthropoid primates, there is a clear grade shift between apes and monkeys, indicating that apes have larger cerebella for any given cerebrocortical volume (Figure 6), and this difference is concentrated in the cerebellar hemispheres as opposed to the vermis (MacLeod *et al.*, 2003). Important differences between apes and monkeys are also found in cerebellar components. Matano and Hirasaki (1997) regressed the volume of the dentate nucleus on the volume of the medulla oblongata for a sample of 26 anthropoid primate species (from Stephan *et al.*, 1981) and found that apes have relatively larger dentate nuclei than monkeys. This was equally true of greater and lesser apes; however, there was an interesting difference between the two in the relative size of the interposed nucleus, which

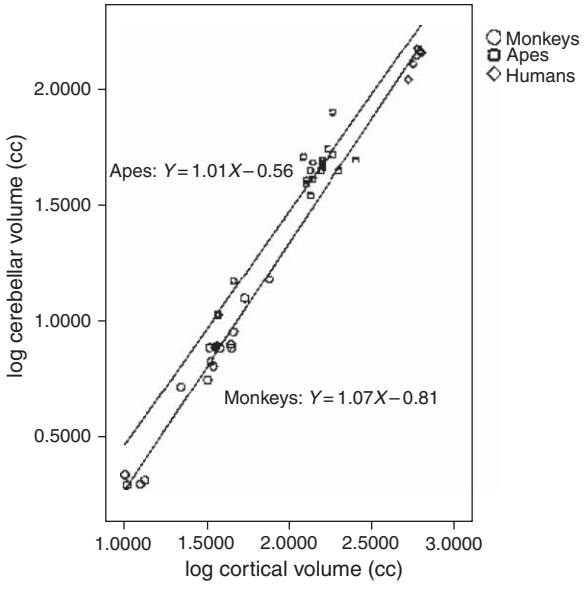

Figure 6 Comparison of cerebellum volume relative to cerebral cortical volume in anthropoid primates. Regression of log cerebellum volume on log cerebral cortical volume, with separate least-squares regression lines fit through the monkey and ape data. Human data points are plotted but not included in either regression. Data are from Rilling and Insel (1998, 1999).

was found to be markedly larger in lesser apes. This raises the possibility that Hylobatidae and Pongidae independently evolved to a similar grade of overall cerebellar development.

Given the anatomical connections between the cortex of the lateral cerebellar hemisphere and the dentate nuclei, the larger relative size of the ape dentate suggests that the expansion of the cerebellar hemispheres in apes may be concentrated in the lateral, rather than intermediate portion of the hemisphere. Detailed comparisons of the dentate nuclei reveal further differences between monkey and hominoid cerebella. On the basis of morphological, histological, embryological, histochemical, and pathological evidence, the dentate nucleus is thought to consist of two parts: an older dorsomedial and newer ventrolateral part (Dow, 1988) (Figure 7). Among anthropoid primates, the ventrolateral part is unique to humans and apes (Leiner *et al.*, 1991). Based on neuropsychological and neurophysiological evidence, Leiner *et al.* (1991) argue that the ventrolateral dentate sends output to nonmotor regions of the frontal lobe by way of the ventrolateral thalamus. This hypothesis will require further testing, perhaps with noninvasive *in vivo* methods such as diffusion tensor imaging (DTI) that allow visualization of white matter fiber tracts. However, if the hypothesis proves accurate, then the emergence of the ventrolateral dentate in hominoids could reflect a qualitative shift toward increased cerebellar involvement with cognition by virtue of connections with nonmotor frontal lobe regions.

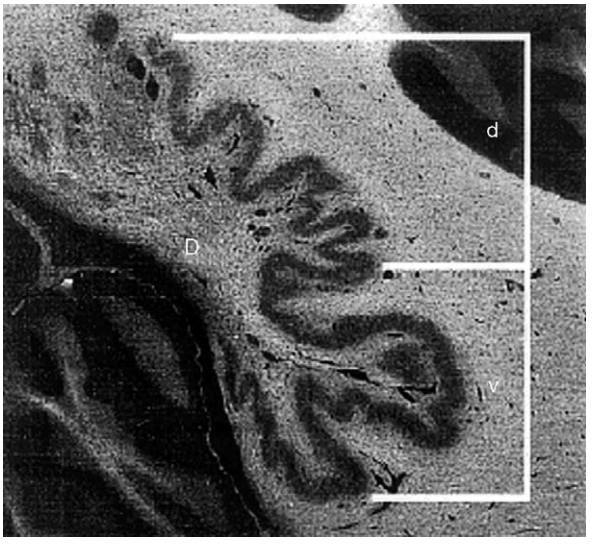

Figure 7 Dorsal and ventral aspects of the cerebellar dentate nucleus. Coronal section through dentate nucleus, showing dorsal and ventral halves. d, dorsal; v, ventral. Reprinted from Matano, S. 2001. Brief communication: Proportions of the ventral half of the cerebellar dentate nucleus in humans and great apes. *Am. J. Phys. Anthropol.* 114, 163–165. Copyright © 2001, Wiley-Liss. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

What might be the functional significance of the elaborated cerebellum in hominoids? As suggested above, the enlarged ape cerebellum might improve the functioning of all cortical regions with which it is connected. One cortical target of lateral cerebellar efferents is motor and premotor cortex, and it has been argued that apes have a greater complexity of movement than monkeys (Povinelli and Cant, 1995; MacLeod *et al.*, 2003). Also compatible with this augmentation of function in motor and premotor cortex is Ott's observation that, in contrast to apes and humans, baboons apparently lack presyntactical motor planning, the ability to modify current movements based on awareness of movements to follow (Ott *et al.*, 1994). Other skills that apes excel at relative to monkeys are also likely to be dependent on the motor and premotor cortex. For example, when reaching for an object, apes and humans exhibit more complex preshaping of their hand compared with monkeys (Christel, 1993; Christel *et al.*, 1998). In addition to these differences in motor-related functions, apes and monkeys also possess numerous cognitive differences. It is conceivable that cerebellar augmentation of prefrontal function could be involved in apes' putative capacity for self-awareness (Gallup, 1970), components of theory of mind (Tomasello *et al.*, 2003), and capacity for symbolic thought (Tomasello and Call, 1997). Each of these abilities is known to depend upon prefrontal cortex in humans (Deacon, 1997; Frith and Frith, 1999; Gusnard *et al.*, 2001; Gallagher and Frith, 2003).

4.10.5 Is the Human Cerebellum an Allometrically Enlarged Ape Cerebellum?

The human cerebellum is remarkably larger than the ape cerebellum, even after adjusting for differences in body weight (Figure 5). In fact, it is 2.8 times larger than expected for a nonhuman primate of equivalent body weight. Humans also have a larger dentate nucleus for their body size than apes (Matano and Hirasaki, 1997), and the difference is concentrated in the ventrolateral portion of the nucleus (Matano, 2001). Matano (2001) measured the area of the dorsal and ventral halves of the dentate in gorillas, chimps, and humans and calculated a ratio of ventral to dorsal areas in each species. The average human ratio (2.11) was much larger than the average ape ratio (1.64), indicating expansion of the ventral half in humans.

Although there are certainly absolutely more cerebrocerebellar connections in humans compared with apes, the degree of connectivity in humans is

probably less than what would be expected for an ape brain of human size. This is because humans fall below the ape regression line of cerebellar volume against cortical volume (Figure 6). This indicates that either (1) humans have small cerebella for their cortex size or (2) humans have large cortices for their cerebellar size. In fact, the existing data support the latter possibility given that the human cerebral cortex is disproportionately large when regressed on other brain structures, and the cerebellum is not disproportionately small when regressed on other brain structures (Deacon, 1988; Rilling and Insel, 1999). This result suggests that some of the cortical regions that expanded in humans did not maintain the degree of connectivity with the cerebellum that is found in apes or that cortical expansion was concentrated in regions that do not receive many cerebellar efferents. A likely candidate is temporal cortex, which is not intimately connected with the cerebellum and known to have expanded extra-allometrically in humans (Rilling and Seligman, 2002). Prefrontal cortex, when defined cytoarchitectonically, is also disproportionately large in humans (Passingham, 1973; Deacon, 1997; Preuss, 2000).

What could be the functional significance of the enlarged cerebellar hemispheres and ventrolateral dentate in humans? One possibility is accurate overhand throwing, which likely represented a strong selective pressure throughout our hunting and gathering past (Isaac, 1987; Calvin, 1993). Accurately throwing rocks and projectiles may have been crucial for hunting and scavenging prey, predator defense, and intergroup hostilities. Another possibility is that the enlarged human cerebellum supports fine motor coordination involved in the manufacture and use of tools (see Neurological Specializations for Manual Gesture and Tool Use in Humans). Humans are superior to apes in terms of manual dexterity. However, it is also clear that the cerebellum does indeed take on a cognitive role in humans, being involved in a wide range of mental operations (as discussed above). Leiner *et al.* (1993) have emphasized its role in language in view of neuropsychological evidence and hypothesized connections with BA44 (Broca's area). One attractive possibility is that the human cerebellum is involved in a more global augmentation of frontal lobe function that extends to cognitive domains beyond language.

4.10.6 Conclusion

1. Compared with monkeys, apes have larger cerebellar hemispheres for their brain size.
2. The dentate nucleus, the cerebellar deep nucleus that receives input from lateral cerebellar cortex, is also relatively larger in apes than in monkeys and relatively larger in humans than in apes.
3. The ventrolateral portion of the dentate, which may project to nonmotor regions of frontal cortex that are involved in higher cognition, is reportedly absent in monkeys and more developed in humans than in apes.
4. Although the human cerebellum is large relative to body size, the human cerebral cortex is even larger. Consequently, we have a large cerebral cortex for our cerebellum size, and the relative degree of cerebrocerebellar connectivity is probably reduced in humans compared with apes.
5. Given evidence for cerebellar connections with both motor and higher-order association cortex, the above differences in cerebellar anatomy among monkeys, apes, and humans could support motor and cognitive specializations of the three groups.

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4.11 Evolution of Ventral Premotor Cortex and the Primate Way of Reaching

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Glossary

anthropoids Monkeys, apes, and humans, but not prosimians.

catarrhines Old World primates, including Old World monkeys, apes, and humans.

corticobulbar Axonal projections from the cerebral cortex to the brainstem.

corticofugal Axonal projections from the cerebral cortex to subcortical targets.

corticospinal Axonal projections from the cerebral cortex to the spinal cord.

interaction torques Undesired forces generated by the movement of one body part on another part.

motor cortex (1) Primary motor cortex; (2) any part of frontal cortex directly involved in motor control, including premotor cortex.

motor pool A group of motor neurons in the spinal cord or brainstem projecting to a muscle.

nonprimary motor cortex Parts of the motor cortex other than the primary motor cortex, sometimes synonymous with premotor cortex.

orofacial Of the mouth and face, including the lips and jaw.

platyrrhines New World primates.

premotor cortex Cortex between the prefrontal cortex and the primary motor cortex, sometimes (but not always) excludes medial motor areas, such as the supplementary motor cortex.

primary motor cortex Abbreviated M1, sometimes called area 4, the motor strip, the precentral motor cortex, or simply the motor cortex.

propriospinal Systems of axonal projections connecting different segments of the spinal cord.

prosimians A group of primates that includes lemurs, lorises, bush babies, and tarsiers.

shoulder girdle A group of muscles that position the shoulder joint relative to the vertebral column.

4.11.1 Cortex for Central Control of Movement

The primate motor cortex comprises the primary motor cortex (M1) and several nonprimary motor areas (Figure 1; see The Evolution of Motor Cortex and Motor Systems). Among the latter, the ventral premotor cortex (PMv) appears to occupy a particularly pivotal place (Dum and Strick, 2005). Of the areas projecting directly to both the spinal cord and M1, PMv has the most interconnections with the prefrontal cortex (red arrow in Figure 1b). Much current thinking about PMv focuses on the neurophysiology of mirror neurons, cells that show similar activity regardless of whether a monkey performs an action or observes a similar one (Ferrari *et al*., 2003). This article, however, concentrates on information from other disciplines: comparative and connectional neuroanatomy, cortical stimulation, comparative morphology, and comparative psychology. Sections below briefly outline some findings from each of these fields in turn, followed by a summary, discussion, and conclusion.

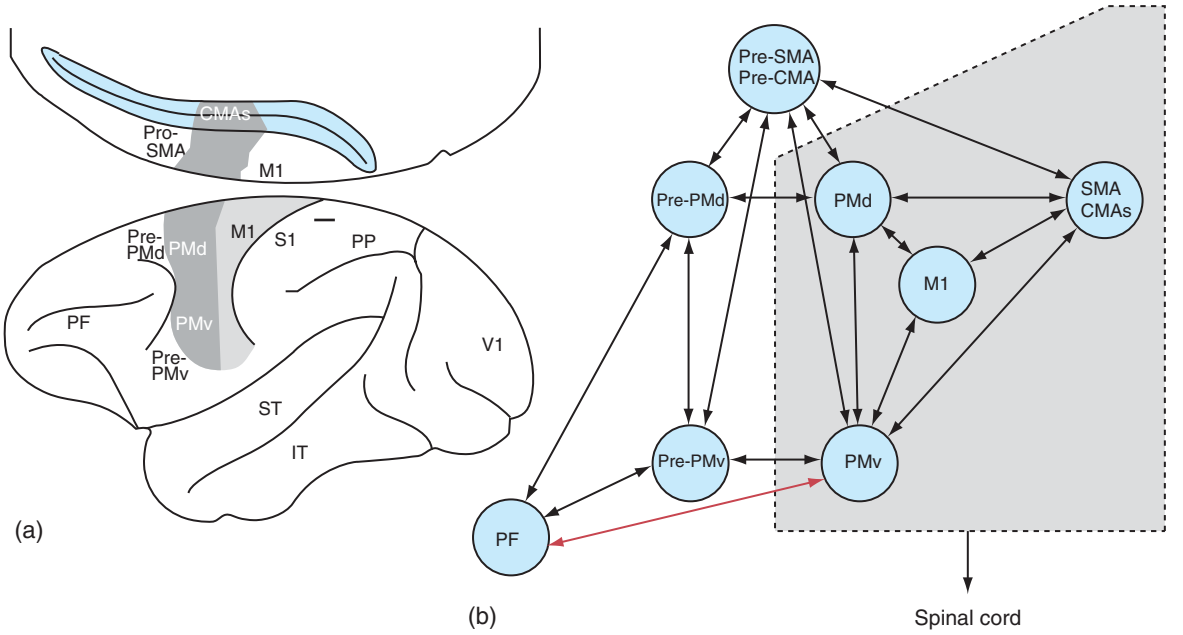

Figure 1 Motor cortical areas in macaque monkeys, their interconnections, and their direct outputs to the spinal cord. a, Brain drawing showing a left hemisphere; rostral is to the left. Ventral is down for the lateral surface of the hemisphere, but up for the medial surface, shown as if reflected at the top. The cingulate sulcus is shown opened to its fundus. b, The connectivity chart has thick lines suggesting stronger and functionally more important connections. The red arrow emphasizes the direct projection from the prefrontal cortex to PMv, which other spinally projecting motor areas (those in the shaded box) lack, at least to the same extent. CMAs, cingulate motor areas; IT, inferior temporal cortex; M1, primary motor cortex; PF, prefrontal cortex; PMd, dorsal premotor cortex; PMv, ventral premotor cortex; PP, posterior parietal cortex; Pre-PMd and Pre-PMv, areas rostral to PMd and PMv, which do not project directly either to the spinal cord or to M1; S1, somatosensory areas; SMA, supplementary motor area; ST, superior temporal cortex; V1, primary visual cortex. Data mainly from Dum, R. P. and Strick, P. L. 2005. Motor areas in the frontal lobe: The anatomical substrate for the central control of movement. In: Motor Cortex in Voluntary Movements (*eds.* A. Richle and E. Vadia), pp. 3–47. CRC Press.

4.11.2 Comparative Neuroanatomy

In their comparative study of the corticospinal system, Nudo and Masterton (1990a) found that corticospinal projections originate from three zones, which they called regions A, B, and C. Figure 2 shows the distribution of retrogradely labeled corticospinal neurons in a prosimian, a platyrrhine (New World) primate, and a catarrhine (Old World) primate. Nudo and Masterton examined two prosimians – lesser bush babies (Figure 2, top) and slow lorises; two New World monkeys – squirrel monkeys (Figure 2, middle) and common marmosets; and three Old World monkeys – green, cynomolgous, and rhesus (Figure 2, bottom) monkeys. They could identify region C, which corresponds to PMv, in each of the seven primates species they studied, but not in any of the other 15 mammals. Accordingly, they concluded that PMv's corticospinal projection evolved in stem primates and was a primate innovation.

When Nudo and Masterton (1990b) examined some possible functional correlates of region C, they found little relationship with manual dexterity and only a slightly stronger one with hand–eye coordination, evaluated in terms of whether an animal can easily view its hand or forepaw. Instead, they identified a robust relationship between the relative extent of region C and an animal's lifestyle, i.e., where it typically lives, sleeps, and eats. Nudo and Masterton (1990b) concluded that "the relative size of Region C depends mostly on an arboreal habit, both in nesting and in foraging" and suggested that

> It is the arboreal life-style of primates that may have provided the selective pressure for the origin and expansion of Region C . . .. The best clue is to be found in some motor trait necessitated by an arboreal life and more closely related to hand–eye coordination than to digital dexterity (Nudo and Masterson, 1990b, pp. 596–597).

4.11.3 Connectional Neuroanatomy

Corticospinal projections originate from neurons in several frontal and parietal areas (Figures 1 and 2) and PMv contains approximately 5% of these neurons in both macaque and capuchin monkeys (Dum and Strick, 2005). By far, most of PMv's corticospinal axons terminate in upper segments of

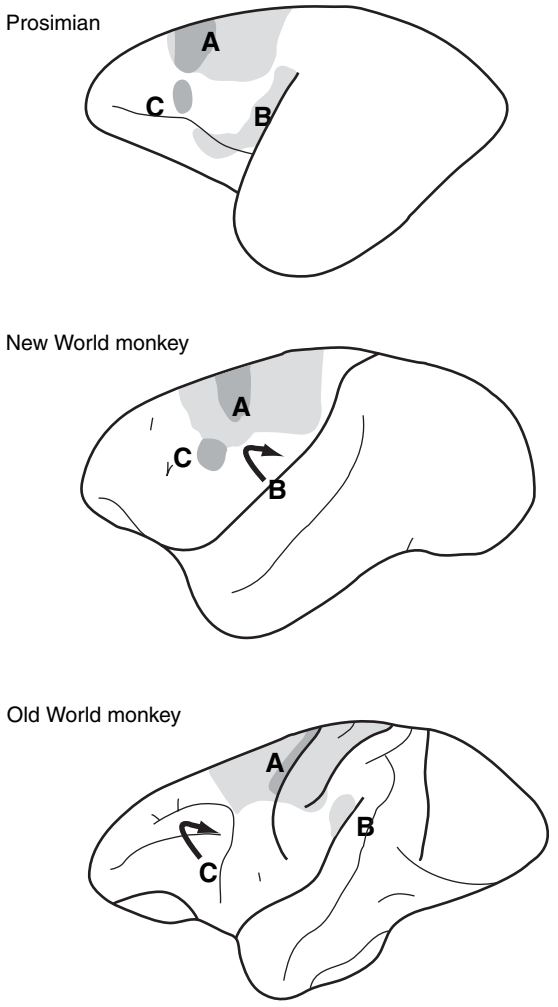

Figure 2 Corticospinal region C. Drawings of left hemispheres show the locations of three major zones of corticospinal neurons: regions A, B, and C. Rostral, left; dorsal, up. Region C, which corresponds to PMv, appears only in primates and is situated lateral to the main source of corticospinal projections, region A. Region A corresponds to the primary motor cortex, the dorsal premotor cortex, certain somatosensory areas, and posterior parietal area 5. Region B corresponds to other somatosensory areas. Reproduced from Nudo, R. J. and Masterton, R. B. 1990. Descending pathways to the spinal cord. III: Sites of origin of the corticospinal tract. *J. Comp. Neurol.* 296, 559–583, with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

the cervical spinal cord, which have motor neurons with four principal functions: control of head orientation via neck muscles, control of the shoulder girdle, regulation of inspiration, and tongue stabilization.

In addition to its projection to the spinal cord, PMv sends strong corticofugal projections to the brainstem, including to the facial nucleus, the medullary reticular formation, and the parvocellular red nucleus. PMv's projection to the facial nucleus targets motor pools controlling lower face muscles, specifically those of the lip and jaw. PMv, M1, and the caudal cingulate motor areas share a similar pattern of terminations in the facial nucleus, with PMv and M1 sending the densest projections. Neurons projecting to the facial nucleus and spinal cord are intermingled within PMv, with a predominance of the former (Morecraft *et al*., 2004). Thus, PMv projects predominantly to the facial nucleus, less but still significantly to the upper cervical spinal cord, and only weakly to other spinal segments.

Several neuroanatomists have studied connections among the various motor areas, mostly in macaques, but also in capuchins, owl monkeys, and galagos (Kaas, 2004). In capuchins and macaques, six premotor areas have been identified on the basis of connections with M1, including PMv. In galagos and owl monkeys, four to six premotor areas have been identified, also including PMv. An overview of the connections among the motor areas (Figure 1b) indicates that PMv serves as a key node in the network connecting the prefrontal areas, on the one hand, with M1 and the dorsal premotor areas, on the other. The identification of a ventral premotor area projecting to M1 in a prosimian primate, in two New World monkeys, and in Old World monkeys suggests that PMv had evolved by the time of the earliest primates (Preuss, 1993).

4.11.4 Cortical Stimulation

In rhesus monkeys, Graziano *et al*. (2002) inserted electrodes into PMv to electrically stimulate parts of it for periods of up to 1 s. This stimulation evoked a coordinated set of movements that included closure of the hand, smooth movement of the hand to the mouth at a speed appropriate for feeding, and mouth opening (Figure 3b). When the monkey's head could move, it rotated into alignment with the hand. Figure 3a shows the region from which such movements were evoked (blue shading), as well as the nearby regions generating other complex actions (gray shading). Graziano *et al*. concluded that PMv controls a particular class of behavior, one related to bringing the hand to the mouth during feeding.

4.11.5 Comparative Morphology

On the basis of fossil evidence, Bloch and Boyer (2002) extended earlier work by Cartmill (1974) and Martin (1990) to conclude that the first true primates appeared approximately 55 Mya as specialized graspers. In their view, stem primates evolved these and other specializations to feed on flowers, nectar, and tender leaves, which develop on the

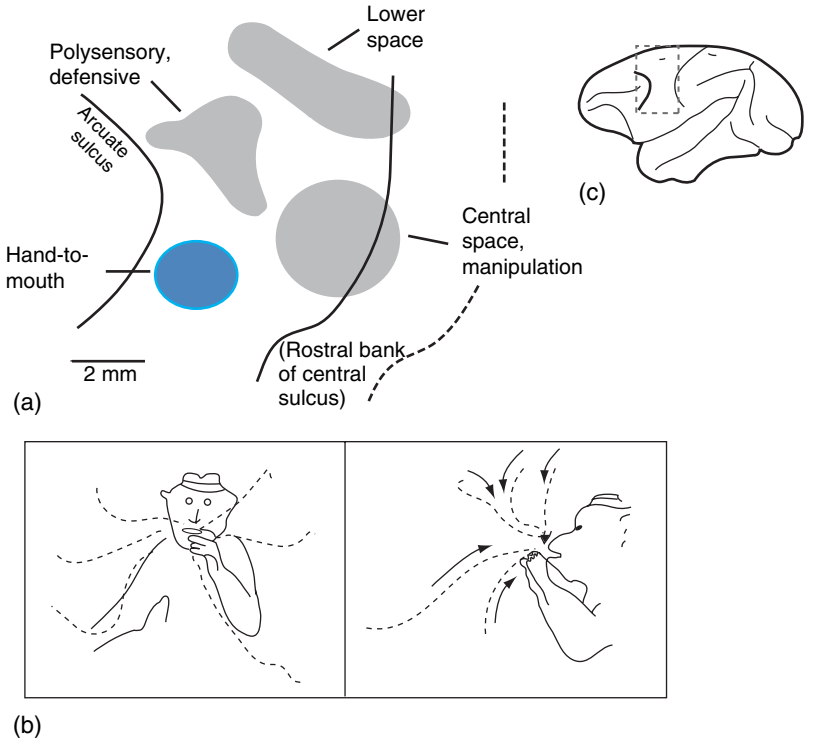

Figure 3 a, Regions of the frontal cortex from which complex, ethologically relevant movements can be evoked by long trains of electrical pulses. Note particularly the region, shaded in blue, ventral to the arcuate sulcus labeled hand-to-mouth. This region corresponds to PMv and contains the corticospinal neurons illustrated in Figures 1 and 2. The solid curved lines indicate the locations of the arcuate sulcus (left) and the central sulcus (right). The dashed line to the right represents the fundus of the central sulcus. b, Each dash shows the hand's position at a given point in time, as the electrical stimulation brings the monkey's hand to its mouth. c, Left hemisphere of a macaque brain; the boxed region (dashed lines) is shown expanded in (a). a, Courtesy of M. S. Graziano. b, Reproduced from Graziano, M. S. A., Taylor, C. S., and Moore, T. 2002. Complex movements evoked by microstimulation of precentral cortex. *Neuron* 34, 841–851, copyright 2002, Cell Press, with permission from Elsevier.

distal branches of trees. Bloch and Boyer also concluded that this specialized reaching and grasping ability preceded the capacity for advanced leaping and frontally directed eyes. Figure 4 shows their image of these extinct animals. Other paleontologists view the first true primates as more insectivorous and more visually oriented than the frugivorous species studied by Bloch and Boyer. Notwithstanding this ongoing debate, the feeding technique of the earliest primates probably involved visually guided reaching and grasping in an arboreal niche, whether primarily for insects, as emphasized by some, or for food items mainly available in the fine-branch niche, as emphasized by Bloch and Boyer.

The work of Radinsky (1979) also bears on these issues. He prepared fossil endocasts, which allowed him to examine sulcal patterns in extinct primates, and concluded that

> The fossil record of primate endocasts suggests that expansion of neocortex in general, expansion of visual cortex, and

Figure 4 A depiction of visually guided reaching movements in an early primate. Reconstruction of an extinct primate, genus *Carpolestes* (meaning fruit robber), drawn by Douglas M. Boyer. Reproduced from Sargis, E. J. 2002. Paleontology: Primate origins nailed. *Science* 298, 1564–1565, with permission from D. M. Boyer.

> possibly also reduction in the olfactory bulbs, had begun by 55 million years ago. ... Endocasts of some of the oldest known anthropoids suggest that by 25 to 40 million years ago, expansion of visual cortex and reduction in olfactory bulb size had progressed further, and was within the modern anthropoid range. Further, ... the central sulcus had appeared by that time, at least in Old World anthropoids. In both anthropoids and prosimians, expansion of frontal lobes lagged behind that of the rest of the brain (Radinsky, 1979, p. 22).

According to Radinsky, then, when the first true primates appeared approximately 55 Mya, the expansion of visual cortex had begun, but it took another 15–30 million years to reach an approximation of its modern state (see The Evolution of Visual Cortex and Visual Systems). Several researchers have suggested that binocular vision drove the expansion of the visual system in primates, and, if this is so, then the findings of Bloch and Boyer (2002) led to the idea that unimanual reaching preceded the rotation of the orbits into the more frontal orientation of anthropoid primates. This later development, then, probably led to increasing reliance on binocular depth information for reaching and a concomitant expansion of the visual cortex.

4.11.6 Comparative Psychology

MacNeilage *et al.* (1987), using the methods of comparative psychology, also proposed particular ways in which the earliest primates adapted their motor system to an arboreal environment (see The Evolution of Sensory and Motor Systems in Primates). They noted that modern prosimians are specialized for unimanual predation and supported the idea that life in a fine-branch, arboreal niche led to this adaptation. MacNeilage *et al.* further suggested that adaptation to this niche involved hemispheric specialization. Specifically, they argued for a left-hand (right hemisphere) specialization for visually guided movements, along with a right-hand (left hemisphere) specialization for postural support, as illustrated in Figure 4.

4.11.7 Consilience

To sum up the preceding sections, evidence from comparative morphology and psychology indicates that the earliest primates adapted to a fine-branch, arboreal niche that involved unimanual feeding (Figure 4). Cortical stimulation of PMv results in a coordinated movement of the hand toward the mouth, along with reorientation of the head (Figure 3). Evidence from comparative neuroanatomy indicates that PMv and its corticospinal projection first appeared in the earliest primates and correlates in extent with an arboreal life (Figure 2). And evidence from connectional neuroanatomy shows that PMv preferentially projects to spinal segments that control head and shoulder movements, brainstem nuclei that control the lips and jaw, and key cortical areas involved in making decisions and taking action, including the prefrontal cortex, the dorsal and medial premotor areas, and M1 (Figure 1).

4.11.8 Command and Control

If PMv evolved as an adaptation to an arboreal life of grasping fine branches and unimanual feeding, to which aspects of that life did it contribute most? The findings summarized here militate against certain possibilities. For example, specializations for hind limb grasping, leaping, and postural support would likely involve projections to the lumbar spinal cord. PMv sends very few axons there in macaque monkeys. Instead, PMv's corticofugal projections point to a role in controlling mouth, face, and head movements. If propriospinal systems in the upper cervical spinal cord contribute to reaching and grasping, then PMv's corticospinal efferents might also be important for these functions. Thus, the neuroanatomy suggests that PMv's contribution to movement has more to do with controlling the upper body than with controlling either the legs or the trunk and perhaps this was the case in the earliest primates, as well.

What does the upper part of the body need to do for unimanual feeding in a fine-branch niche? Two factors seem especially important: (1) coordination of head orientation with the hand movements that bring food to the mouth and (2) compensation for passive movements caused by the instability of distal tree branches and the food items on those branches, compensation for interaction torques produced by reaching and the forces involved in separating food items from their source, and compensation for Coriolis forces that accompany rotational movements as the hand reaches to grasp food.

As for the first factor, PMv's corticofugal projections – to the facial nucleus and to the upper cervical spinal cord – seem well suited to coordinate head and mouth movements with those of the hand. Unlike terrestrial quadrupeds, which often use whole-body movements to bring their mouths to food, unimanual feeding on a platform composed of fine, distal branches would require more flexible control of head orientation during feeding. Among other advantages, the ability for flexible reorientation of the head during feeding would minimize changes in the body's center of mass and the resulting instability that such body shifts would cause.

As for the second factor, consider the motor-control problems posed by swaying branches, undulating food items, and Coriolis forces. The last concept requires some explanation. When primates reach to a target in the outside world, as their body axis rotates, their body's rotation appears to cause a force on the limb that is proportional to the velocity of the hand's movement toward the target and the angular velocity of the rotation. To make a straight movement relative to the outside world, a reacher will have to curve its hand trajectory relative to itself, in the direction opposite to the body's rotation, and thus must exert the forces necessary to do so. When Coriolis forces are considered together with swaying branches that displace the reacher's body and targets that also move, it seems clear that unimanual reachers in an arboreal niche face major problems in motor control. One possible solution to these problems is to keep the position of the shoulder joint stable relative to the outside world. PMv might control movements of the shoulder girdle through its projections to the upper cervical spinal cord and thus compensate for body displacements, at least to some extent. This solution seems less important and less robust than a second one: continuously compute the location of the hand relative to the target and modify ongoing motor commands as the movement progresses – until the hand reaches the target.

PMv appears to play an important role in the latter solution. In a model of reaching and pointing in primates, Shadmehr and Wise (2005) pointed to neurophysiological studies indicating that PMv computes a difference vector, which corresponds to the distance and direction of the hand from the target of a reaching movement. The hand represents one among many kinds of controlled objects, collectively called end effectors, which include not only hands or parts of hands, but also tools such as remotely controlled cursors. Kakei *et al*. (2001) have shown that when macaque monkeys use wrist movements to control a cursor on a video screen, PMv's neuronal activity reflects the direction from the cursor's current position to the target. Importantly, it does so in terms of an extrinsic coordinate frame, based on vision, rather than an intrinsic coordinate frame, based on the joint-angle changes needed to reach the target. Along the same lines, Schwartz *et al*. (2004) have shown that a population of PMv cells reflects what the monkey sees in terms of a target's trajectory more than what the monkey does in terms of its hand's trajectory. These and other data support the idea that PMv contributes to the computation of a difference vector, which the motor system uses to drive the hand (or some other end effector) to a target. This computation appears to be fundamental to the primate way of reaching.

The primate way of reaching also involves using the difference vector to compute the next motor command and doing so in vision-based coordinates. Shadmehr and Wise (2005) called this component the next-state planner and the available evidence indicates that the primate motor system uses vision-based coordinates not only for guiding movements to visible targets, but also for guiding movements to acoustically localized targets and targets that cannot – even in principle – be seen, such as the back of a reacher's head. The dependence on vision-based coordinates leads to the counterintuitive prediction that the primate motor system must update its estimation of both hand and target position whenever the reacher makes an eye movement and there is evidence for such updating (see Shadmehr and Wise, 2005). Furthermore, if the target drifts as the movement progresses or if some external force passively repositions the reacher – both likely occurrences in a fine-branch niche – a motor system based on the difference vector and a next-state planner will adjust the hand's trajectory smoothly and accurately by continuously updating its computation of the difference vector until the hand reaches its target. This mechanism sometimes goes by the name autopilot control and it is fundamental to the primate way of reaching.

At first glance, the idea that PMv represents a difference vector for reaching movements seems at odds with a role in grasping or unimanual feeding. Indeed, orienting the head, bringing food to the mouth, reaching, and grasping differ in many important ways. They may, however, share a mechanism involving the difference vector. For example, when the head or face serves as the end effector, it could be pointed with a mechanism much like that involved in reaching. The fingers also are end effectors and grasping might involve similar computations, at least in part.

4.11.9 Communication

The capability for enhanced control of the head and orofacial musculature for feeding might also have served social signaling. MacNeilage (1998) has proposed that Broca's area and nearby regions such as PMv originally evolved to control feeding movements, but later came to underlie social communication, including language. According to his idea, motor programs that

evolved to control chewing and licking were later modified for use in lip smacks, tongue movements, and teeth-based noises that many primates use for social signaling. Along these lines, Ferrari *et al.* (2003) have reported that PMv neurons discharge during the observation or production of ingestive and communicative oral movements. PMv's projection to the motor nuclei that control the lips and jaw accords with MacNeilage's idea. Its projection to the upper cervical spinal cord does so, as well, because of the relationship between head orientation and social communication. The sender benefits from pointing his or her head toward an intended recipient – for both acoustic and orofacial signals – to focus acoustic energy, present the sender's face in the desired orientation, and avoid sight-line obstructions.

MacNeilage (1998) has further proposed that motor programs used in feeding served as a medium for the messages conveyed by voiced syllables framed in cycles of mandibular oscillations. PMv's projection to the upper cervical spinal cord, which has motor pools that regulate inspiration and stabilize the tongue, could have contributed to that development. In macaque monkeys, electrical stimulation of PMv evokes responses in muscles that lengthen and either tense or relax the larynx (Hast *et al.*, 1974), which supports the idea that PMv contributes to the control of vocal gestures.

4.11.10 Conclusions

The findings and ideas summarized here (see also Preuss, 1993) suggest that PMv evolved in the earliest primates as an adaptation for unimanual feeding in a fine-branch, arboreal niche. As such, its functions probably involved coordinating head orientation and orofacial movements with bringing food to the mouth, visually guided reaching and grasping, and postural support with the nonreaching arm and hand. PMv's projections to the facial nucleus and upper cervical spinal cord probably served these functions from the first.

Later, these same neural systems may have supported various forms of conspecific communication, as well as tool use. Reaching, when transformed into pointing, is fundamentally a communicative gesture. The control of head orientation contributes to both voiced and unvoiced social signaling and primates employ orofacial movements for a wide variety of signals. Finally, a mechanism for controlling reaching and grasping movements based on the computation of a difference vector provides a straightforward preadaptation for tool use, and neuroimaging studies by Chao and Martin (2000) point to PMv as a key contributor to such praxic functions in humans.

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4.12 Specializations of the Cortical Microstructure of Humans

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Glossary

aspiny or sparsely spiny nonpyramidal cells Constitute the typical interneurons but show a great variety of morphological, biochemical, and physiological characteristics. Their axons are found near the parent cell, but some have prominent axonal collaterals that run in the horizontal or vertical dimension (ascending and/or descending). Most of them appear to be GABAergic. They are found in all cortical layers and constitute the majority of interneurons and approximately 15–30% of the total neuron population.

basic microcircuit Repeating stereotypical circuit of neurons, composed by elementary operative units in which all elements of the cortex are represented.

GABA γ-amino-butyric acid, is the most abundant inhibitory transmitter in the CNS.

interneurons Short-axon cells or neurons whose axon does not leave the cortex (15–30% of the total neuron population).

macrocolumn The basic processing unit consisting of a cylinder of cortical tissue with a width that varies according to the spread of the specific individual afferent fibers entering the cortex (300–600 μm in diameter, depending on the cortical area and/or species). This macrocolumn is commonly referred to simply as a 'column'.

minicolumn The basic modular subunit in the cortex representing the smallest functional unit of cortical organization, the ensemble of several minicolumns making up the macrocolumns. These minicolumns are formed by a group of vertically oriented interconnected cells that are contained in a vertical cylinder of tissue of approximately 25–50 μm in diameter (depending on the cortical area and/or species).

pyramidal cells Constitute the vast majority of projection neurons, and located in all layers except layer I. They are characterized by the pyramidal or triangular shape of the soma and the presence of a prominent apical dendrite (70–85% of the total population). These neurons are glutamatergic and represent the main source of cortical excitatory synapses.

spiny nonpyramidal cells Constitute the typical neurons of the middle cortical layers (especially layer IV), comprising a morphologically heterogeneous group of neurons. Spiny nonpyramidal cells are excitatory (probably glutamatergic)

neurons whose axons are distributed within layer IV or in the layers above or below that in which their parent cell bodies are located.

4.12.1 Introduction

The great expansion and the differentiation of the neocortex constitute two major events during the evolution of the mammalian brain. Thus, it is particularly interesting to ascertain how the human neocortex has evolved to endow us with the capacity of speech and thought – faculties that distinguish humans from other mammals. In other words, the crucial question remains: what is special about the neocortex of humans and how does it differ from that of other species? Since the very earliest studies of the neocortex two main streams of thought were established. The first holds that the differences in the human neocortex are only quantitative, while the second holds that in addition to quantitative differences, important qualitative variations also exist. Santiago Ramón y Cajal made some of the most important earlier contributions to the study of the cerebral cortex organization. He beautifully summarized this subject as follows:

> At that time, the generally accepted idea that the differences between the brain of [nonhuman] mammals (cat, dog, monkey, etc.) and that of man are only quantitative, seemed to me unlikely and even a little offensive to human dignity [...] language, the capability of abstraction, the ability to create concepts and finally, the art of inventing ingenious instruments [...] do [these facets] not seem to indicate (even admitting fundamental structural correspondences with the animals) the existence of original resources, of something qualitatively new which justifies the psychological nobility of *Homo sapiens*? Microscope at the ready, I then launched with my usual ardor to conquer the supposed anatomical characteristic of the king of Creation, to reveal these enigmatic strictly human neurons upon which our zoological superiority is founded (Cajal, 1917; translated by J. DeFelipe).

Among other authors, Cajal tried to apply the same reasoning that was followed when comparing the structures in different functional regions of the cerebral cortex to the study of comparative anatomy between species. In this way he hoped to determine whether it was possible to explain functional specialization through structural specialization:

> [...] for example, if an organizational detail is found exclusively in or is particularly exaggerated in the visual cortex, we will be justified in suspecting that it has something to do with [cerebral visual function]. Conversely, if an anatomical detail is repeated equally in all cortical regions, we will be justified in assuming that it is devoid of specific functional significance and instead is of more general [significance] (Cajal, 1899a).

Thus, Cajal and other authors thought that it was essential to carry out comparative histological studies to see whether any structural peculiarities existed in the human cerebral cortex that might yield a key to specific human behaviors. How Cajal changed his general scheme of neuronal classification when he studied the cortex of small mammals (rabbit, rat, and mouse) and after examining the human cortex is shown in Figure 1 (Cajal, 1917). In this way he reached the following conclusion:

> [...] my investigations showed that the functional excellence of the human brain is intimately linked to the prodigious abundance and unwonted wealth of forms of the so-called neurons with short axon (Cajal, 1917).

However, as stated above, other researchers did not necessarily support these observations. A good

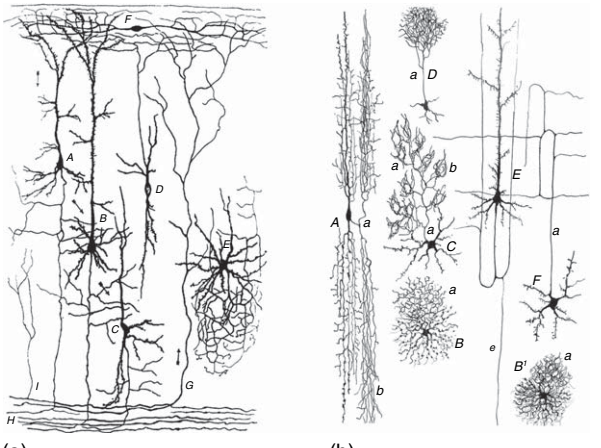

Figure 1 a, The principal cellular types based on the works of Cajal (1890–1894) on the cerebral cortex of small mammals (rabbit, guinea pig, rat, and mouse). A, pyramidal cell of medium size; B, giant pyramidal cell; C, polymorphic cell; D, cell whose axon is ascending; E, cell of Golgi; F, special cell of the molecular layer; G, fiber that terminates freely in the thickness of the cortex; H, white matter; I, collateral of the white matter. b, The principal cellular types based on the works of Cajal (1899–1902) on the human cerebral cortex. A, bitufted cell; B, dwarf cell with short axon; C, basket cell; D, dwarf cell with axon resolving into a tuft; E, pyramidal cell with arciform collateral branches ("small pyramidal cell characterized by an axon that is almost completely exhausted in giving rise to very long arciform and recurrent collaterals"); F, cell with ascending axon dividing into very long horizontal branches. Cajal (1917); referring to this figure, he says, "These cellular elements, particularly the first [A], second [B], fourth [E] and sixth [F], are extremely numerous and can be considered unique to the human cerebrum." – a, Reproduced from Cajal, S. R. 1894. The Croonian Lecture: La fine structure des centres nerveux. *Proc. R. Soc. Lond.* 55, 444–468, with permission. Copyright Herederos de Santiago Ramón y Cajal. b, Reproduced from Cajal, S. R. 1917. Recuerdos de mi vida, vol. 2. Historia de mi labor científica. Moya, with permission. Copyright Herederos de Santiago Ramón y Cajal.

reflection of the scientific thought at the time (against the idea of the qualitative differences between humans and other species) is provided by a disciple of Cajal, Rafael Lorente de Nó, who wrote the following:

> And by comparing our illustrations [regarding the mouse cerebral cortex] with those of our master (Cajal, 1899a, 1899b, 1900, 1901, 1911) that refer to the human cerebral cortex, it is difficult to find a detail that characterizes the latter. Neither the existence of special cells nor more [cortical] layers – qualities which are attributed to the human cortex – can be verified. Rather, we only note that in the [human cortex] there are a greater number of cells, but this in no way departs from the fundamental architectural plan (Lorente de Nó, 1922).

An important factor underlying the idea of the basic uniformity of the neocortex is its rather uniform appearance in Nissl stained sections, despite its functional diversity. Indeed, with the exception of some cortical regions such as the primary motor and visual areas, most of the areas are difficult to distinguish. This point was emphasized by Bailey and von Bonin (1951): "After long and careful study of the human isocortex the main impression we have retained is that vast areas are so closely similar in structure as to make any attempt at subdivision unprofitable, if not impossible."

Therefore, the notion that cortical information processing should be performed through ensembles of neurons organized into small, multiple, repeating microcircuits is both reasonable and attractive. The functional differences between the various cortical areas in different species or within the same species could be explained as the result of their different connections with afferent projection systems and efferent target structures. Thus, it is generally thought that the complexity of the neocortex increases in larger brains during evolution due to the addition of microcircuits with the same basic structure. As a result, it has been considered unlikely that new types of neurons or other fundamental differences would exist. In this article we shall discuss certain features of the neocortex that have been identified through comparative microanatomical studies that emphasize the differences between the human neocortex and that of other mammals. We will see that the human neocortex shows unique specialization, likely to be crucial for human cortical function.

4.12.2 Neuronal Components of the Neocortex

According to the collective work of a number of laboratories (reviewed in Peters and Jones, 1984), the neuronal components of the neocortex can be summarized as follows. The neurons can be divided into three major groups: pyramidal cells, spiny nonpyramidal cells, and aspiny nonpyramidal neurons. 'Pyramidal cells' are the most abundant cortical neurons (70–85% of the total population), constituting the vast majority of projection neurons, and they are located in all layers except layer I. These neurons are glutamatergic and represent the main source of cortical excitatory synapses. They are frequently subdivided according to their projection site. 'Spiny nonpyramidal cells' are, with some exceptions, short-axon cells (spiny interneurons) that are located in the middle layers (especially in layer IV). Various types of spiny nonpyramidal cells are recognized on the basis of their dendrite morphology, axonal arbors, and laminar connections. The typical spiny nonpyramidal cell (granule cells of layer IV) is an excitatory spiny stellate cell, and this cell type is considered by a number of authors to be a modified pyramidal cell. However, there is relatively little information about this group of cells and we will not deal with these cells in detail in the present article. 'Aspiny nonpyramidal neurons' are short-axon cells with smooth dendrites or dendrites that have only sparse spines (smooth interneurons). They are found in all layers, and represent the vast majority of short-axon cells and 15–30% of the total population of neurons although they show a wide morphological variety. Most smooth interneurons express GABA and are the main source of cortical inhibitory synapses. The different types of smooth interneurons are characterized by their particular synaptic connectivity and the expression of a variety of other neurotransmitters, neuroactive peptides, and calcium-binding proteins (reviewed in DeFelipe, 1993; Kawaguchi and Kubota, 1997; Somogyi *et al.*, 1998; Gupta *et al.*, 2000; Markram *et al.*, 2004; DeFelipe *et al.*, 2005). These attributes confer great functional diversity on them.

4.12.3 Cortical Circuits: Widely Shared Features of Connectivity

At present, there is little information on the detailed synaptic connectivity of pyramidal neurons and smooth interneurons (reviewed in DeFelipe *et al.*, 2005). However, these connections follow relatively simple rules: pyramidal cells establish synapses with the somata and dendrites of smooth interneurons, and with the dendritic spines (mainly) and shafts of pyramidal cells, avoiding the somata of these neurons. Smooth interneurons establish synapses with the somata and dendrites of other smooth

interneurons, and with all regions of pyramidal cells (dendrites, soma and, in some cases, the axon initial segment). Furthermore, interneurons do not necessarily form synapses exclusively with other particular types of neurons, nor are their synapses restricted to pyramidal or nonpyramidal cells, or to a single postsynaptic region. However, they do generally show a preference for certain postsynaptic partners. Thus, on the basis of the preferred postsynaptic region of the pyramidal neurons, four different groups of interneurons can be recognized (Figure 2):

- *Axo-dendritic cells (AD cells).* Cells forming synapses only (or almost only) with dendrites (shafts and spines).
- *Axo-somatodendritic cells (ASD cells).* Cells forming multiple synapses with both the dendrites and somata, but with a preference for somata.
- *Axo-dendrosomatic cells (ADS cells).* Cells forming multiple synapses with both the dendrites and somata, but with a preference for dendrites.
- *Axo-axonic or chandelier cells (CH cells).* Cells forming synapses with only the initial axon segment.

With the exception of chandelier cells that establish synapses exclusively with the axon initial segment of pyramidal cells, the other types of interneurons examined so far form synapses with both pyramidal cells and other smooth interneurons (Figure 2). The synaptic connections of large or classical basket cells, chandelier cells and double bouquet cells are three examples of smooth interneuron types that are morphologically and neurochemically well characterized, and that can be considered as common components of cortical circuits in primates (Figure 3). Each type innervates different regions of the pyramidal cell: large basket cells form synapses with the soma and proximal and distal dendrites (shafts and spines); chandelier cells with the axon initial segment; and double bouquet cells with the basal and apical collateral dendrites (shafts and spines). Regarding the subcortical and long-range corticocortical afferent systems, they generally establish synapses with dendrites of both pyramidal and smooth interneurons, avoiding the cell bodies of pyramidal neurons (reviewed in White, 1989). However, it has been shown that some subcortical afferents may form multiple contacts (synapses or appositions) with the somata and

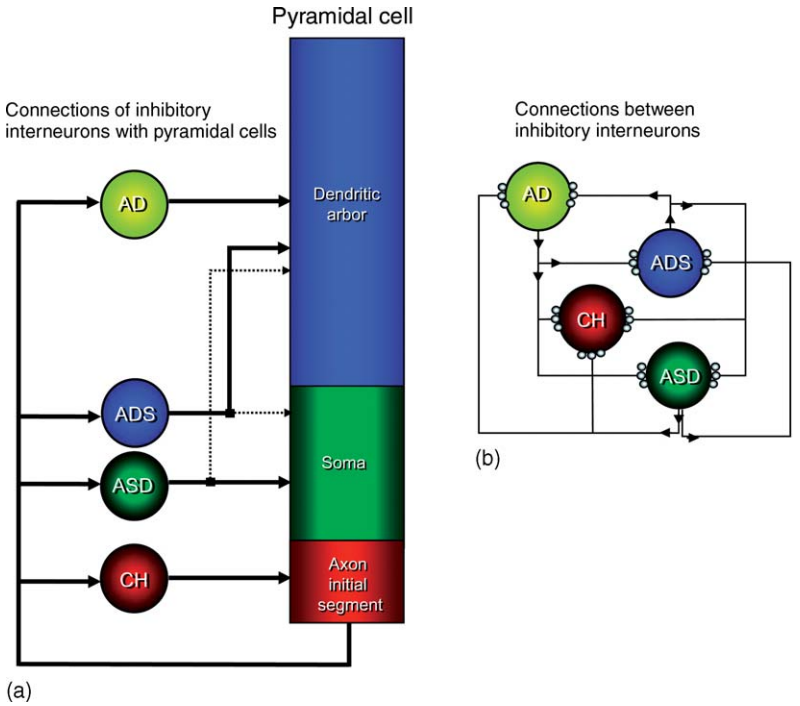

Figure 2 a, Illustration of the synaptic relationships between interneurons and pyramidal cells. Four different groups of interneurons can be recognized: axo-dendritic cells (AD cells); axo-dendrosomatic cells (ADS cells); axo-somatodendritic cells (ASD cells); and chandelier cells (CH cells). b, Illustration of the connections between interneurons. All interneurons are presumably connected with other interneurons, except CH cells, which only connect with pyramidal neurons. The connections between the same types of cells (for example, AD cells with AD cells) are not included for simplicity.

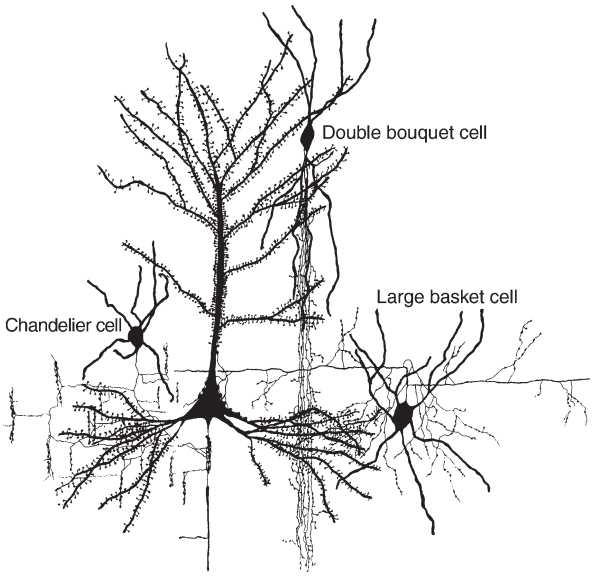

Figure 3 Illustration of the synaptic relationships between double bouquet cells, chandelier cells, and large basket cells with pyramidal cells. These cells constitute the best morphologically and chemically characterized types of aspiny nonpyramidal neurons. From DeFelipe, J. and Fariñas, I. 1992. The pyramidal neuron of the cerebral cortex: Morphological and chemical characteristics of the synaptic inputs. *Prog. Neurobiol.* 39, 563–607.

proximal dendrites of certain smooth interneurons (DeFelipe *et al.*, 1991; Freund and Gulyás, 1991).

4.12.4 Elementary Units of Operation: Macrocolumns and Minicolumns

Rafael Lorente de Nó was the first to propose that the cerebral cortex is composed of elementary operative units in which all elements of the cortex are represented. He conceived these units as small cylinders formed by vertical chains of neurons that crossed all cortical layers and had specific afferent fibers along their axes (Lorente de Nó, 1938). In the 1950s, Mountcastle was the first to provide physiological evidence for the columnar organization in the somatic sensory cortex of the cat and monkey (Mountcastle, 1957; Powell and Mountcastle, 1959). This organization was later investigated both anatomically and physiologically, and confirmed in a number of cortical areas by numerous investigators. Of these, the studies of Hubel and Wiesel in the visual cortex of the cat and monkey were the most detailed and convincing, corroborating the notion of this organization (for reviews see Hubel and Wiesel, 1977; Mountcastle, 1978, 1997; Jones, 1983, 2000a; Buxhoeveden and Casanova, 2002). Mountcastle proposed two types of vertical organization: the macrocolumn and the minicolumn (Mountcastle, 1978, 1997). The macrocolumn is the basic processing unit and consists of a cylinder of cortical tissue the width of which varies according to the spread of the specific individual afferent fibers entering the cortex (300–600 μm in diameter, depending on the cortical area and/or species). This macrocolumn is commonly referred to simply as a 'column' and is therefore the equivalent to the elementary cortical unit of operation of Lorente de Nó. In contrast, the minicolumn is the basic modular subunit in the cortex, representing the smallest functional unit of cortical organization, the ensemble of several minicolumns making up the macrocolumns. These minicolumns are formed by a group of vertically oriented interconnected cells that are contained in a vertical cylinder of tissue of approximately 25–50 μm in diameter (depending on the cortical area and/or species). Accordingly, Mountcastle (1997) defined the macrocolumn as a complex information processing and distributing unit that links a number of inputs to a number of outputs via overlapping internal processing chains (minicolumns).

The number of neurons within a strip of tissue with similar dimensions to a functional minicolumn (30 μm wide by 25 μm thick from the pial surface to the white matter) was counted by Rockel *et al.* (1980) in the motor, somatic sensory, visual, frontal, parietal, and temporal regions of the mouse, rat, cat, monkey, and human. The absolute number of neurons found in all areas and in all species was approximately 110, with the exception of the binocular part of area 17 of the monkey and human where there were approximately 2.5 times more neurons. In addition, earlier electron microscope studies had found that the proportion of neurons that could be characterized as pyramidal and nonpyramidal neurons through their ultrastructure was the same in various cortical areas of the monkey, rat, and cat (Sloper, 1973; Tömböl, 1974; Sloper *et al.*, 1979; Winfield *et al.*, 1980). These observations led Rockel and his colleagues to propose that the intrinsic structure of the neocortex is essentially more uniform than previously thought, and that differences in cytoarchitecture and function reflect different extrinsic connections. To date, these studies have had a strong influence and have provided much of the support for the dominant idea that the cerebral cortex is composed of multiple, small, repeating microcircuits and that the variations between cortical areas and species are insignificant, if we disregard the differences in the afferent and efferent connections (e.g., Douglas and Martin, 2004). However, we will see that there are a number of findings that strongly suggest this to be a gross oversimplification.

4.12.5 Observations That Challenge the Uniformity of the Neocortex

4.12.5.1 Distribution of Neurons

The uniform number of neurons within a narrow vertical piece of neocortical tissue from the pial surface to the white matter reported by Rockel *et al.* (1980) could not be confirmed in the mouse, rat, cat, or human (e.g., Beaulieu and Colonnier, 1989; Beaulieu, 1993; Skoglund *et al.*, 1996; DeFelipe *et al.*, 2002a). These later studies took advantage of more powerful quantitative methods that had been developed, such as the dissector method of Sterio (1984). In this way, differences in the number of neurons in cubes of 50 μm wide by 50 μm thick were identified from layer I to layer VI in the human, rat, and mouse (Figure 4). Furthermore, the conclusion that the proportion of pyramidal and nonpyramidal neurons was the same in various cortical areas of the monkey, rat, and cat based on electron microscope studies (Sloper, 1973; Tömböl, 1974; Sloper *et al.*, 1979; Winfield *et al.*, 1980) has been challenged. This is because it is frequently difficult to unequivocally identify pyramidal and nonpyramidal cells at the electron microscope level and also because the size of the samples analyzed with the electron microscope was relatively small. Since most nonpyramidal neurons are smooth GABAergic interneurons (Houser *et al.*, 1984; DeFelipe, 1993), the introduction of immunocytochemical techniques to visualize neurons expressing GABA or its synthesizing enzyme GAD has made it possible to obtain a more accurate picture of the proportion of aspiny nonpyramidal neurons. Using this approach in the rat, it was found that GABAergic cells represent no more than 15% of the total population in all cortical areas and layers II–VI (e.g., Meinecke and Peters, 1987; Beaulieu, 1993; Micheva and Beaulieu, 1995; Gabbott *et al.*, 1997). In the macaque monkey, they constitute 20% in the visual cortex and up to 25% in other cortical areas, even reaching up to 34–44% in supragranular layers of certain areas of the macaque and human (Hendry *et al.*, 1987; Beaulieu *et al.*, 1992; Gabbott and Bacon, 1996; del Río and DeFelipe, 1996). Therefore, there are clear differences between the rats and primates in terms of the proportion of smooth interneurons.

It is important to emphasize that there are more than 4500 mammalian species, but the vast majority of

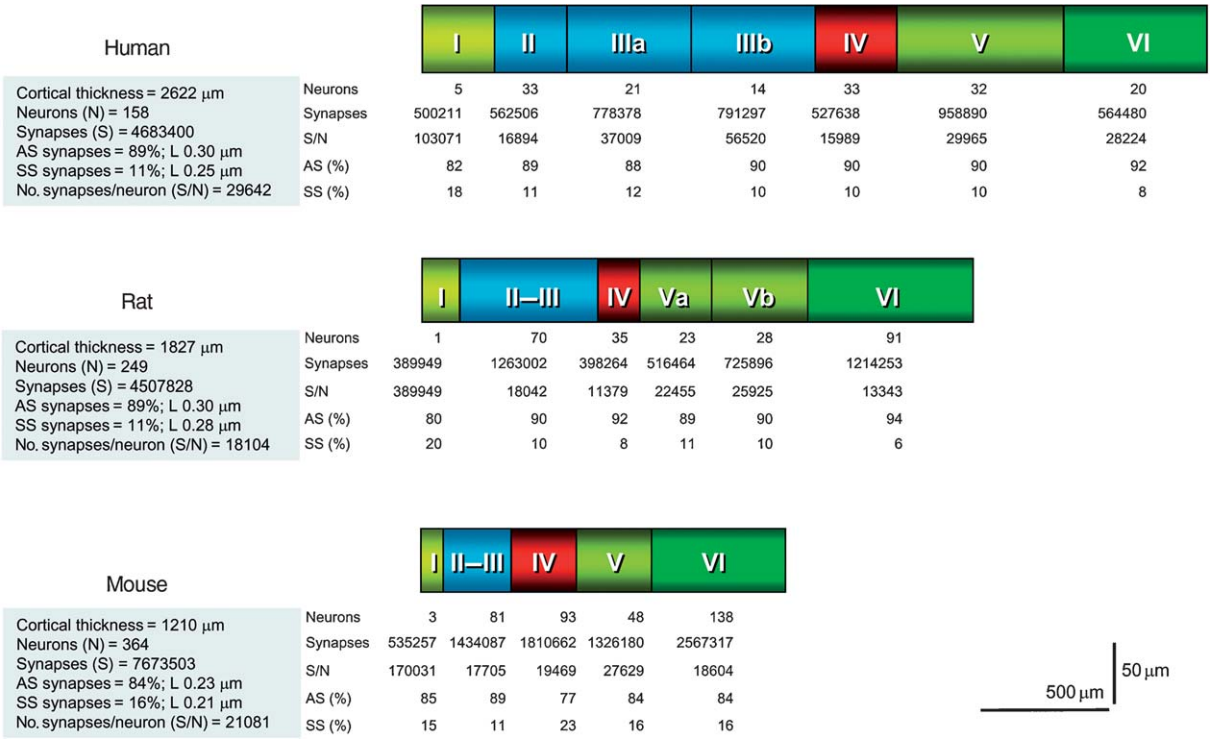

Figure 4 Comparison of the thickness of layers, number of neurons, and of the synaptic profiles within cubes of cortical tissue (50 μm wide by 50 μm thick) from the pial surface to the white matter in the human (anterolateral temporal cortex; T2-T3), rat (hindlimb area of the somatosensory cortex), and mouse (barrel cortex). L, cross-sectional length of synaptic junctions; AS, asymmetrical synapses; SS, symmetrical synapses. Values taken from DeFelipe, J., Alonso-Nanclares, L., and Arellano, J. I. 2002a. Microstructure of the neocortex: Comparative aspects. *J. Neurocytol.* 31, 299–316.

studies of cortical microstructure have been performed in the mouse, rat, cat, and monkey, and to a lesser extent in the human. Furthermore, researchers are often amazed, not only by the great variety of brain sizes and external topographies (Figure 5), but also by the variety of different cortical structures observed in the neocortex of some more 'exotic' species such as elephants, pigmy shrew, manatees, platypus, dolphins, giraffes, or apes (e.g., Haug, 1987; Reep *et al.*, 1989; Stolzenburg *et al.*, 1989; Glezer *et al.*, 1988, 1993; Hof

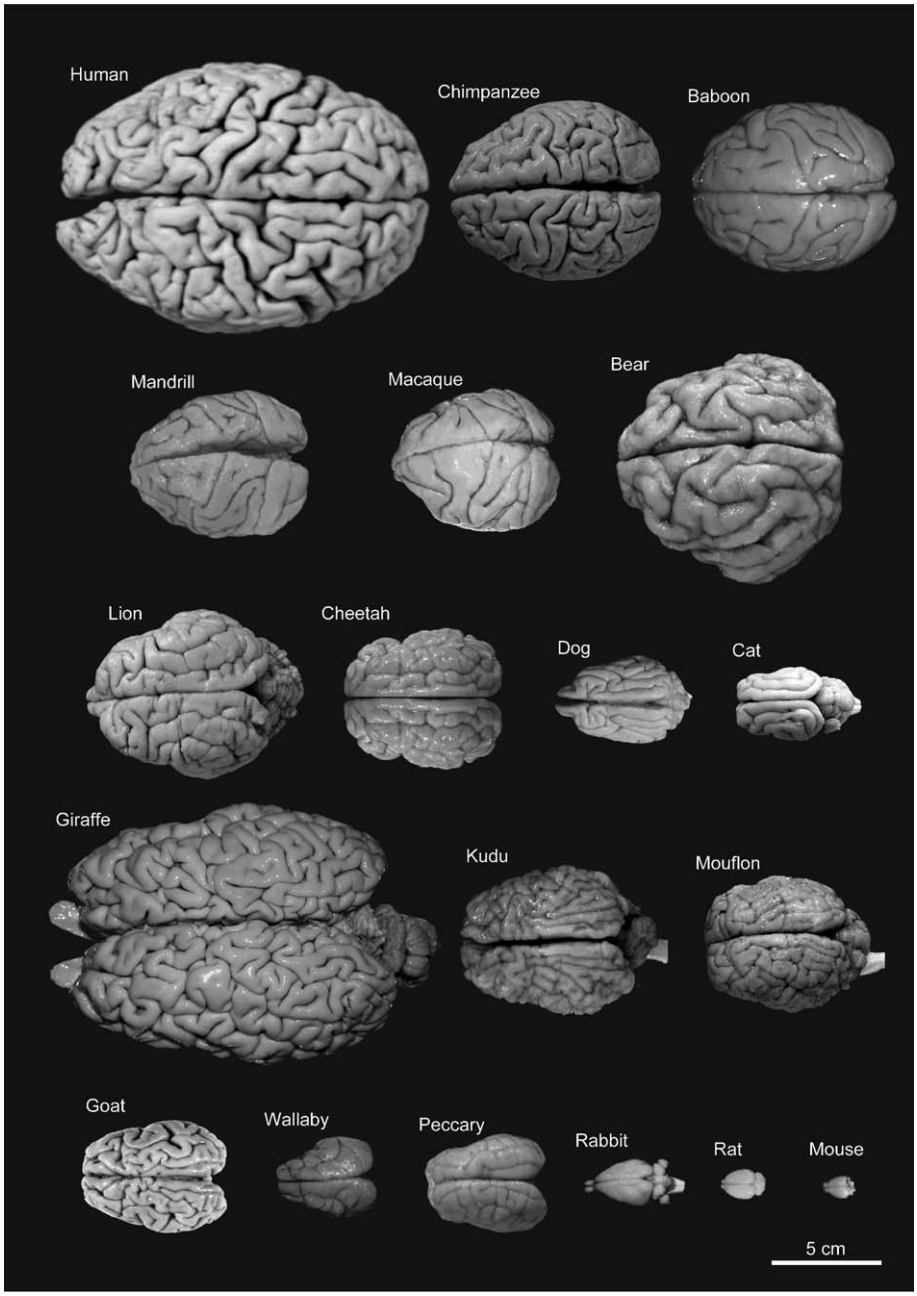

Figure 5 Photographs and weights in grams of the brains of different species. Primates: human (*Homo sapiens*, 1176 g), chimpanzee (*Pan troglodytes*, 273 g), baboon (*Papio cynocephalus*, 151 g), mandrill (*Mandrillus sphinx*, 123 g), macaque (*Macaca tonkeana*, 110 g). Carnivores: bear (*Ursus arctus*, 289 g), lion (*Panthera leo*,165 g), cheetah (*Acinonix jubatus*, 119 g), dog (*Canis familiaris*, 95 g), cat (*Felis catus*, 32 g). Artiodactyls: giraffe (*Giraffa camelopardilis*, 700 g), kudu (*Tragelaphus strepsiceros*, 166 g), mouflon (*Ovis musimon*, 118 g), ibex (goat, *Capra pyrenaica*, 115 g), peccary (*Tayassu pecari*, 41 g). Marsupials: wallaby (*Protemnodon rufogrisea*, 28 g). Lagomorphs: rabbit (*Oryctolagus cuniculus*, 5.2 g). Rodents: rat (*Rattus rattus*, 2.6 g), mouse (*Mus musculus*, 0.5 g). Scale bar: 5 cm. The chimpanzee brain was kindly supplied by Dr. Dean Falk. The rest of nonhuman brains were from material used in Ballesteros-Yáñez, I., Muñoz, A., Contreras, J., Gonzalez, J., Rodriguez-Veiga, E., and DeFelipe, J. 2005. The double bouquet cell in the human cerebral cortex and a comparison with other mammals. *J. Comp. Neurol.* 486, 344–360.

et al., 1999, 2000; Preuss, 2000, 2001; Preuss and Coleman, 2002; DeFelipe *et al.*, 2002a; DeFelipe, 2005). For example, Dexler (1913) found aggregations of neuronal cell bodies that he called 'basal Rindenkerne' (deep cortical clusters) in layer VI of the cerebral cortex of dugongs. Similar clusters of neurons were also found in the cerebral cortex of manatees in a region that Reep *et al.* (1989) called the 'cluster cortex'. This atypical cytoarchitecture has not been described in any other species, suggesting that it is a unique trait of the Sirenia (Reep *et al.*, 1989; see also Johnson *et al.*, 1994). Similarly, in layer II of the giraffe, instead of the typical, more or less homogeneous distribution of neurons, prominent small clusters of neurons exist, whereas this layer is very dense and thin in the platypus (DeFelipe *et al.*, 2002a; DeFelipe, 2005). Indeed, considerable differences have been observed in the neuron density between species. For instance, in the somatosensory cortex of the insectivorous white-toothed pygmy shrew (*Suncus etruscus*), whose brain weight is only 0.062 g, the neocortex is only 409 μm thick and there are approximately 170 000 neurons per mm^3. In contrast, the brain of the European mole (*Talpa europaea*) weighs 1.02 g, the neocortex is 1192 μm thick and there are only 40 000 neurons per mm^3 (Stolzenburg *et al.*, 1989). These densities are 7 and 1.6 times greater than in the human temporal cortex (24 186 neurons per mm^3; DeFelipe *et al.*, 2002a). It is sometimes frustrating to note how little attention has been paid to such comparative studies in spite of their importance for understanding the organization of the neocortex.

In conclusion, there is no 'basic' number or proportion of pyramidal cells and smooth interneurons, shared by all mammals, in a given minicolumn of tissue from layer I to layer VI. In addition, it is likely that as more studies are performed in different mammalian species, more differences will be found with regard to what we currently conceive as basic aspects of neocortical organization. This is important to bear in mind, particularly when one attempts to apply the data regarding cortical circuitry obtained from a given area of a particular species to another species.

4.12.5.2 Proportion of Interneurons

The variety of smooth interneurons is largely based on the diverse morphological, molecular, and physiological characteristics that they show (e.g., Cajal, 1911; Lorente de Nó, 1922; Jones, 1975; Szentágothai, 1975; Fairén *et al.*, 1984; Valverde, 1985; DeFelipe, 1993; Lund *et al.*, 1994; Kawaguchi and Kubota, 1997; Somogyi *et al.*, 1998; Gupta *et al.*, 2000; Markram *et al.*, 2004; DeFelipe *et al.*, 2005). Indeed, we still do not know how many different types of smooth interneurons exist but it is likely to reach the hundreds. If we consider the size of the minicolumn and estimate the number of neurons within a vertical strip of tissue 50 μm wide by 50 μm thick from the pial surface to the white matter (the approximate size of a minicolumn), we obtain a total number of 158 neurons in the human temporal cortex, and 249 and 364 neurons in the rat and mouse somatosensory cortex, respectively (Figure 4; DeFelipe *et al.*, 2002a). As discussed above, in primates and rodents approximately 25% and 15% of neurons are GABAergic and consequently, there would be 40, 37, and 55 smooth interneurons in these minicolumns, respectively. These numbers appear to be too low to include all types of such interneurons. Therefore, it seems likely that several types of microcircuits must exist that can be distinguished by their neuronal components.

As discussed elsewhere (DeFelipe, 2005), the axonal arborization of the vast majority of smooth interneurons surpasses the size limit of the minicolumn, and thus it would perhaps be more appropriate to consider the number of smooth interneurons within macrocolumns. The macrocolumn is approximately 500 μm wide by 500 μm thick from the pial surface to the white matter and within this, there would be a total number of 15 854, 24 885, and 36 395 neurons (DeFelipe *et al.*, 2002a). Considering the percentages of GABAergic neurons outlined above, in these macrocolumns there would be a total of 3963, 3733, and 5459 smooth interneurons in the human, rat, and mouse neocortex, respectively. These numbers do appear to be sufficiently large to include all types of smooth interneurons within the macrocolumn. Now the question arises as to whether the same types of smooth interneurons can be found in all macrocolumns or whether certain types are excluded from some macrocolumns at the expense of an increased number and proportion of other types.

Since the proportion of GABAergic neurons in the primate cortex is higher than in nonprimate mammals, three nonmutually exclusive events may have occurred during evolution (DeFelipe *et al.*, 2002a). First, the number of all types of interneurons already present in nonprimate mammals might have increased in general. Alternatively, an increase in the number of certain types of interneurons may have occurred. Third, new types of interneurons may have been generated during the evolution of the primate cortex.

There are a number of studies that have demonstrated interspecies variation in the distribution, number, morphology, and neurochemical characteristics of both pyramidal cells and smooth

interneurons, supporting the theory of the evolutionary diversity of neurons (e.g., Campbell and Morrison, 1989; Lewis and Lund, 1990; Glezer *et al.*, 1993, 1998; del Río and DeFelipe, 1997a; Nimchinski *et al.*, 1999; Hof *et al.*, 1999, 2000; Elston *et al.*, 1999a, 1999b, 2001, 2005a, 2005b; Dombrowski *et al.*, 2001; Preuss and Coleman, 2002; Ballesteros-Yáñez *et al.*, 2005). For example, the basal dendritic arbor of pyramidal cells displays remarkable differences in terms of size, number of bifurcations, and spine density between homologous cortical areas of different species (reviewed in Elston, 2003a, 2003b; see also Specialization of the Neocortical Pyramidal Cell during Primate Evolution). Since each dendritic spine appears to establish at least one excitatory synapse, these differences indicate the existence of species variations in the cortical circuits established by pyramidal cells. Regarding interneurons, there are a number of interesting examples (DeFelipe, 2002) of which we have selected three cases: the neurons expressing tyrosine hydroxylase (TH), the chandelier cells, and the double bouquet cells.

4.12.5.2.1 Neurons expressing TH TH is the rate-limiting enzyme in catecholamine synthesis. Catecholaminergic fibers are widely distributed throughout the nervous system, yet neurons expressing TH are restricted to certain regions of the brain and spinal cord, including the cerebral cortex (reviewed in Smeets and Gonzalez, 2000). In the neocortex, the laminar distribution of TH-positive neurons is distinct between different species (Figure 6), as is the co-expression of GABA (or GAD). These TH-positive neurons are relatively numerous and are found mostly in layers V and VI of the human neocortex (Benavides-Piccione and DeFelipe, 2003). In contrast, there are no or very few neurons, depending on the cortical area, which express TH in the macaque monkey neocortex (Kohler *et al.*, 1983; Lewis *et al.*, 1988) (Figures 6a and 6b). In the neocortex of certain cetaceans, TH-positive neurons are mainly confined to layer I (Hof *et al.*, 1995). The relatively few TH-positive neurons in the rat can be found in all the cortical layers, yet are most abundant in layers II and III. In addition, the majority of TH-positive neurons in the rat cortex co-express GABA or GAD (Kosaka *et al.*, 1987), whereas only approximately 50% contain GABA in the human neocortex (Gaspar *et al.*, 1987; Hornung *et al.*, 1989; Kuljis *et al.*, 1989; Trottier *et al.*, 1989). This finding is striking because it is thought that most smooth interneurons are GABAergic (Houser *et al.*, 1984) and, therefore, they appear to represent a special type of interneuron particularly abundant in the human neocortex. TH-positive neurons in the human neocortex constitute a subpopulation of smooth interneurons that may include Martinotti cells (or cells with an ascending axon) but not double bouquet cells, chandelier cells, or basket cells (Benavides-Piccione and DeFelipe, 2003).

4.12.5.2.2 Chandelier cells The chandelier cell is a type of smooth interneuron that is distinguished by the short vertical rows of boutons at the terminal portions of its axon (Ch terminals) that resemble candlesticks (Szentágothai and Arbib, 1974). In a variety of species including humans, Ch terminals have been shown to only innervate the axon initial segments of pyramidal cells with which they form symmetrical synapses. Indeed, they represent the major or sole source of synapses on the axon initial segments of pyramidal cells (e.g., Somogyi, 1977; Fairén and Valverde, 1980; Peters *et al.*, 1982; Somogyi *et al.*, 1982; Freund *et al.*, 1983; DeFelipe *et al.*, 1985, 1989a; del Río and DeFelipe, 1994; Gonchar *et al.*, 2002; for a review see DeFelipe and Fariñas, 1992). It seems clear that a given chandelier cell does not form synapses with the initial axon segment of any pyramidal cell it comes across, since the number of pyramidal neurons within the axonal plexus of a chandelier cell is higher than the number of chandelier cell axon terminals. Indeed, only a small proportion of pyramidal cells were innervated by impregnated chandelier cells in layers II and III of area 4 of the monkey, up to 20% (DeFelipe *et al.*, 1985). Until 1989, it was only possible to visualize these neurons by the Golgi method or using intracellular injections of horseradish peroxidase (e.g., Szentágothai, 1975; Jones, 1975; Somogyi, 1977; Fairén and Valverde, 1980; Somogyi *et al.*, 1982; Peters *et al.*, 1982; Freund *et al.*, 1983; DeFelipe *et al.*, 1985; Marin-Padilla, 1987). Because of the inconsistency of the Golgi method and the difficulties in performing intracellular injections in these neurons, their distribution across the cerebral cortex was not studied. However, since they can now be identified by immunocytochemical detection of the calcium-binding protein, parvalbumin (DeFelipe *et al.*, 1989a), their distribution, synaptic connectivity, and neurochemical characteristics can be investigated in detail. As a result, chandelier cells or Ch terminals have been visualized using a variety of antibodies, including antibodies against calbindin (del Río and DeFelipe, 1997a; Arellano *et al.*, 2004), corticotropin-releasing factor (Lewis *et al.*, 1989; Lewis and Lund, 1990), GABA transporter GAT-1 (DeFelipe and González-Albo, 1998; Woo *et al.*, 1998), or against the polysialylated forms of the cell-surface glycoprotein NCAM (PSA-NCAM) (Arellano *et al.*, 2002).

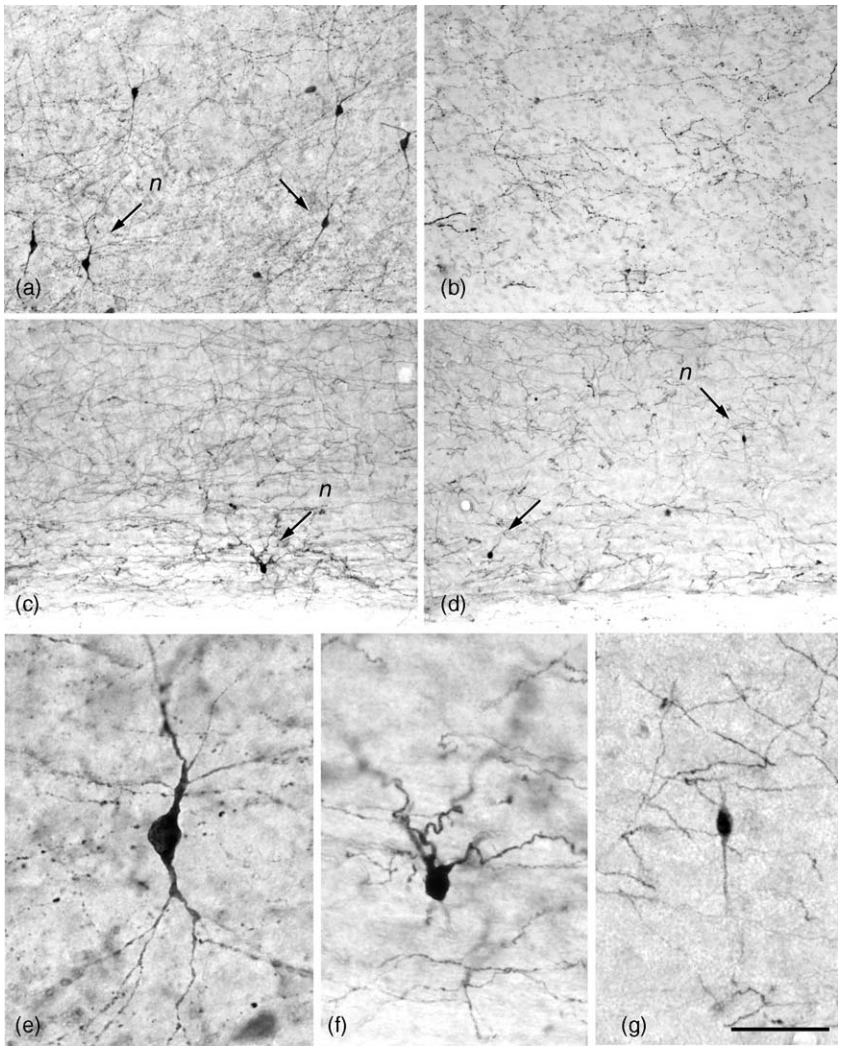

Figure 6 Low-power photomicrographs of TH immunostaining in the temporal cortex of the human (anterolateral temporal lobe) (a), macaque (area TE) (b), somatosensory cortex of the gerbil (c), and somatosensory cortex of rat (d), extending from lower part of layer V to the white matter. e–g, High-power magnification of the TH-positive neurons indicated (*n*) in (a), (c), and (d), respectively. TH-immunoreactive neurons are relatively abundant in the human compared to other species where only occasional cells were found except in the macaque monkey, where no TH-positive neurons were observed in the lateral temporal cortex. Scale bar: a–d, 160 μm; e–g, 40 μm.

On the basis of these and other immunocytochemical studies, chandelier cells can be chemically defined as GABAergic cells that contain parvalbumin and calbindin but not the calcium-binding protein calretinin, and which contain the neuropeptide corticotropin-releasing factor but not other neuropeptides such as cholecystokinin, somatostatin, neuropeptide Y, vasoactive intestinal polypeptide, and tachykinins (reviewed in DeFelipe and Fariñas, 1992; DeFelipe, 1997, 1999).

The most general, prominent and complete staining of Ch terminals is obtained with immunocytochemistry for parvalbumin and GAT-1 (DeFelipe and González-Albo, 1998). Parvalbumin-positive Ch terminals are present throughout layers II–VI of the human temporal neocortex and they are particularly prominent in layers V and VI (del Río and DeFelipe, 1994; Arellano *et al.*, 2002). However, the expression of other substances varies across species and across cortical areas and layers. Therefore, chandelier cells are chemically heterogeneous. For example, using antibodies against corticotropin-releasing factor to examine the prefrontal and occipital cortex of *Macaca mulatta, Macaca fascicularis*, and *Saimiri sciureus*, Lewis and Lund (1990) only found labeled Ch terminals in the *S. sciureus* and mainly in layer IV of the prefrontal cortex. Calbindin immunocytochemistry

labels a small subpopulation of Ch terminals located mainly in layers V and VI of the human neocortex, but not in other species including the macaque monkey (del Río and DeFelipe, 1997a; DeFelipe *et al.*, 1999b). Furthermore, in the human temporal neocortex the majority of parvalbumin-positive Ch terminals are immunostained for PSA-NCAM in layers II and III, whereas in deeper layers Ch terminals expressing PSA-NCAM are only occasionally found (Arellano *et al.*, 2002). In the temporal cortex of the macaque (area TE), the distribution of Ch terminals containing parvalbumin is different to that observed in the human temporal neocortex, since they are observed mainly in layers II–IV in the macaque and only very sparsely in layers V and VI (DeFelipe *et al.*, 1999b; Figure 7). But what is the meaning of this lack of immunostaining? In some cases, it certainly does not mean that chandelier cells are not present since double-labeling immunocytochemistry clearly shows the contrary. For example, the lack of PSA-NCAM immunostaining in layer VI in the human temporal neocortex does not mean that there are no Ch terminals in this layer because they are prominently stained for parvalbumin. Rather, these observations suggest that the neurochemical characteristics, and therefore the functional properties of chandelier cells, vary considerably between cortical layers, areas, and species.

Nevertheless, differences of a more fundamental nature regarding chandelier cells exist. Since chandelier cells innervate only the axon initial segment of pyramidal cells, the existence of pyramidal cells whose axon initial segment lacks synapses indicates that they are not innervated by chandelier cells. By examining the synaptology of the axon initial segment of corticothalamic projecting pyramidal cells in layer VI of the cat visual cortex, only one or two axo-axonic synapses were found to be established and, therefore, they were not postsynaptic targets of chandelier cells (Fariñas and DeFelipe, 1991). In summary, it appears that the axon initial segment of pyramidal cells may or may not be innervated by chandelier cells and that when it is innervated, chandelier cells may show a wide variety of molecular characteristics depending on the cortical layer, area, and species.

4.12.5.2.3 Double bouquet cells The French term 'double bouquet cell' (bitufted cell in English) was given by Cajal (1899a) to describe a variety of morphologically distinct interneurons in the human cerebral cortex. One of these cells is characterized by its long, descending, vertically bundled axon, which crosses several cortical layers. However, instead of giving a different name to this particular cell type, such as, for example, Szentágothai who called them "cells with horsetail-shaped axons," Jones "type 3," and Valverde who used "cells with axons forming vertical bundles" (Szentágothai, 1973, 1975; Jones, 1975; Valverde, 1978, 1985), a number of authors, including ourselves, have preferred to apply the term 'double bouquet cell' only to those neurons with axons forming such vertical bundles irrespective of their somato-dendritic morphology (DeFelipe, 2002). The axonal arbors of these cells, generally termed double bouquet cell axonal 'bundles' or 'horse-tails', are the source of a large number of GABAergic inhibitory synapses on small dendritic shafts and dendritic spines within a very narrow column of cortical tissue (Somogyi and Cowey, 1981; DeFelipe *et al.*, 1989b, 1990; de Lima and Morrison, 1989; Peters and Sethares, 1997).

As with chandelier cells, neither the Golgi method nor intracellular injections are adequate techniques for examining the distribution or chemical characteristics of these cells. However, thanks to the introduction of calbidin immunocytochemistry (Hendry *et al.*, 1989; DeFelipe *et al.*, 1989b, 1990), it is possible to study their distribution, synaptic connectivity, and neurochemical features in detail. At present, double bouquet cells can be

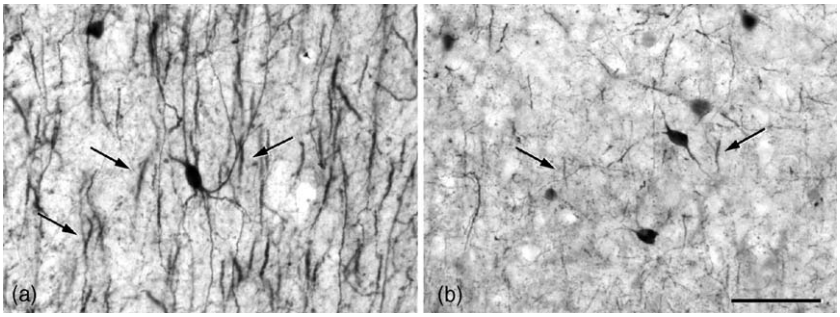

Figure 7 Low-power photomicrographs from human (a) and macaque (b) temporal cortex to illustrate the pattern of immunostaining for parvoalbumin in layer VI. Note how numerous the parvoalbumin-positive chandelier cell axon terminals are (some of them indicated by arrows) in the human compared to the macaque. Scale bar: 70 μm.

neurochemically defined as GABAergic interneurons that are immunoreactive for calbindin, certain subpopulations also expressing the calcium-binding protein calretinin and the peptides somatostatin and tachykinin. In contrast, these cells never contain other peptides or parvalbumin, nor do they appear to express markers for nitric oxide expressing neurons (Somogyi *et al.*, 1981; de Lima and Morrison, 1989; DeFelipe *et al.*, 1989b, 1990, 1999b; DeFelipe and Jones, 1992; del Río and DeFelipe, 1995, 1997b; DeFelipe, 1997; Benavides-Piccione and DeFelipe, 2003).

Double bouquet cells in the human and macaque monkey are very numerous and regularly distributed (Figure 8). Therefore, they are considered to be a key element in the microcolumnar organization of the cerebral cortex, acting on groups of pyramidal cells located in different layers within the minicolumns (DeFelipe *et al.*, 1990; Favorov and Kelly, 1994a, 1994b; del Río and DeFelipe, 1995; Peters and Sethares, 1997; DeFelipe, 1997, 2002, 2005; Jones, 2000a). Indeed, it has been shown that the radial fasciculi (bundles of myelinated axons originated from vertical aggregates of pyramidal cells or pyramidal cell modules) and double bouquet axons overlap on a one-to-one basis (del Río and DeFelipe, 1997b; Peters and Sethares, 1997; Ballesteros-Yáñez *et al.*, 2005). This microcolumnar organization has been demonstrated in the visual, somatosensorial, auditory, and temporal cortex of the macaque monkey, as well as in the human prefrontal, motor, somatosensory, temporal, and visual cortex (DeFelipe *et al.*, 1990, 1999b; Peters and Sethares, 1997; del Río and DeFelipe, 1997b; Ballesteros-Yáñez *et al.*, 2005). In addition, using immunocytochemistry for calbindin we found double bouquet cell horse-tails in a variety of nonprimate species but not in rodents (mouse and rat), lagomorphs (rabbit), and artiodactyls (goat) (Ballesteros-Yáñez *et al.*, 2005). Double bouquet cell axonal horse-tails were identified in the frontal, parietal, and occipital regions of carnivores (cat, cheetah, lion, and dog), although that they were mainly restricted to the occipital cortex, rarely existing in the parietal and frontal cortical regions studied (Ballesteros-Yáñez *et al.*, 2005). We also found that there are many fewer of these neurons in carnivores than in humans (and monkeys). In the neocortex of many species, including rodents, lagomorphs, and artiodactyls, some vertically oriented calbindin-positive axons have been identified but these did not present the typical axonal arbors of double bouquet cells. While it is possible that these cells are present but not stained for calbindin in these species and cortical regions, it is unlikely since they have not been observed using any of the other known markers for double bouquet cell axons as far as we are aware.

Since the proportion of GABAergic neurons is higher in primates than in rodents, we proposed that the presence of double bouquet cells may be at least partly responsible for the higher proportion of GABAergic cells in primates (DeFelipe, 2002; DeFelipe *et al.*, 2002a). It is known that distinct

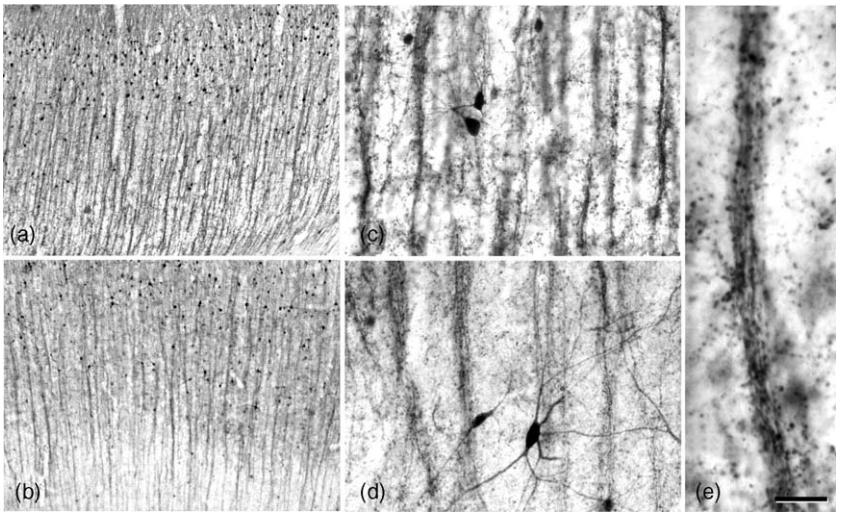

Figure 8 Low-power photomicrographs from the human occipital (area V2) (a) and temporal (anterolateral temporal lobe) (b) cortex immunostained for calbindin, illustrating how numerous double bouquet cell axons (horse-tails) are in the human cortex. (c) and (d) are higher magnifications of (a) and (b), respectively, to show immunoreactive neurons and double bouquet cell horse-tails. e, Example of a double bouquet cell horse-tail (temporal cortex) at a high magnification. Scale bar: a and b, 250 μm; c and d, 50 μm; e, 13 μm.

subtypes of interneurons may derive from different telencephalic subdivisions (Anderson *et al.*, 2002; Xu *et al.*, 2004; for a review see Xu *et al.*, 2003) and it is possible that the interspecies differences in the types of interneuron might reflect species-specific programs of GABAergic neurogenesis. For example, the existence of two lineages of human cortical GABAergic neurons has been proposed (Letinic *et al.*, 2002). One lineage, which expresses the transcription factors Dlx1/2 and Mash1, makes up 65% of the GABAergic neurons. These cells originate in the ventricular and subventricular zones of the dorsal telencephalon and migrate radially across the intermediate zone to the cerebral cortex. The other lineage, the remaining 35% of GABAergic neurons, express Dlx1/2 but not Mash1, and these cells originate in the ganglionic eminence of the ventral telencephalon from where they migrate tangentially across the intermediate zone to the cerebral cortex. However, all or almost all GABAergic neurons in rodents originate in the ganglionic eminence and migrate tangentially to the cerebral cortex (Tan *et al.*, 1998; Anderson *et al.*, 1999; Marin and Rubenstein, 2003; Xu *et al.*, 2003). As double bouquet cells are very numerous, to the extent that double bouquet axons and pyramidal cell modules generally overlap on a one-to-one basis, it is possible that double bouquet cells of the primate cortex might originate in the ventricular/subventricular zone from the GABAergic lineage expressing Dlx1/2 and Mash1. These neurons may migrate radially along common pathways as radial units, or as the ontogenetic columns that form the pyramidal cell modules (Rakic, 2002). Thus, we suggest that this could be a mechanism by which the complementary microcolumnar organization of double bouquet cells and pyramidal cell modules might originate in the neocortex of primates (DeFelipe, 2002, 2005). In conclusion, while double bouquet cells may be an important element in the microcolumnar organization of primates, this is not necessarily the case in other mammalian species. Differences in the abundance and distribution of double bouquet cells appear to reflect fundamental differences in the cortical microorganization between primates and other species.

4.12.5.3 Synaptology of the Neuropil

Differences and similarities in synaptic density have been reported in various cortical areas and species (see below). However, it is often difficult to interpret these findings because the number of specimens studied and the extension of the cortex examined is frequently relatively small. Moreover, in many studies the different cortical layers have not been examined systematically. Other considerations, such as the age of the individuals may also differ considerably between studies and this information is often not available. This is particularly important since changes in the density of synapses may occur during puberty and throughout adulthood, depending on the cortical area, layer, and species examined (reviewed in Rakic *et al.*, 1994; see also Bourgeois *et al.*, 1994; Poe *et al.*, 2001; DeFelipe *et al.*, 1997, 2002a, 2002b). Furthermore, there is no general consensus for the method of counting synapses and, therefore, it is often difficult to compare data obtained in different laboratories (DeFelipe *et al.*, 1999a). Nevertheless, we shall review some of these issues and compare them with the data we have obtained from the study of the morphology and density of synapses in the thin neuropil (i.e., avoiding the somata of neurons and glia, blood vessels, large dendrites, and myelinated axons) of the mouse visual and somatosensory cortex (area 17 and barrel cortex, respectively), the rat hindlimb area, and the human anterolateral temporal cortex (Brodmann's areas 20 and 21; DeFelipe *et al.*, 2002a). In later studies, we also examined the morphology and density of synapses in relation to their depth in the cortex (from layer I to layer VI), in the adult mouse (3 months old) and rat (5 months old), and in human (normal cerebral cortex obtained from surgically resected brain tissue from three male individuals of 24, 27, and 36 years of age). We applied the same methods to quantify synapses in the thin neuropil, and the neuronal density was also calculated to compare and estimate the number of synapses per neuron in each cortical layer and species. Data from the mouse visual cortex were not used for statistical comparisons and, therefore, unless otherwise specified, the mouse cortex refers to the barrel cortex. In addition, we have included data from unpublished electron microscope studies of the temporal cortex (area TE) of two female adult macaque monkeys in this article. This material was only used to estimate the percentage of multiple synapses.

4.12.5.3.1 Morphology of synapses A consistent finding in all cortical areas and species is that there are two major morphological types of synapses, Gray's (1959) type I and type II synapses that correspond to the asymmetrical and symmetrical types of Colonnier, respectively (Colonnier, 1968; see also Colonnier, 1981; Peters, 1987; Peters *et al.*, 1991; Peters and Palay, 1996). The hallmark for distinguishing these two types of synapses is the postsynaptic density, which is prominent in

asymmetrical synapses and thin in symmetrical synapses. In general, the axons of pyramidal cells (and to a lesser extent spiny stellate cells) are the major source of asymmetrical synapses, as well as the thalamocortical fibers and other main subcortical afferent systems. Most symmetrical synapses originate from the GABAergic interneuron population. Since pyramidal and spiny stellate cells, thalamocortical fibers and most extrathalamic subcortical afferent fibers are excitatory and GABA is an inhibitory neurotransmitter, asymmetrical synapses are excitatory and symmetrical synapses inhibitory (for reviews, see Houser *et al.*, 1984; White, 1989; Peters *et al.*, 1991; DeFelipe and Fariñas, 1992; Peters and Palay, 1996; Conti and Weinberg, 1999; Jones, 2000b; Amitai, 2001). Synaptic size also plays an important role in determining the functional properties of synapses (Mackenzie *et al.*, 1999; Schikorski and Stevens, 1999; Takumi *et al.*, 1999; Kubota and Kawaguchi, 2000; Lüscher *et al.*, 2000). For example, larger synapses seem to contain a greater number of postsynaptic receptors (Mackenzie *et al.*, 1999) and are associated with a greater number of docked synaptic vesicles (Schikorski and Stevens, 1999).

It is important to bear in mind that the vast majority of asymmetrical synapses originate from the cerebral cortex itself, through the local axonal arborization of pyramidal cells (and spiny stellate cells), and from the distal axonal arborization of corticocortical projecting pyramidal cells. This idea comes mainly from the study of thalamocortical axon terminals, which contribute only a small proportion of asymmetrical synapses although they represent a major subcortical afferent system to the cortex. It has been estimated that even in the layers to which the thalamocortical fibers predominantly project, they only represent 5–10% of asymmetrical synapses (Peters, 2002), although other quantitative studies showed this percentage to be higher. For example, LeVay and Gilbert (1976) used electron microscope autoradiography to quantify geniculocortical terminals and synapses in the cat visual cortex and they found the percentage of thalamocortical synapses in layer IV to be about 20%. Similarly, following lesion-induced degeneration (White, 1978) or in studies of anterograde transport of the lectin, *Phaseolus vulgaris leucoagglutinin*, to label and quantify thalamocortical terminals in the mouse barrel cortex (Keller *et al.*, 1985), approximately 20% of the asymmetrical synapses in layer IV were formed by thalamocortical axon terminals (reviewed in White, 1989). Thus, the differences between species in the density and the size of asymmetrical and symmetrical synapses reflect how similar or dissimilar their intrinsic excitatory and inhibitory circuits are in general.

In most electron microscope studies, the percentage of asymmetrical and symmetrical synapses varied between 80–90% and 20–10%, respectively in all the cortical areas and species examined (e.g., Blue and Parnavelas, 1983; Rakic *et al.*, 1986; Zecevic *et al.*, 1989; Schüz and Palm, 1989; Zecevic and Rakic, 1991; Beaulieu *et al.*, 1992, 1994; Bourgeois *et al.*, 1994; Beaulieu and Colonnier, 1985, 1989; Micheva and Beaulieu, 1996; DeFelipe *et al.*, 1997, 2002a, 2002b; White *et al.*, 1997; Marco and DeFelipe, 1997). Therefore, as a general rule excitatory circuits predominate over inhibitory circuits, a predominance that is imposed mainly by the axons of pyramidal cells. The fact that there are approximately 10% more inhibitory neurons in the macaque and human compared to the rat, but the percentage of asymmetrical and symmetrical synapses in the human and rat cortex is the same (89% asymmetrical and 11% symmetrical; DeFelipe *et al.*, 2002a), suggests that human inhibitory interneurons (the main source of symmetrical synapses) and pyramidal neurons (the main source of asymmetrical synapses) have axons with fewer and more synaptic boutons, respectively.

A given axon terminal forms an asymmetrical or symmetrical synapse with either one (single synapse), or with two or more postsynaptic elements (multiple synapses). As shown in Table 1, it appears that axon terminals originating from different types of cortical cells or from the thalamus give rise to different proportions of multiple synapses. Therefore, we have examined the proportion of multiple synapses in the neuropil of the human, macaque, rat, and mouse. Whereas the large majority of axon terminals establish single synapses with a postsynaptic element in the four species examined (human, 99.7%; macaque, 99.3%; rat, 99%; mouse, 97.6%), notable differences between species were found in certain layers (Table 2). For example, the greatest proportion of multiple synapses in the human, rat, and mouse were observed in layer IV (0.7% in the human, 1.6% in the rat, and 4.5% in the mouse), whereas in the macaque the highest proportion was found in layer IIIb (0.9%). In layer I, no axon terminals were seen to establish multiple synapses in the human cortex, whereas in the macaque, rat, and mouse multiple synapses contributed 0.8%, 0.7%, and 0.9%, respectively. Although small, these percentages represent a large number of synapses. For instance, in layer IV of the human temporal cortex, rat hindlimb area, and mouse barrel cortex there are 8 million, 29 million, and 173

Table 1 Examples of multiple synapses formed by identified axons in various species and cortical areas

Type of cell or axon	*Species, cortical area*	*No. synaptic axon terminals examined, type of synapse*	*% multiple synapses*[a]	*Reference*
Golgi-impregnated interneuron with axonal arcades (layers II–III)	Cat, visual cortex (area 17)	152, symmetrical	7	DeFelipe and Fairén (1988)
Tachykinin-immunoreactive terminals (layers II, III, V, VI)	Macaque monkey, somatic sensory cortex (areas 1 and 2), motor cortex (area 4)	312, symmetrical	11	Jones *et al.* (1988)
Intraaxonally injected geniculocortical axon (parvicellular axons; layer IV C)	Macaque monkey, visual cortex (area 17)	150, asymmetrical	16–37	Freund *et al.* (1989)
Intraaxonally injected geniculocortical axon (magnocellular axons; layer IV C)	Macaque monkey, visual cortex (area 17)	173, asymmetrical	35	Freund *et al.* (1989)
Calbindin-immunoreactive double bouquet cell axons (layer III)	Macaque monkey, somatic sensory cortex (areas 3a and 1)	237, symmetrical	16	DeFelipe *et al.* (1989b)
Tachykinin-immunoreactive double bouquet cell axons (layer III)	Macaque monkey, primary auditory cortex	277, symmetrical	16	DeFelipe *et al.* (1990)
Intracellularly injected cluth cells (layers IV, V)	Cat, visual cortex (area 17)	269, symmetrical	22	Kisvárday *et al.* (1985)
PHA-L labeled corticocortical axons (layers III, IV)	Cat, visual cortex (area PMLS)	182, asymmetrical	4	Lowenstein and Somogyi (1991)
Intracellularly injected large basket cells (layer III)	Cat, visual cortex (area 17)	155, symmetrical	11	Somogyi *et al.* (1983)

[a]Number of single axon terminals forming synapses with two or more postsynaptic profiles (percentage). These numbers must be considered as an underestimate since the percentages were obtained from single electron microscope sections: a synaptic junction can be observed in approximately 5–8 sections, whereas an axon terminal of 1 μm of diameter for example, can be followed in approximately 16 sections (60 nm thick).

Table 2 Percentages of multiple synapses in the neocortex of the human, macaque, rat, and mouse

Human temporal cortex (2911 axon terminals examined)		*Macaque monkey temporal cortex (3365 axon terminals examined)*		*Rat somatosensory cortex (4865 axon terminals examined)*		*Mouse somatosensory cortex (3401 axon terminals examined)*	
I	0	I	0.8	I	0.7	I	2.2
II	0.2	II	0.8	II–III	0.9	II–III	1.8
IIIa	0.5	IIIa	0.5	IV	1.6	IV	4.5
IIIb	0	IIIb	0.9	Va	1.3	V	1.5
IV	0.7	IV	0.6	Vb	1.5	VI	1.4
V	0.5	V	0.5	VI	0.3		
VI	0.3	VI	1.1				
I–VI	0.3	I–VI	0.7	I–VI	1	I–VI	2.4

million multiple synapses per mm^3, respectively. These quantitative observations suggest that there are significant differences in the proportion or types of axons that establish multiple synapses in the different cortical areas or species.

4.12.5.3.2 Cross-sectional length of synaptic junctions In most electron microscope studies, the cross-sectional length of synaptic junctions in all cortical areas and species studied so far varies between 0.20 and 0.40 μm. (e.g., Blue and Parnavelas, 1983; Beaulieu and Colonnier, 1985, 1989; Schüz and Palm, 1989; Glezer and Morgane, 1990; Beaulieu *et al.*, 1992, 1994; Huttenlocher and Dabholkar, 1997; White *et al.*, 1997; DeFelipe *et al.*, 1997, 2002a,

2002b; Marco and DeFelipe, 1997). A comparison of the mean cross-sectional length of synaptic junctions in the various cortical layers between the human, rat, and mouse (DeFelipe *et al.*, 2002a), revealed that the mean cross-sectional length when all layers were considered was 0.30, 0.30, and 0.23 μm for asymmetrical synapses, and 0.25, 0.28, and 0.21 μm for symmetrical synapses, respectively (Figure 9 and Table 3). Nevertheless, the mean cross-sectional length of asymmetrical synapses was significantly shorter in all layers of the mouse cortex when compared to the human and rat. No significant differences were found between humans and rats except in layer V of the human where asymmetrical synapses were shorter (0.27 μm) compared to layer Va (0.31 μm) and Vb (0.30 μm) of the rat. With regards to symmetrical synapses,

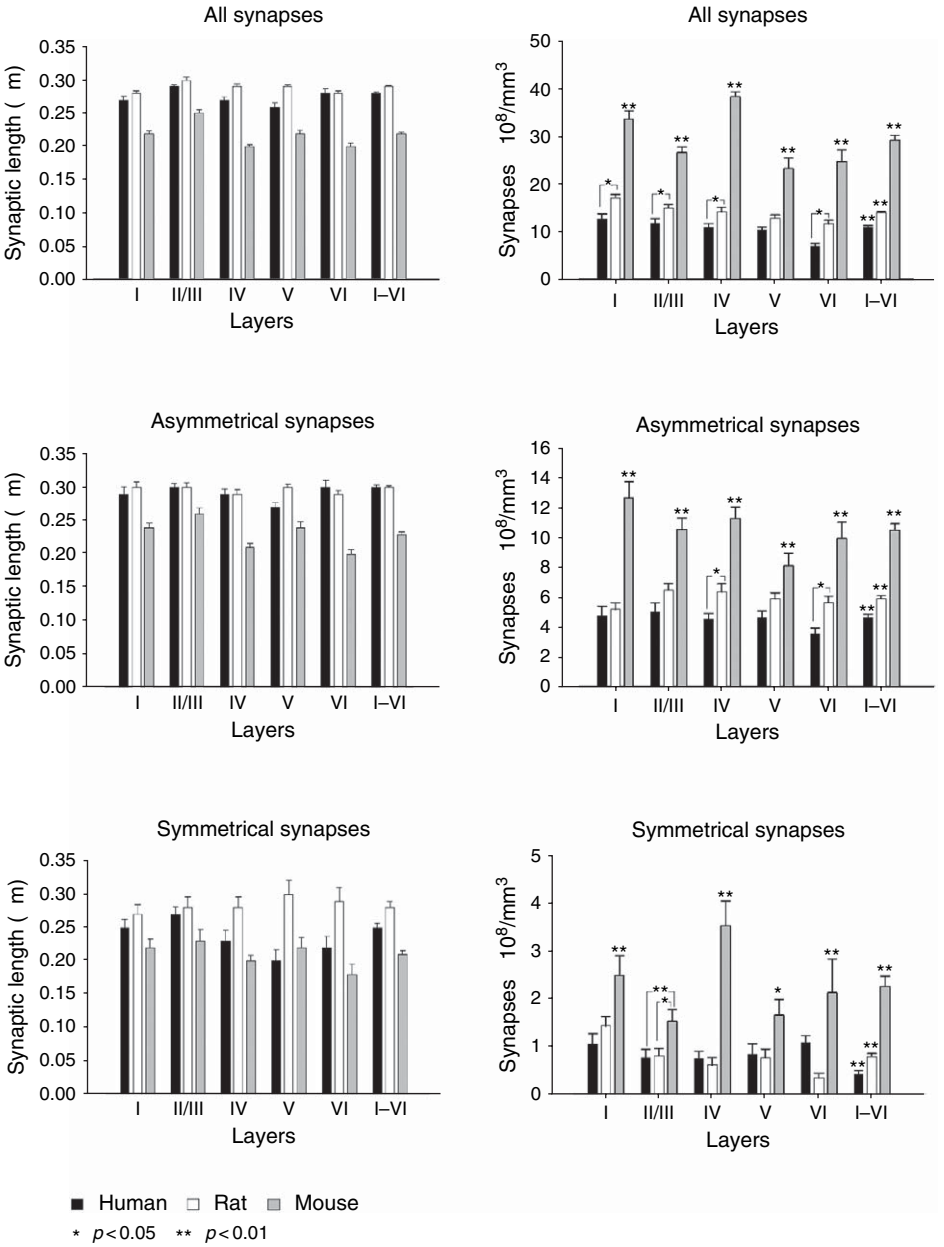

Figure 9 Comparison of the mean cross-sectional lengths (± s.e.m.) and densities of synaptic profiles between human, rat, and mouse. Density values obtained in layers II, IIIa, and IIIb of human, and layers Va and Vb of rat were recalculated according to the relative thickness of these layers to estimate the representative values of layers II–III and V, respectively. Reproduced from *J. Neurocytol.*, vol. 31, 2002a, pp. 299–316, Microstructure of the neocortex: Comparative aspects, Defelipe, J., Alonso-Nanclares, L., and Arellano, J. I. With kind permission of Springer Science and Business Media.

Table 3 Synapse sizes, synapse numbers, and neuron numbers across all cortical layers

	Human temporal cortex ($n = 3$)	*Rat hindlimb somatosensory cortex* ($n = 3$)	*Mouse barrel cortex* ($n = 2$)	*Mouse visual cortex* ($n = 1$)
Total number of synaptic profiles studied	2195	2523	2054	897
Range of asymmetrical and symmetrical synaptic profiles per 100 μm^2 (mean $\pm$ s.e.m.)	11–18 (14.9 ± 0.7)	9–21 (17.9 ± 2.2)	22–35 (28.2 ± 1.4)	24–38 (29.4 ± 1.8)
Range of all synaptic profiles per 100 μm^2 (mean $\pm$ s.e.m.)	20–36 (29.9 ± 0.79)	32–46 (38.9 ± 0.95)	50–75 (63.1 ± 1.96)	40–64 (51.3 ± 2.1)
Mean cross-sectional lengths of all synapses ($\mu m \pm$ s.e.m.)	0.28 ± 0.01	0.29 ± 0.01	0.22 ± 0.01	0.21 ± 0.09
Synaptic density of asymmetrical and symmetrical synaptic profiles per $10^8/mm^3$ (mean $\pm$ s.e.m.)	5.42 ± 0.28	6.46 ± 0.26	12.83 ± 0.65	14.46 ± 0.93
Synaptic density of all synapses per $10^8/mm^3$ (mean $\pm$ s.e.m.)	10.94 ± 0.34	13.97 ± 0.33	29.31 ± 1.02	25.19 ± 1.16
% of asymmetrical synapses	89	89	84	89
% of symmetrical synapses	11	11	16	11
Number of neurons per mm^3	24 186	54 468	120 315	
Number of neurons per 30×25 μm column through the depth of the cortex	48	75	109	
Number of synapses per neuron	29 821	18 015	21 983	

Data from mouse visual cortex were not used for statistical comparisons with data from mouse barrel cortex, nor with the rat and human cortex.

All synapses include asymmetrical, symmetrical, and uncharacterized synapses.

Reproduced from *J. Neurocytol.*, vol. 31, 2002a, pp. 299–316, Microstructure of the neocortex: Comparative aspects, DeFelipe, J., Alonso-Nanclares, L., and Arellano, J. I., table 1. With kind permission of Springer Science and Buisness Media.

significant differences were found between human and rat in layers IV, V, and VI (Figure 9). In summary, it is remarkable that in spite of the great differences in brain size between human, rat, and mouse, there are relatively small differences in the mean cross-sectional length of synaptic junctions, although significant laminar differences can be observed between the different species.

4.12.5.3.3 Density of synapses As far as we know, Cragg (1967) was the first author to perform comparative ultrastructural studies of the neocortex. He pointed out that there was relatively little variation in synaptic density between cytoarchitectonically and functionally different regions such as the motor and visual cortex of the mouse and monkey (between 6 and 9×10^8 synapses per mm^3). Later, Colonnier and colleagues also argued that the numerical density of synapses was relatively constant throughout the cortical layers, as well between different cortical areas and different species (see O'Kusky and Colonnier, 1982; Beaulieu and Colonnier, 1989). These observations were confirmed by others, such as Schüz and Palm (1989) who failed to detect systematic differences between layers or different cortical regions in the mouse, including areas 6, 8, and 17. Similarly, Rakic and colleagues emphasized the remarkable small variation they found in the neuropil of all cortical layers in diverse regions of the rhesus monkey neocortex (15–20 synaptic profiles per 100 μm^2), including the motor cortex, somatosensory cortex, prefrontal cortex, and visual cortex (Rakic *et al.*, 1986; see also Zecevic and Rakic, 1991). Furthermore, similar data was obtained from the human visual, auditory, and prefrontal cortex by Huttenlocher and Dabholkar (1997).

O'Kusky and Colonnier (1982) hypothesized that this uniformity in synaptic density probably reflects an optimal number of synapses, and that it may be due to some limiting metabolic or structural factor (see also Rakic *et al.*, 1986). However, as discussed previously (DeFelipe *et al.*, 2002a), the problem with this hypothesis is that the estimates of synapse number were made with very different techniques and did not always apply stereological methods. In addition, most comparisons are only qualitative and not based on statistical analysis. Therefore, one must be careful when comparing data from different authors because it may lead to uncertain conclusions. This is clearly demonstrated in the study of Beaulieu *et al.* (1992, 1994), who found significant differences in the number of synapses per volume between certain layers of both the rat and monkey visual cortex by applying the dissector method and statistical comparisons.

In our comparative studies of human, rat, and mouse tissue (DeFelipe *et al.*, 2002a), we detected a lower synaptic density in the human (1094 million per mm^3), than in the rat (1397 million per mm^3), and mouse (2931 million per mm^3) when all layers were considered (Table 3). In most layers, these differences between the three species were statistically significant (Figure 9). The amazingly high density of synapses found in the mouse barrel cortex when compared to the human (Figure 10) and rat was not due to a peculiarity of the barrel cortex as the mouse visual cortex also had a very high density (2519 million per mm^3; Table 3). The transmitter released at synaptic or nonsynaptic sites may diffuse a certain distance and act on other synaptic contacts or on extra-synaptic receptors ('volume transmission'; for a review see Agnati and Fuxe, 2000). Hence, the density of synapses may represent a very significant factor in the diffusion of chemical signals and, therefore, species differences in synapse density may indicate differences in volume transmission. In general, a number of significant differences have been found in the density of synapses depending on the layer and species, particularly when asymmetrical and symmetrical synapses are analyzed separately (Figure 9). We conclude that the density of synapses is not uniform throughout the cortex and that there are laminar specific differences between different cortical areas in different species. Thus, if an optimal number of synapses for cortical circuits does exist, it must be species and laminar specific.

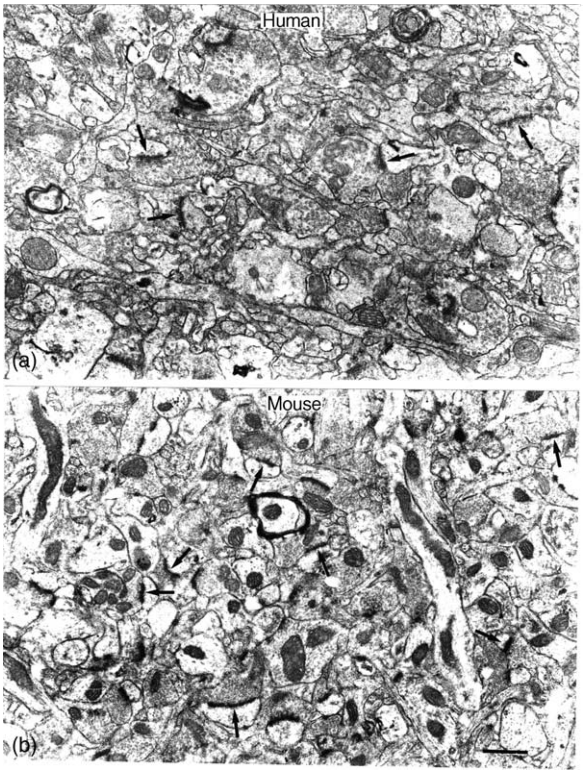

Figure 10 Electron micrographs illustrating the thin neuropil from layer IIIa in the human temporal cortex (a), and layer II/III of the mouse barrel cortex (b). Note the higher density of synapses (some of them indicated by arrows) in the mouse cortex. Scale bar: 0.5 μm. Reproduced from *J. Neurocytol.*, vol. 31, 2002a, pp. 299–316, Microstructure of the neocortex: Comparative aspects, DeFelipe, J., Alonso-Nanclares, L., and Arellano, J. I. With kind permission of Springer Science and Buisness Media.

4.12.5.4 Number of Synapses per Neuron

In terms of connectivity, it is difficult to interpret the comparisons of synaptic density between species because the thickness and neuronal density in different layers and species differ (Table 3; Figure 4). Therefore, to examine possible differences in connectivity between species it is more appropriate to compare the values obtained by dividing the synaptic density by the corresponding neuronal density. Nevertheless, there are a number of considerations that must be borne in mind. First, the number of synapses per neuron in a given layer is not an accurate estimate of the number of synapses received by neurons in that layer, since dendrites, particularly of pyramidal cells, may cross several layers. Second, axon terminals in a given layer do not necessarily originate from local neurons; they may come from neurons located in other layers, or from other cortical areas or subcortical nuclei. Third, the number of synapses per neuron obtained simply by dividing the synaptic density of the neuropil by the neuronal density is an overestimate because the volume occupied by neurons, glia cells, blood vessels, and neuropil is usually not taken into account. Thus, the synapse/neuron ratio should be taken as a useful parameter to compare between cortical layer, areas, and species in terms of 'general' connectivity (DeFelipe *et al.*, 2002a).

Cragg (1967) compared the number of synapses per neuron in the motor and visual areas of the mouse and macaque monkey, and found species differences in the relationship between neuronal density and number of synapses per neuron. For example, in the motor cortex, the density of neurons was lower and the number of synapses per neuron was much greater in the monkey than in the mouse (16.1×10^6 neurons per cm^3; 60 000 synapses per neuron vs. 64.4×10^6 neurons per cm^3; 13 000 synapses per neuron). On the other hand, the neuron density was higher in the visual cortex where the synapse per neuron ratio was

lower in the monkey than in the mouse (110.3×10^6 neurons per cm^3; 5600 synapses per neuron vs. 92.4×10^6 neurons per cm^3; 7000 synapses per neuron). An inverse relationship between neuronal density and the number of synapses per neuron was also observed for individual layers of the macaque visual cortex (O'Kusky and Colonnier, 1982), corroborating the findings of Cragg. However, O'Kusky and Colonnier's estimates were approximately 60% lower than Cragg (2300 synapses per neuron) in the macaque visual cortex, probably due to the different methods used. O'Kusky and Colonnier suggested that a limiting factor was responsible for this inverse relationship, and that neurons receiving more synapses would have more complex dendritic trees, thereby increasing the distance between their cell bodies. In contrast, neurons receiving fewer synapses would have a less-complex dendritic tree, allowing them to be more densely packed. According to Cragg (1967), the origin of this idea is based on the old histological studies of Franz Nissl (1860–1919) and Constantin Von Economo (1876–1931). Nissl pointed out that cortical neurons were more crowded in the mole and dog than in humans. Based on this observation, Von Economo proposed that the richer the fiber plexus was between neurons the more they would be separated, increasing the opportunity for neuronal interactions. Thus, the wider separation of neurons in humans compared to other species could be taken as an indication of a greater refinement of the connections between neurons.

We examined this issue in the human, rat, and mouse and found that the principle of the inverse relationship between neuronal density and the number of synapses per neuron in general held true in the human when compared with the rat and mouse, but not when comparing the rat with the mouse (Figure 11). Additionally, when specific layers were compared, this rule was applicable to all layers in the rat, but not to the human or mouse. Similarly, there was no common pattern between species or within a given species with respect to the distribution of synapses per neuron when considering asymmetrical and symmetrical synapses (Figure 11). Instead, there were impressive laminar differences not only between certain layers of the human and mouse when compared to the rat, but also between the human and mouse.

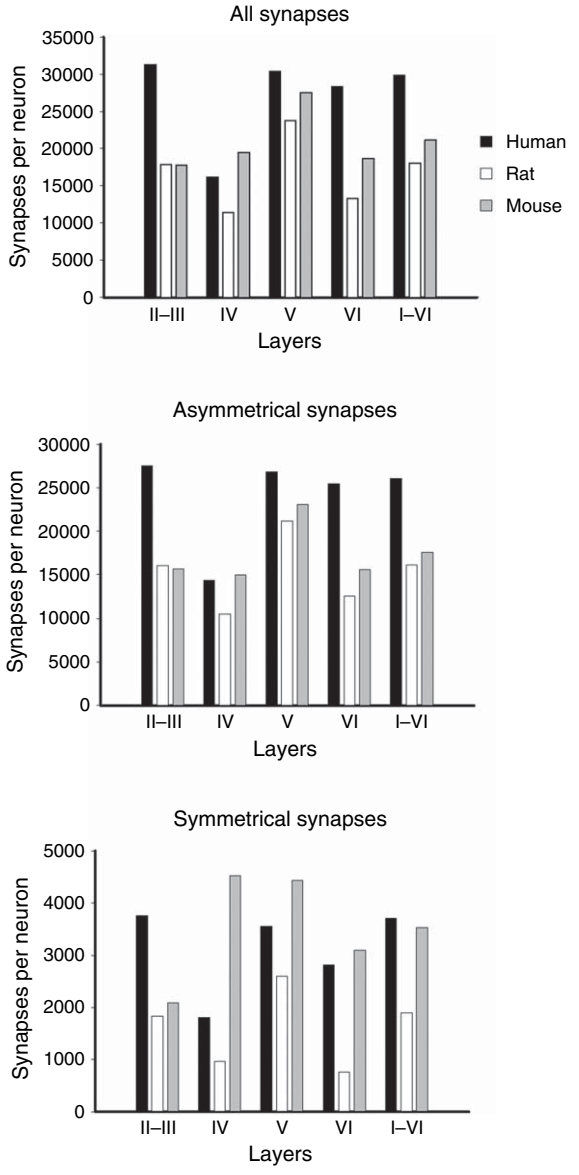

Figure 11 Graphs showing the number of synapses per neuron in the human, rat, and mouse. The values obtained in layers II, IIIa, and IIIb of human, and layers Va and Vb of rat were recalculated according to the relative thickness of these layers to estimate the representative values of layers II, III, and V, respectively. Reproduced from *J. Neurocytol.*, vol. 31, 2002a, pp. 299–316, Microstructure of the neocortex: Comparative aspects, DeFelipe, J., Alonso-Nanclares, L., and Arellano, J. I. With kind permission of Springer Science and Buisness Media.

4.12.6 Concluding Remarks

In summary, there are variations between species in terms of the density of neurons, as well as in the proportion of GABAergic interneurons, the synaptology in the neuropil and in the number of synapses per neuron. Furthermore, certain subtypes of interneurons are lacking or greatly modified in some species. The specific similarities between the human, rat, and mouse, and other species with respect to the types of cortical neurons and the percentage, length, and density of asymmetrical and symmetrical synapses, and in the number of

synapses per neuron, might be considered as the common features of cortical organization. The differences that can be observed probably indicate evolutionary adaptations of excitatory and inhibitory circuits to particular functions.

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4.13 Specialization of the Neocortical Pyramidal Cell during Primate Evolution

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4.13.1 Introduction

4.13.1.1 The Study of Brain Evolution

The study of the evolution of the brain poses an innate problem: soft tissue is rarely preserved in fossil specimens. Natural endocasts of brains reveal aspects of gross structure, such as overall size, and occasionally it is possible to identify particular sulci – allowing speculation regarding the expansion of the cortical lobes during brain evolution. Differences in the size of brain endocasts and, in particular, the region of neocortex anterior to the lunate sulcus, have been interpreted by some as a measure of the evolution of cognitive abilities of hominids (Figure 1; see Oakley, 1949; Dart, 1956; Eiseley, 1957; Tobias, 1981; Gray and Thompson, 2004 for reviews). However, there has been a dramatic increase in complexity in material culture with little change in brain size during the past 300 000 years or so. Moreover, there is some evidence to suggest that the cerebrum in modern humans is actually smaller than in his predecessors (see Tobias, 1995; Henneberg, 1998; Manger, 2005a for reviews). Thus, advances in cognitive abilities of humans, as evidenced by the increasing complexity of material culture, may be explained by something that cannot be gleaned from the fossil record – specializations in microstructure of the brain.

The study of the evolution of the primate brain is thus largely restricted to extant species. By systematically investigating internal features of the brains of different species, inferences can be made about the evolution of these structures (see Kaas, 1989, 1995; Northcutt and Kaas, 1995; Northcutt, 2002 for reviews). We have adopted such an approach to study the evolution of microcircuitry in the primate brain. Here we review presently available data on pyramidal cell structure in the primate cortex, how regional and species differences in pyramidal cell structure may influence patterns of connectivity and, in turn, the functional complexity and computational abilities of the circuits they form. Data sampled from homologous cortical areas among species reveal interesting trends in the evolution of the pyramidal cell phenotype in different regions of brain. The structure of pyramidal cells in the primary visual area (V1) is relatively similar in prosimian galagos, New World and Old World monkeys, and the baboon, despite more than a fivefold difference in the cortical surface area occupied by V1 in these primates (Brodmann, 1913; see Elston and Garey, 2004 for a translation). Pyramidal cells in granular prefrontal cortex (gPFC), on the other hand, have markedly different phenotypes in the different primate species: those

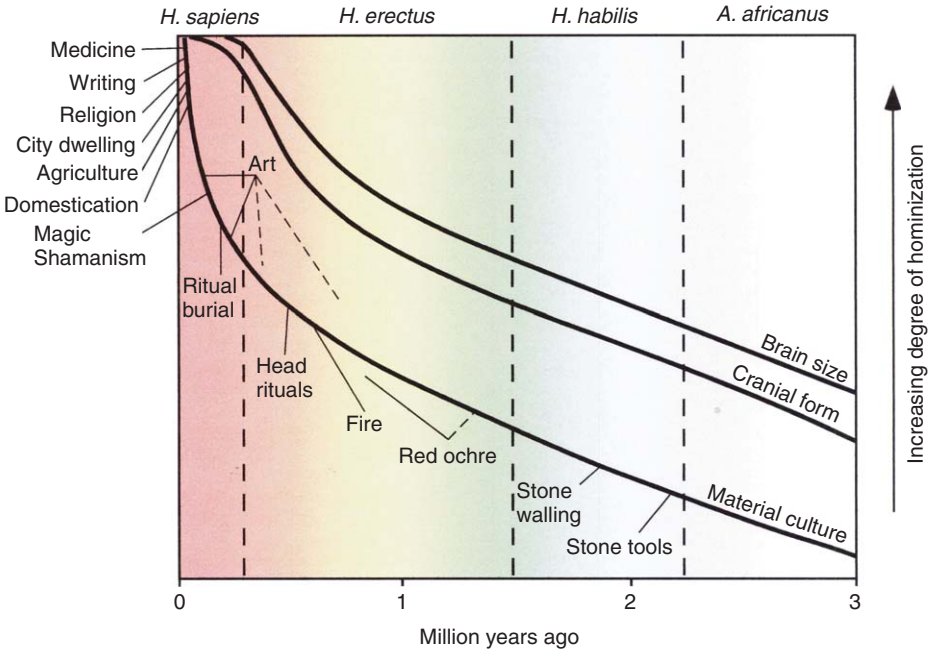

Figure 1 Schematic illustrating the evolution of cranial form and brain size in relation to the level of sophistication of cognitive abilities during the past three million years of human evolution. Note in particular that there was a sharp increase in brain size in *H. erectus* beginning ~500 kya, which coincided with the first record of the mastery of fire, ritualization, and the beginnings of art. However, more recently, there has been a dramatic increase in the level of sophistication of cognitive abilities in *H. sapiens*, as evidenced by the domestication of animals, implementation of agricultural practices, city dwelling, writing, and the practice of medicine, which has occurred without notable increase in brain size. Modified from Tobias, P. V. 1981. Evolution of the human brain, intellect and spirit. In: 1st Abbie Memorial Lecture, pp. 5–70. The University of Adelaide.

in the prosimian galago are relatively simple, in monkeys are progressively more complex, in the baboon yet more complex, and those in human are the most complex of all pyramidal cells studied to date. Based on regional and species differences in the pyramidal cell phenotype, it is possible to postulate some new theories on specialization of circuit structure in neocortical evolution. The data suggest not one simple rule, but various trends, which may differ according to the cortical region in question or which species are included for comparison. A better understanding of the evolution of specialized cortical function in primates will only be possible by encompassing this diversity in structure and function. While this 'unwelcome revolution' will no doubt be unpalatable to some (see Preuss, 2001 for a review), the inevitability of the necessity to perform systematic quantitative comparative studies will, no doubt, become increasingly more apparent with each new species included for study. This becomes even more obvious when comparing the evolution of cortical circuitry between primates and nonprimates, for which some preliminary data are presented. Differences in the size and location of specific cortical areas within a given species (Figure 2, Table 1), and possible differences in cortical microstructure, further complicate the story but are not considered further here. For a translation of Brodmann's (1913) in-depth review of individual variation in cortical organization, the reader is referred to Elston and Garey (2004).

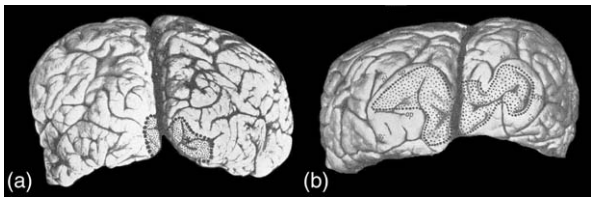

Figure 2 Photomicrographs of two brains of contemporary humans (from the late 1800s) studied by Brodmann. Note the difference in the relative size and location of the primary visual area (stipple), and differences in the sulcal patterns in the two brains. Modified from Brodmann, K. 1913. Neue Forschungsergebnisse der Grosshirnrindenanatomie mit besonderer Berücksichtigung anthropologischer Fragen. *Gesselsch. Deuts. Naturf. Artze.* 85, 200–240. Translated by Elston, G. N. and Garey, L. J. 2004. New Research Findings on the Anatomy of the Cerebral Cortex of Special Relevance to Anthropological Questions. University of Queensland Printery.

Table 1 Surface areas of different human striate cortices

Case	Size of hemisphere (mm^2)	Size of V1 (mm^2) = percentage of total hemisphere	Size and percentage of V1 located in sulci	Size and percentage of V1 located on the medial surface	Brain weight (hemisphere) measure
K.B. ♂, 28 Y ...		4416	3091 = 70.0		?
A.H. ♂, 24 Y ...	135 470	3761 = 2.77	2531 = 67.3	2984 = 79.3	1590 (707)
J.K. ♂, 40 Y. L		3080	1955 = 63.5	2770 = 89.9	1240 (530)
R		3405	2249 = 66.0	3405 = 100	
A.M. ♂, 43 Y. R		R 3036	2105 = 69.4	2815 = 92.7	1470 (640)
L		L 3070	2140 = 69.7	2665 = 86.8	
E.St. ♀, 36 Y. R		R 2958	1661 = 56.2		
L	101 918	L 2843 = 2.79	1609 = 56.6	2636 = 92.3	
Herero		4234	2020 = 61.8	3298 = 77.9	1360 (540)
Cameroonian I	94 089	2768 = 2.94	2046 = 73.9	2143 = 77.4	1210 (530)
Cameroonian II	87 642	2512 = 2.87	1563 = 62.2	1855 = 73.8	1170 (515)
Cameroonian III	81 133	2130 = 2.62	1193 = 56.0	1330 = 62.4	950 (420)

Modified from Brodmann, K. 1913. Neue Forschungsergebnisse der Grosshirnrindenanatomie mit besonderer Berücksichtigung anthropologischer Fragen. *Gesselsch. Deuts. Naturf. Artze.* 85, 200–240.

4.13.1.2 Cortical Microcircuitry

As students we are often told that the mammalian cerebral cortex comprises distinguishable cortical layers, six in total. Within these cortical layers reside particular populations of neurons (glia are often neglected), which make particular types of projections. Patterns of afferent inputs are usually stereotyped, inevitably terminating in layer IV. The mammalian cerebral cortex is clearly far more diverse than this standard 'plan' would suggest (see Rockland, 1997 for a critical review; see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates). The most eminent authorities in comparative cytoarchitecture disputed the number and nomenclature of cortical layers for decades, yet this is poorly acknowledged by many contemporary scientists (see Garey, 1994 for a translation of Brodmann's, 1909 original monograph). This is more than just a matter of historical accuracy, as acknowledgment of the diversity in cytoarchitecture of the mammalian cerebral cortex is a fundamental step if the discipline of neuroscience is to forge ahead. Without this most basic of acceptances, we cannot hope to make any inferences regarding the evolution of the mammalian cerebral cortex, much less the evolution of specialized cortical functions such as vision, somatosensation, and intellect. To accept the cortical uniformity hypothesis is to accept that cortex has not evolved. In the following sections we set out how we have attempted, and only just begun, to quantify aspects of cortical microstructure systematically across the cerebral cortex of mammals. We have done so by focusing on the pyramidal cell, the most abundant neuronal type in the cerebral cortex.

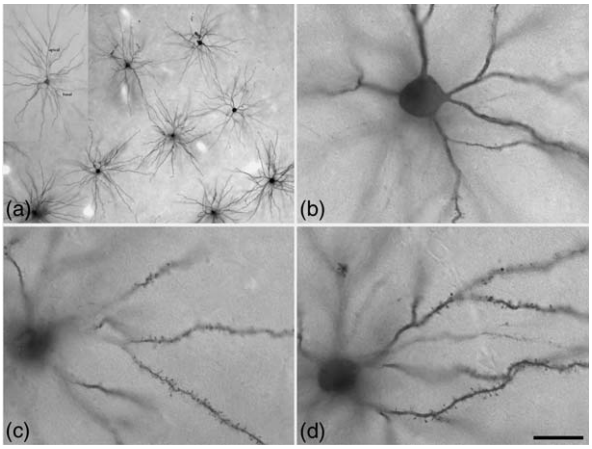

Figure 3 Photomicrographs of neocortical pyramidal cells that were injected with Lucifer Yellow and processed for a diaminobenzadine (DAB) reaction product. Note the characteristic features of a pyramidal cell as seen in the transverse section (inset in (a)), including the prominent apical dendrite and the basal skirt of dendrites. Cells in panels (a–d) were injected in tangential sections. In each instance, cells are injected close to the top of the section so as to include the entire basal skirt of dendrites (250 μm thick sections), while at the same time leaving enough space between the top of the section and the cell body to allow visualization of the proximal portion of the apical dendrite. Upward of 100 cells can be injected in individual sections (a), which can then be studied at high power (b–d) to quantify dendritic branching pattern, spine density, and the size of the cell bodies.

4.13.1.3 What Is a Pyramidal Cell?

Pyramidal cells are distinguished by their prominent apical dendrite and basal dendritic tree (Figure 3). They comprise ~70–90% of all neurons in cortex. Pyramidal cells form rich plexuses of connections, often called intrinsic lattices or

horizontal patches, within cortical areas. They form nearly all corticocortical connections, both ipsi- and contralateral, as well as most subcortical connections. Pyramidal cells also contain the excitatory neurotransmitter glutamate: their discharge directly facilitates cortical activity, rather than inhibiting it. Arguably, pyramidal cells are the principal neurons of the cerebral cortex, generating nearly all cortically initiated excitation (see Feldman, 1984; Jones, 1984; DeFelipe and Fariñas, 1992; Nieuwenhuys, 1994; Elston and DeFelipe, 2002; Valverde, 2002 for reviews).

Different subtypes of pyramidal cells have been reported based on different aspects of their morphology, for example, inverted pyramidal cells, those whose axons project into the white matter, and those whose axons are restricted to the gray matter. In addition, pyramidal cells have been subdivided according to their neurochemical content and receptor subunit profiles (del Rio and DeFelipe, 1994; Preuss *et al.*, 1997; Gabernet *et al.*, 1999; González-Albo *et al.*, 2001; Hof *et al.*, 2001). It has also been proposed that pyramidal cells are not genetically fated to have their characteristic morphology. According to this theory, all variants of spiny neurons from the typical pyramidal cell to the typical spiny stellate cell are derived from a common precursor (Valverde, 1988). Here, the focus is on the discussion of pyramidal cells to the 'typical' pyramidal cell of unknown neurochemical content observed in mature primate cortex. As a prelude to studying specializations in pyramidal cell structure that have occurred during the evolution of different primate species we first set out how pyramidal cell structure varies in the cerebral cortex in a single species, the macaque monkey, and outline how specialization in cell structure may influence functional capabilities and, in turn, behavioral complexity.

4.13.2 Phenotypic Variation of Neocortical Pyramidal Cells

4.13.2.1 The Macaque Monkey

The macaque monkey is the most widely studied primate model in neuroscience. The primary objective of the study of the macaque brain is to better our understanding of the human brain. The normal mature macaque brain is clearly smaller than the normal mature human brain, and its cortex is arguably less differentiated. Estimates vary somewhat, but Old World monkeys, to which the macaque monkeys belong, separated from the lineage that led to contemporary humans ~25 Mya (Figure 4). Since this time sakis and a host of monkey species (e.g., titi, marmoset, tamarin, spider, howler, woolly, capuchin, squirrel, and owl monkeys) have branched from a common New World ancestor, while colobus monkeys, langurs, guenons, and

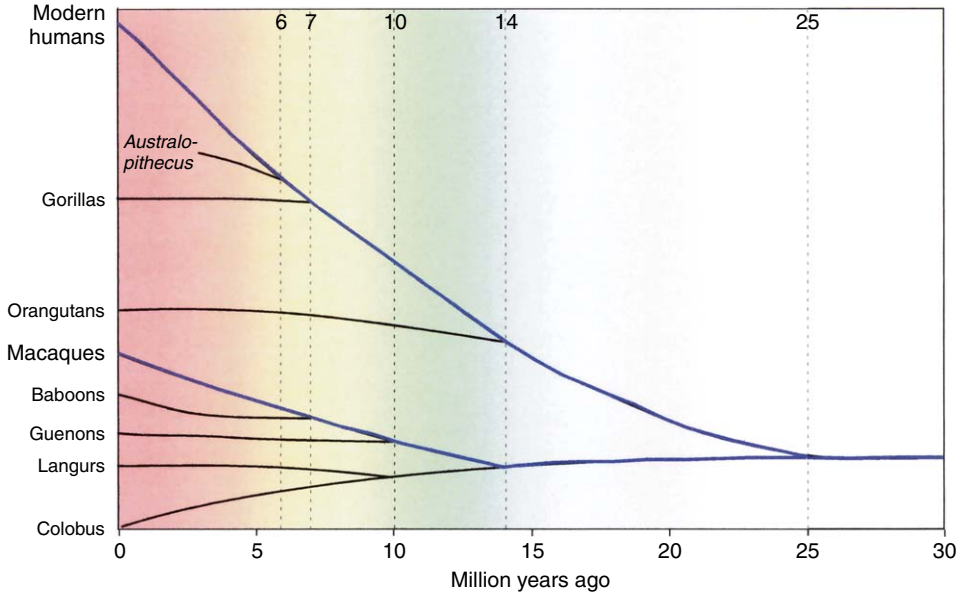

Figure 4 Schematic illustrating the evolution of different groups of primates. Note in particular the time at which the lineages leading to present-day humans and macaque monkeys (solid blue lines) diverged – 25 Mya. Note also that since this time all Old World and New World anthropoids have evolved from a common ancestor (~14 Mya), present-day orangutans and gorillas diverged from the human lineage (~14 and 7 Mya, respectively), and various hominids have evolved and become extinct (labeled *Australopithecus*). Modified from Dawkins, R. 2004. The Ancestor's Tale. A Pilgrimage to the Dawn of Life. Weidenfeld & Nicolson.

macaques have branched from a common Old World ancestor. Gibbons, chimpanzees, and gorillas have also evolved in this time. During a much shorter period of time (~6 million years), various hominids have evolved and become extinct (e.g., *Homo habilis, H. erectus, H. habilis, Africanis africanis, A. afarensis, A. biosei,* and *A. robustus*). Clearly enough time has passed for aspects of both the macaque and human brains to evolve their own unique specialized structure and functions. Indeed, studies of macaque monkeys (of which there are 20 species) reveal differences in their preferred habitats and behavior. Some macaques are reportedly highly social, gregarious foragers, whereas others are more circumspect in their interactions with conspecifics, some are terrestrial while others are arboreal, and some are obligate rainforest dwellers while others have adapted to living in human urbanizations (Nowak, 1999). Macaques that live on the Rock of Gibraltar have become expert robbers, relieving unsuspecting tourists of breakfast, lunch, and dinner. So adept have these monkeys become in their newfound trade that they are able to survive atop an otherwise barren rock almost completely devoid of food. Others, such as the Japanese macaque, have learned the pleasure of indulging in hot springs, spending hours bathing in steaming hot water while snow falls around them. Even more pronounced differences are seen in human behavior: hundreds of volumes have been published by anthropologists in which differences in behavior among human populations/cultures have been documented in painstaking detail. Nonetheless, in terms of the structure and function of the neocortex, the macaque monkey has become the most widely studied and best understood of primates. While acknowledging the problems associated with transferring findings from the macaque brain to that of humans, it is because the macaque monkey brain is the most extensively researched and best understood of all primate brains that much of my earlier work was focused on this animal.

4.13.2.1.1 Visual cortex The areas of the cerebral cortex that contain neurons involved in some form of visual processing are perhaps the most thoroughly explored in the macaque brain (see The Evolution of Visual Cortex and Visual Systems). Prior to the 1970s, most studies were restricted to the striate, or primary (V1), visual area. Since then, there has been an explosion in the number of studies in, and our understanding of, extrastriate visual cortex. Application of new techniques such as electrophysiological mapping and imaging, and the development of specialized tracers, have revealed that visual processing is much more complex than previously thought, involving up to half of the cortex and as many as 30 different areas (Figure 5). Various theories have been presented for the existence of so many visual cortical areas, and how visual stimuli are processed by neurons within these areas (see Kaas, 1987, 2000; Weller, 1988; Felleman and Van Essen, 1991; Rosa, 1997 for reviews). In addition, many theories have been proposed regarding the recruitment and interaction of neurons in these different cortical areas during particular visual tasks. Two of the most popular models include the quasihierarchical model and the distributed processing model. In the quasihierarchical model, visual inputs to cortex are processed through a series of cortical areas. These areas are not necessarily organized into a strict hierarchy, but there is some form of serial processing through select visual areas. In the distributed processing model, visual processing occurs in multiple cortical areas, but not necessarily in any form of hierarchy. That is not to say, however, that the two theories are mutually exclusive. Mountcastle, for example, highlighted how hierarchies may exist within a distributed system (Mountcastle, 1995).

New methods of quantification (e.g., Elston and Rosa, 1997, 1998, 2000; Elston, 2001) have revealed marked, systematic differences in pyramidal cell structure (cortical circuitry) in these different visual areas (Figures 6–8). Briefly, there is a trend for increasingly more complex pyramidal cells with progression from V1 to the second visual area (V2) and visual areas in the parietal (the lateral intraparietal area, LIP, and cytoarchitectonic area 7a) and temporal (the fourth visual area, V4, the middle temporal area, MT, cytoarchitectonic areas, TEO and TE, and the superior temporal polysensory area, STP) lobes. The increase in the size of the dendritic tree, coupled with a concomitant increase in the number of dendritic branches and spine density, results in a progressive doubling in our estimates of the total number of spines in the basal dendritic trees of pyramidal cells through V1, V2, V4, and TEO (Figure 8). The functional implications of these specializations in pyramidal cell structure in functionally related cortical areas are discussed in Section 4.13.2.2.

4.13.2.1.2 Sensorimotor cortex Like visual cortex, somatosensory and motor cortex in the macaque monkey has been divided into a number of highly interconnected cortical areas (Figure 9). Based on patterns of connectivity, neuronal response properties, and, more recently, imaging studies, several theories have been put forward regarding normal function across, and cooperativity

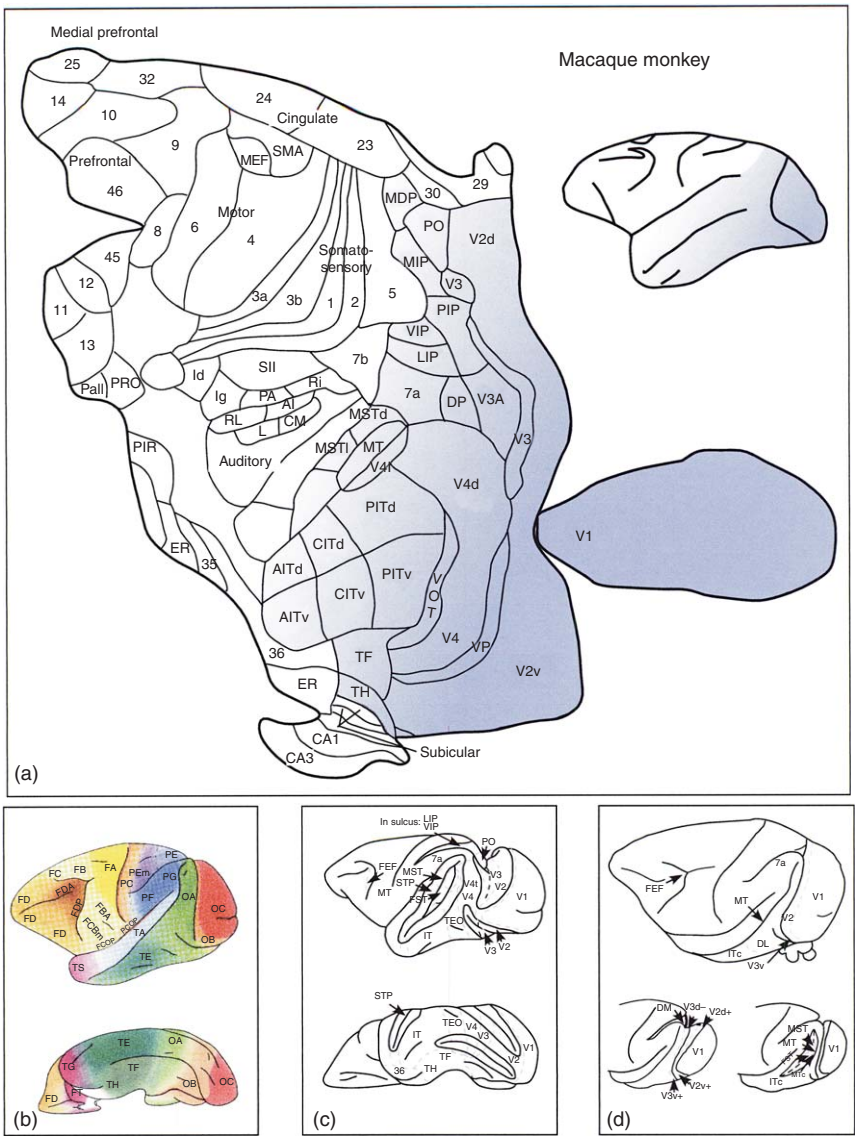

Figure 5 Schematic illustrating how different authors have divided visual cortex of the macaque monkey (blue shading). Note that the entire occipital lobe and a large portion of the temporal and parietal lobes contain neurons responsive to visual stimuli. Neurons in PFC, such as area 8 (the FEF) are also involved in visual processing (not shaded).1, 2, 3(a, b), 4, 5, 6, 7(a, b), 8, 9, 10, 11, 12, 13, 14, 23, 24, 25, 29, 30, 32, 35, 36, 45, 46, cytoarchitechtonic areas; 1 and 2, somatosensory cortex; 3b, primary somatosensory area (SI); 4, primary motor area (MI); 5, somatosensory association; 6, premotor cortex; 7a, visual association; 7b, somatosensory association; 8, includes the frontal eye field; 9, dorsolateral prefrontal cortex; 10, medial prefrontal cortex; 12 and 46, dorsolateral prefrontal cortex; 13, orbital prefrontal cortex (12 orbital); 23, posterior cingulate cortex; 24 and 32, anterior cingulate cortex; A, auditory; A1, primary auditory; AIT, anterior inferotemporal; CA1 and CA3, hippocampus; CIT, central inferotemporal; CM, caudomedial; d, dorsal; DL, dorsolateral area (caudal division); DM, dorsomedial; DP, dorsoparietal; ER, entorhinal; FA, FB, FC, FD, FBA, FCBM, PEM, FDA, FDP, FCOP, PCOP, TG, TS, PC, FF, PG, PF, cytoarchitectonic areas; CAs architechtonic areas; FEF, frontal eye field; FST, fundus of the superior temporal area; IT, inferotemporal cortex; Id, insular dysgranular; Ig, insular granular; L, lateral auditory; LIP, lateral intraparietal; MDP, mediodorsal parietal area; MEF, medial eye field; MIP, medial intraparietal area; MSTd, dorsal middle superior temporal; MSTl, lateral middle superior temporal; MT, middle temporal area; OA, OB, OC, and PE, cytoarchitectonic areas; PA, periamygdaloid; Pall, periallocortex; PIP, posterior intraparietal area; PIR, piriform; PIT, posterior inferotemporal; PO, parieto-occipital; PrCO, precentral opercular; PRO, proisocortex; Ri, retroinsular; RL, rostrolateral auditory cortex; SII, second somatosensory area; SMA, supplementary motor area; STP, superior temporal polysensory area; TE, TF, and TH, cytoarchitectonic areas; v, ventral; V1, primary visual area; V2, second visual area; V3, third visual area; V4, fourth visual area; VIP, ventral intraparietal; VOT, ventral occipitotemporal; VP, ventroposterior. a, Modified from Felleman, D. J. and Van Essen, D. C. 1991. Distributed hierarchical processing in primate cerebral cortex. *Cereb. Cortex* 1, 1–47. b, Modified from von Bonin, G. and Bailey, P. 1947. The Neocortex of *Macaca mulatta*. University of Illinois. c, Modified from Gross, C. G., Rodman, H. R., Gochin, P. M., and Colombo, M. W. 1993. Inferior temporal cortex as a pattern recognition device. In: Computational Learning and Recognition: Proceedings of the 3rd NEC Research Symposium (*ed.* E. Baum), pp. 44–73. Society for Industrial and Applied Mathematics. d, Modified from Kaas, J. H. 2003. Early visual areas: V1, V2, V3, DM, DL and MT. In: The Primate Visual System (*eds.* J. H. Kaas and C. Collins), pp. 139–159. CRC Press.

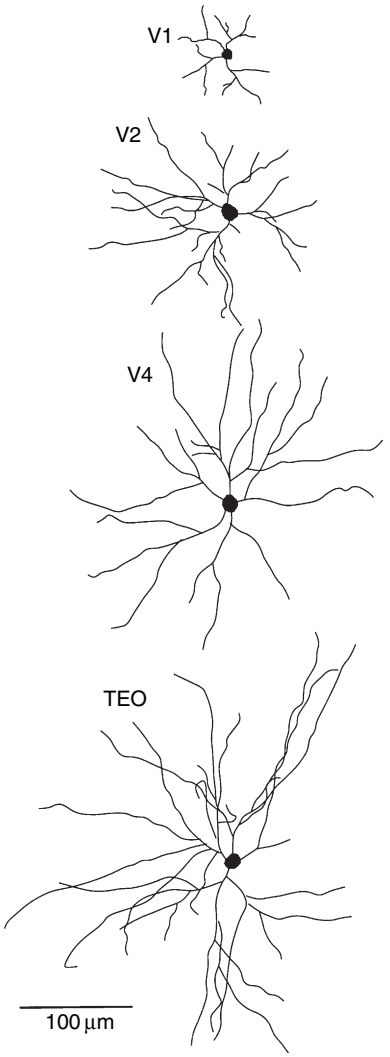

Figure 6 Drawings of the basal dendritic trees of layer III pyramidal cells in the primary (V1), second (V2), and fourth (V4) visual areas, as well as cytoarchitectonic area TEO of the macaque monkey. Each cell has a dendritic tree the size of which approximates the average for all neurons sampled in each cortical area. In addition to differences in the size of the dendritic trees, there are also differences in the number of branches and the total dendritic length for the different cells. Cells are skeletonized, dendritic diameters and spine density are not illustrated. Modified from Elston, G. N. and Rosa, M. G. P. 1998a. Morphological variation of layer III pyramidal neurones in the occipitotemporal pathway of the macaque monkey visual cortex. *Cereb. Cortex* 8, 278–294.

among, these different cortical areas (e.g., Mishkin, 1979; Pons *et al.*, 1987, 1992; Felleman and Van Essen, 1991; Passingham, 1997; Geyer *et al.*, 2000). By injecting large numbers of pyramidal cells in some of these different cortical areas, it has been possible to demonstrate marked and systematic differences in their size, branching complexity, and spine density. More specifically, two trends of increasing morphological complexity have been revealed with progression from the central sulcus to adjacent cortical areas (Figure 10). There is a systematic increase in the size of the dendritic trees of pyramidal cells, their branching complexity, and spine density within their basal dendritic trees, with caudal progression from the primary somatosensory area on the posterior wall of the central sulcus (area 3b) to the rostral bank of the intraparietal sulcus (area 5) and the exposed rostral portion of the inferior parietal lobule (area 7b). There is also an increase in the size of the dendritic trees of pyramidal cells, their branching complexity, and the total number of spines within their basal dendritic trees, with rostral progression from the primary motor area on the anterior wall of the central sulcus (area 4) to the exposed lateral portion of the precentral gyrus (area 6 or premotor cortex). These differences in size, branching complexity, and spine density result in appreciable differences in our estimates of the total number of spines in the basal dendritic tree of the average cell in each cortical area (Figure 11).

4.13.2.1.3 Cingulate cortex A study of the literature reveals little agreement regarding the functions performed in cingulate cortex, nor the number and location of cortical areas contained therein (Figure 12). Some have attributed higher cognitive and emotional functions to the anterior cingulate cortex and vegetative functions to posterior cingulate cortex (Allman *et al.*, 2001), whereas others have claimed the reverse (e.g., Baleydier and Mauguiere, 1980). In a recent series of studies in which cortical activity was recorded in awake behaving monkeys by fMRI, Dreher and colleagues revealed that neurons in anterior cingulate cortex, unlike those in posterior cingulate cortex, are often co-activated with gPFC during cognitive tasks (Dreher and Berman, 2002; Dreher and Grafman, 2003). Various other studies have also demonstrated 'executive' or cognitive function in the anterior cingulate, including error detection and reward-based decision-making (Gemba *et al.*, 1986; Carter *et al.*, 1998; Bush *et al.*, 2002; Shidara and Richmond, 2002; Hadland *et al.*, 2003).

While it is well known that these two different regions of cingulate cortex can be distinguished by their cytoarchitecture, particularly the granular layer, relatively little is known of possible differences in their microcircuitry. In order to investigate this, layer III pyramidal cells were injected in the posterior cingulate gyrus (area 23 of Brodmann). Their structure was compared with that of cells injected in the anterior cingulate gyrus (Brodmann's area 24). These investigations revealed

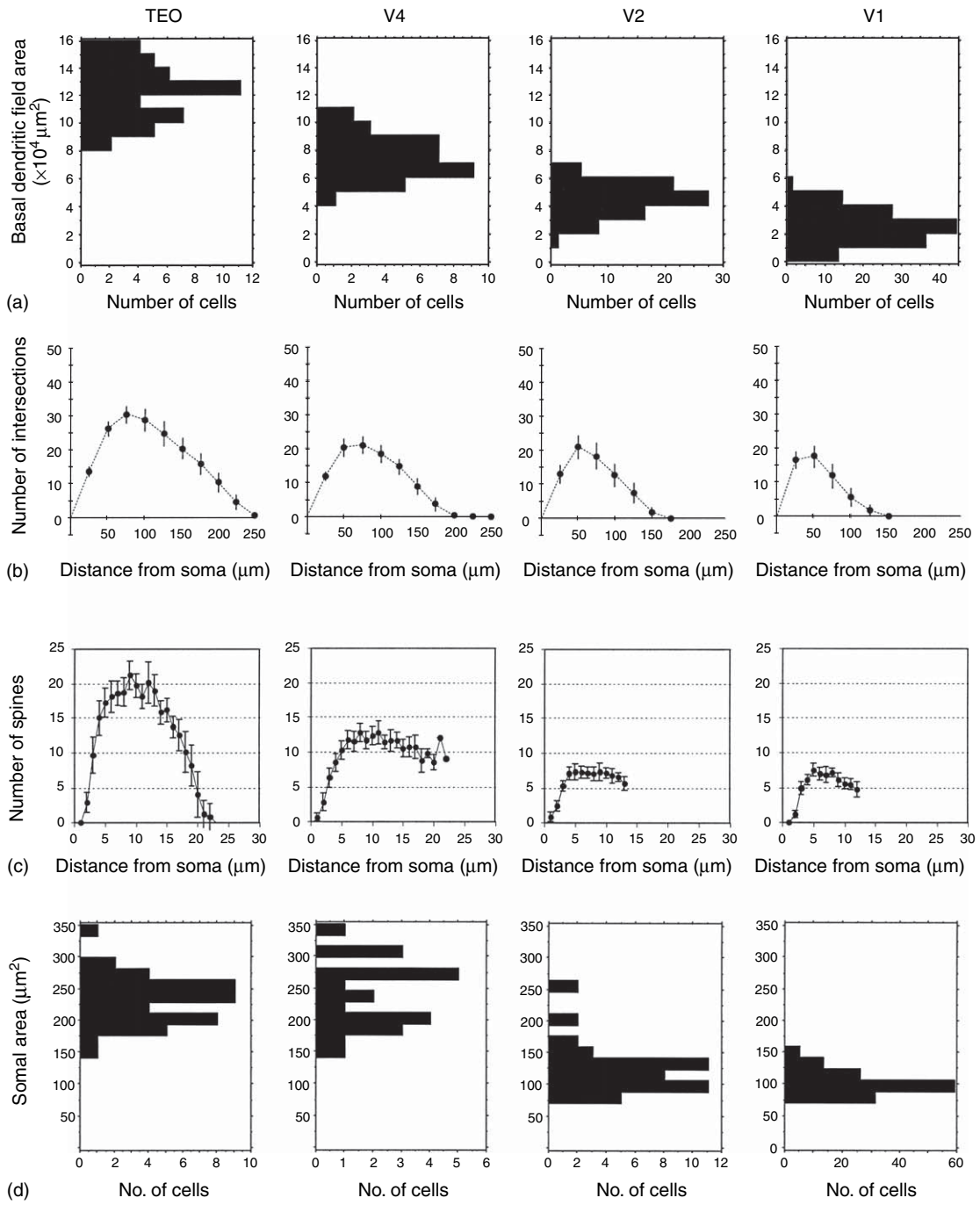

Figure 7 a, Frequency histograms of the size of the basal dendritic trees of layer III pyramidal neurons in the primary (V1), second (V2), and fourth (V4) visual areas, as well as cytoarchitectonic area TEO in the macaque monkey. The average size of the basal dendritic tree of layer III pyramidal neurons increases with rostral progression through the visual areas. b, Sholl analysis of dendritic trees of layer III pyramidal neurons in V1, V2, V4, and TEO yields a graphical representation of the complexity of the basal dendritic fields of layer III pyramidal neurons. Neurons in V1 had a similar branching complexity to those in V2, neurons in V4 had more complex basal dendritic fields than those in V1 or V2, and neurons in area TEO had the most complex basal dendritic trees. c, Plots of spine densities along basal dendrites of pyramidal neurons in V1, V2, V4, and area TEO in the macaque monkey. Twenty horizontally projecting basal dendrites were selected randomly from neurons in each region to determine if there was any difference in spine density. Spine density, which was calculated per 10 μm segment of dendrite, varied as a function of distance from the soma to the distal tips of the dendrites. Peak spine density was relatively low for neurons in V1 and V2, slightly higher in V4, and greatest in area TEO. d, Frequency histograms of somal areas of layer III pyramidal neurons in V1, V2, V4, and area TEO in the macaque monkey. Note there is an overall increase in the size of the cell bodies with rostral progression through these visual areas. Error bars = standard deviations. Modified from Elston, G. N. and Rosa, M. G. P. 1998a. Morphological variation of layer III pyramidal neurones in the occipitotemporal pathway of the macaque monkey visual cortex. *Cereb. Cortex* 8, 278–294.

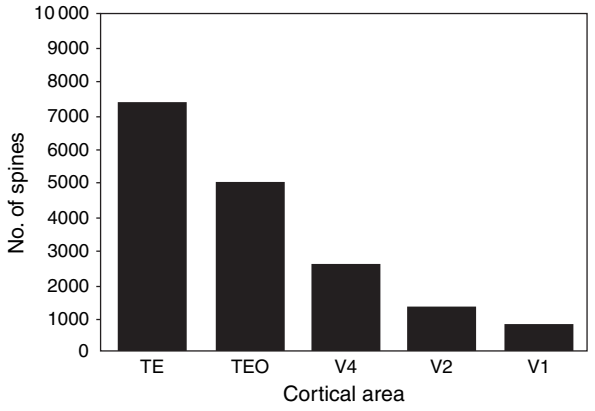

Figure 8 Plot illustrating differences in the estimates of the total number of spines in the basal dendritic trees of layer III pyramidal cells in cortical visual areas of the macaque monkey. Estimates were made by summing the product of average number of dendritic intersections per successive 25 μm annulus derived from Sholl analysis and the average spine density for the corresponding region in the dendritic tree. TE and TEO are cytoarchitectonic areas; V1, primary visual area; V2, second visual area; V4, fourth visual area. Data taken from Elston, G. N. and Rosa, M. G. P. 1998a. Morphological variation of layer III pyramidal neurones in the occipitotemporal pathway of the macaque monkey visual cortex. *Cereb. Cortex* 8, 278–294.

that pyramidal cells in the anterior cingulate gyrus were considerably larger, more branched, and more spinous than those in the posterior cingulate gyrus (Figure 13). Estimates of the total number of spines in the basal dendritic tree of the average cell reveal a twofold difference between cells in the anterior and posterior cingulate gyri (Figure 14). Moreover, pyramidal cell structure in the anterior cingulate gyrus more closely approximates that of cells in the gPFC, than does that of cells in the posterior cingulate (see discussion below).

4.13.2.1.4 Prefrontal cortex Exactly what constitutes 'prefrontal' cortex (PFC) has been interpreted in many ways. Some classify it as cortex that receives projections from the medial dorsal nucleus of the thalamus, whereas others distinguish the PFC by cytoarchitecture (see Fuster, 1997 for a review). Here, as intended by Brodmann (1913), the term is used to only include granular cortex anterior to the central sulcus (Figure 15). To avoid confusion here, this region is referred to as the gPFC. Regional differences in cytoarchitecture and patterns of corticocortical connectivity have been used to subdivide macaque gPFC in various ways (e.g., Vogt and Vogt, 1919; Walker, 1940; Barbas and Pandya, 1989; Preuss and Goldman-Rakic, 1991a, 1991b; Petrides, 1998; Petrides and Pandya, 1999, 2001; Pandya and Yeterian, 2000) (Figure 15). Broadly speaking, these different areas have been grouped into the lateral, medial, and orbital regions.

The gPFC has been the focus of intensive investigation because of its involvement in executive functions such as conceptual thinking, prioritizing and planning (see Goldman-Rakic, 1996; Fuster, 1997; Barbas, 2000; Petrides, 2000; Miller and Cohen, 2001 for reviews). Our understanding of the types of functions performed by neurons in the different regions within gPFC, and how other cortical regions participate in specific tasks, is growing rapidly with the advent of new methodologies (see Quintana and Fuster, 1999; Passingham *et al.*, 2000; Rolls, 2000; Fuster, 2001; Funahashi and Takeda, 2002 for reviews). For example, orbital and medial gPFC are involved in emotional behavior and the processing of taste, reward, memory, affect, and motivation. The lateral gPFC provides cognitive support to the temporal organization of behavior, speech, and reasoning and is involved in executive control of voluntary motor movements. As in other regions, gradients characterized by different patterns of connectivity and functions have also been reported in the gPFC (Goldman-Rakic, 1987; Petrides, 1987, 1991; Barbas, 1992; Wilson *et al.*, 1993; Barbas *et al.*, 1999; Pandya and Yeterian, 2000). What is clear from these studies is that because of the complexity of functions performed by neurons in gPFC, and the difficulty in quantifying neuronal responses to specific executive tasks, our understanding of prefrontal function is likely to lag behind that of sensory cortex for some time to come.

By injecting large numbers of pyramidal cells in different regions of gPFC, and relating these findings back to those of pyramidal cell structure in other cortical regions, new information may emerge related to executive cortical functions performed in different areas in the gPFC. One of the advantages of the cell injection approach is that it is not fraught with the methodological problems of anesthetic state, training, attention, or stimulus specificity associated with electrophysiological mapping and/or imaging studies. The investigations of pyramidal cells in gPFC revealed that they are highly branched and spinous relative to those in many other cortical regions (compare Figures 7, 10, 13, and 16). For example, cells in the frontal eye field (FEF) were the most branched of all cells studied in the cerebral cortex of the macaque monkey (Elston, 2000; Elston *et al.*, 2006b). In addition, estimates of the number of dendritic spines in their dendritic trees revealed that they are more spinous than their counterparts in other cortical areas (Figures 8, 11, 14, and 17). In particular,

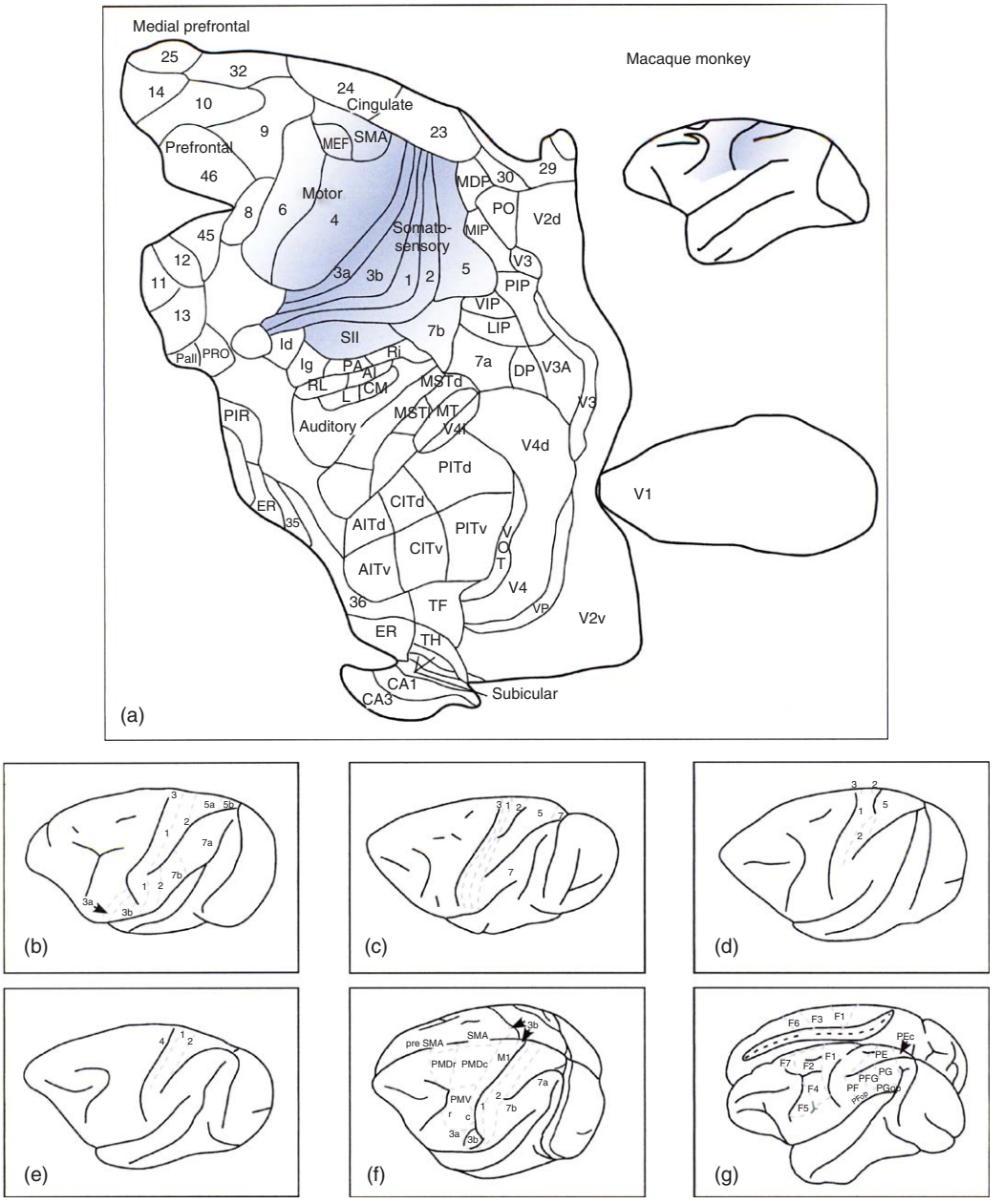

Figure 9 Schematic illustrating divisions in the sensorimotor cortex of the macaque monkey (blue shading) according to different authors. Note the differences in number, location, and nomenclature of cortical areas as presented by these different authors. 1, 2, 3(a, b), 4, 5(a, b), 6, 7(a, b), 8, 9, 10, 11, 12, 13, 14, 23, 24, 25, 29, 30, 32, 35, 36, 45, 46, cytoarchitechtonic areas; 1 and 2, somatosensory cortex; 3b, primary somatosensory area (SI); 4, primary motor area (MI); 5, somatosensory association; 6, premotor cortex; 7a, visual association; 7b, somatosensory association; 8, includes the frontal eye field; 9, dorsolateral prefrontal cortex; 10, medial prefrontal cortex; 12 and 46, dorsolateral prefrontal cortex; 13, orbital prefrontal cortex (12 orbital); 23, posterior cingulate cortex; 24 and 32, anterior cingulate cortex; A1, primary auditory; AIT, anterior inferotemporal; CA1 and CA3, hippocampus; CIT, central inferotemporal; CM, caudomedial; d, dorsal; DP, dorsoparietal; ER, entorhinal; Id, insular dysgranular; Ig, insular granular; L, lateral auditory; LIP, lateral intraparietal; MDP, mediodorsal parietal area; MEF, medial eye field; MIP, medial intraparietal area; MST, middle superior temporal area; MSTd, dorsal middle superior temporal; MSTl, lateral middle superior temporal; MT, middle temporal area; PA, periamygdaloid; Pall, periallocortex; PF, PFG, PFop, PFG, PGop, cytotechtonic areas; PIP, posterior intraparietal area; PIR, piriform; PIT, posterior inferotemporal area; PMD, premotor dorsal area; PO, parieto-occipital; PRO, proisocortex; PMV, premotor ventral area; R, rostral; Ri, retroinsular; RL, rostrolateral auditory cortex; SII, second somatosensory area; SMA, supplementary motor area; TF and TH, cytoarchitectonic area; v, ventral; V1, primary visual area; V2, second visual area; V3, third visual area; V4, fourth visual area; VIP, ventral intraparietal; VOT, ventral occipitotemporal; VP, ventroposterior. a, Modified from Felleman, D. J. and Van Essen, D. C. 1991. Distributed hierarchical processing in primate cerebral cortex. *Cereb. Cortex* 1, 1–47. b, Modified from Vogt, C. and Vogt, O. 1919. Allgemeine ergebnisse unserer hirnforschung. *J. Psychol. Neurol.* 25, 279–462. c, Modified from Peele, T. L. 1942. Cytoarchitecture of individual parietal areas in the monkey (*Macaca mulatta*) and the distribution of the efferent fibers. *J. Comp. Neurol.* 77, 693–738, d, Modified from Powell, T. P. S. and Mountcastle, V. B. 1959. The cytoarchitecture of the postcentral gyrus of the monkey *Macaca mulatta. Bull. Johns Hopkins Hosp.* 105, 108–131. e, Modified from Pons, T. P., Garraghty, P. E., Friedman, D. P., and Mishkin, M. 1987. Physiological evidence for serial processing in somatosensory cortex. *Science* 237, 417–420. f, Modified from Kaas, J. H. 2003. Early visual areas: V1, V2, V3, DM, DL and MT. In: The Primate Visual System (*eds.* J. H. Kaas and C. Collins), pp. 139–159. CRC Press. g, Modified from Geyer, S., Matelli, M., Luppino, G., and Zilles, K. 2000. Functional neuroanatomy of the primate isocortical motor system. *Anat. Embryol.* 202, 443–474.

pyramidal cells in dorsolateral area 9d are considerably more spinous than those in visual, auditory, somatosensory, motor, and cingulate cortex (cf. Elston and Rosa, 1997, 1998a; Elston *et al.*, 1999a, 2002, 2005a, 2006b; Elston and Rockland, 2002). Up to a 16-fold difference has been demonstrated in the number of spines in the basal dendritic trees of populations of pyramidal cells in V1 and the gPFC (Elston, 2001). That is not to say, however, that all pyramidal cells in gPFC are highly branched and spinous relative to those in other cortical regions. For example, those in prefrontal area 10 are less spinous than those in inferotemporal cortex (IT) and the anterior cingulate gyrus (Elston *et al.*, 2005a). Some interpretations of the functional consequences of these regional differences in pyramidal cell structure are discussed in the following section.

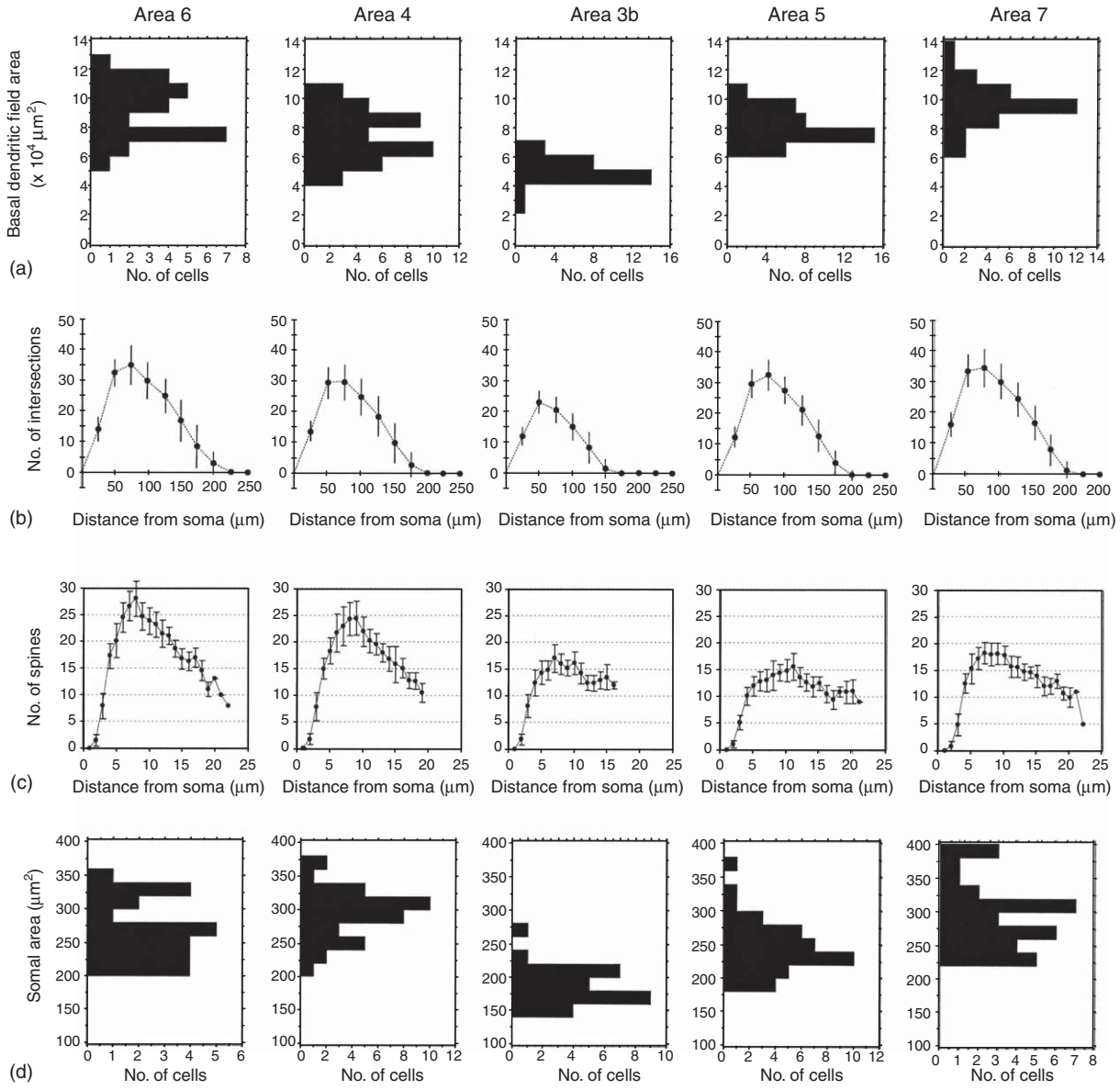

Figure 10 a, Frequency histograms of the size of the basal dendritic trees of layer III pyramidal neurons in the primary somatosensory area (3b), cytoarchitectonic somatosensory areas 5 and 7, the primary motor area (Brodmann's area 4), and premotor cortex (area 6). b, Graphs of the results of Sholl analysis of the basal dendritic trees of pyramidal neurons in sensorimotor areas. c, Plots of the proportion of dendritic spines, as a function of distance, in the basal dendritic trees of layer III pyramidal neurons in the above areas. d, Frequency histograms of somal areas of pyramidal neurons in areas 3b, 4, 5, 6, and 7. Error bars = standard errors. Modified from Elston, G. N. and Rockland, K. 2002. The pyramidal cell of the sensorimotor cortex of the macaque monkey: Phenotypic variation. *Cereb. Cortex* 10, 1071–1078.

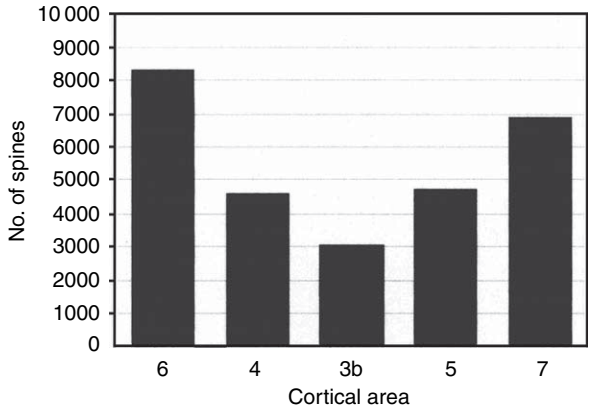

Figure 11 Schematic illustrating differences in the estimates of the total number of spines in the basal dendritic trees of layer III pyramidal cells in sensorimotor cortex of the macaque monkey. Area 3b, primary somatosensory area; area 4, primary motor cortex (also called MI); area 5, cytoarchitectonic somatosensory association area; area 6, premotor cortex; area 7, cytoarchitectonic somatosensory association area. Data taken from Elston, G. N. and Rockland, K. 2002. The pyramidal cell of the sensorimotor cortex of the macaque monkey: Phenotypic variation. *Cereb. Cortex* 10, 1071–1078.

4.13.2.2 Regional Specialization in the Pyramidal Cell Phenotype in the Macaque Monkey Cortex: Functional Interpretations

Neurons in different cortical regions are characterized by different response properties. That is not just to say that neurons in visual cortex discharge when presented with visual stimuli, those in somatosensory cortex discharge when presented with tactile stimuli, or those in auditory cortex discharge when presented with auditory stimuli, and so on. These modality specific aspects of their discharge selectivity are inevitably determined by the source of their inputs, originating from the retina, body surface, and cochlea, respectively. More specifically, neurons in different cortical regions are characterized by different discharge properties when presented with their respective stimuli. For example, while cells in V1 have been classified as 'simple' and 'complex' (Pettigrew and Daniels, 1973; Sillito, 1977; Kato *et al.*, 1978; Nelson and Frost, 1985; Reid *et al.*, 1991; Ferster *et al.*, 1996), in general the temporal duration of neuronal discharge following presentation of a stimulus is short by comparison with neurons in IT. More specifically, neurons in V1 are characterized by phasic discharge, whereas those in IT are characterized by tonic discharge when presented with a visual stimulus (Fuster and Alexander, 1971; Fuster and Jervey, 1981, 1983; Ashford and Fuster, 1985; Miller *et al.*, 1993; Miyashita *et al.*, 1993a, 1993b; Figure 18). Studies of the discharge properties of neurons in parietal cortex, such as somatosensory/visual association area 7, also reveal that the cells continue to discharge tonically for a relatively long time after presentation of a specific stimulus (Gnadt and Andersen, 1988; Koch and Fuster, 1989; Colby *et al.*, 1996; Constantinidis and Steinmetz, 1996; Zhou and Fuster, 1996, 1997; Chafee and Goldman-Rakic, 1998). This tonic activity, however, desists if a distractor is presented. Neurons in dorsolateral gPFC differ from those in association areas of the parietal and temporal lobes in that they continue to discharge tonically even when distractors are presented (Fuster and Alexander, 1971; Fuster, 1973; Fuster *et al.*, 1982; Funahashi *et al.*, 1989, 1993; Miller, 1999; Leung *et al.*, 2002). This persistence in tonic discharge despite presentation of distractors is widely believed to be important for executive functions performed by neurons in gPFC (see Fuster, 1997; Miller and Cohen, 2001; Wang, 2001; Elston, 2003b for reviews). Further evidence for regional variation in the functional characteristics of cortical circuitry comes from the work of Murayama *et al.* (1997), who demonstrated that tetonic stimulation led to long-term potentiation in TE but long-term depression in V1, and Shinomoto *et al.* (2005), who showed that neurons in area TE have characteristically different interspike intervals to those in medial motor areas.

The data on the structural complexity parallel these differences in neuronal discharge properties. Cells in V1, which are characterized by phasic discharge following stimulus presentation, have a relatively simple structure; those in association areas of the parietal and temporal lobes, which are characterized by tonic activity, that is, interrupted by presentation of distractors, have intermediate structural complexity while those in dorsolateral gPFC, which are characterized by tonic discharge properties that are sustained during presentation of distractors, have the most complex structure (Figure 18). The parallel between complexity in pyramidal cell structure and discharge properties makes it likely that the two are related: structure subserving function. Moreover, cortical circuits composed of pyramidal cells of different complexities will be characterized by different patterns of connectivity, resulting in different computational abilities. This theory departs from the popular belief that functional specialization in the cerebral cortex is attributed solely to the source of inputs. How differences in pyramidal cell structure may influence the computational capabilities of the circuits the cells comprise, is discussed below. Different aspects of the structure/function relationship are discussed at the cellular and systems levels.

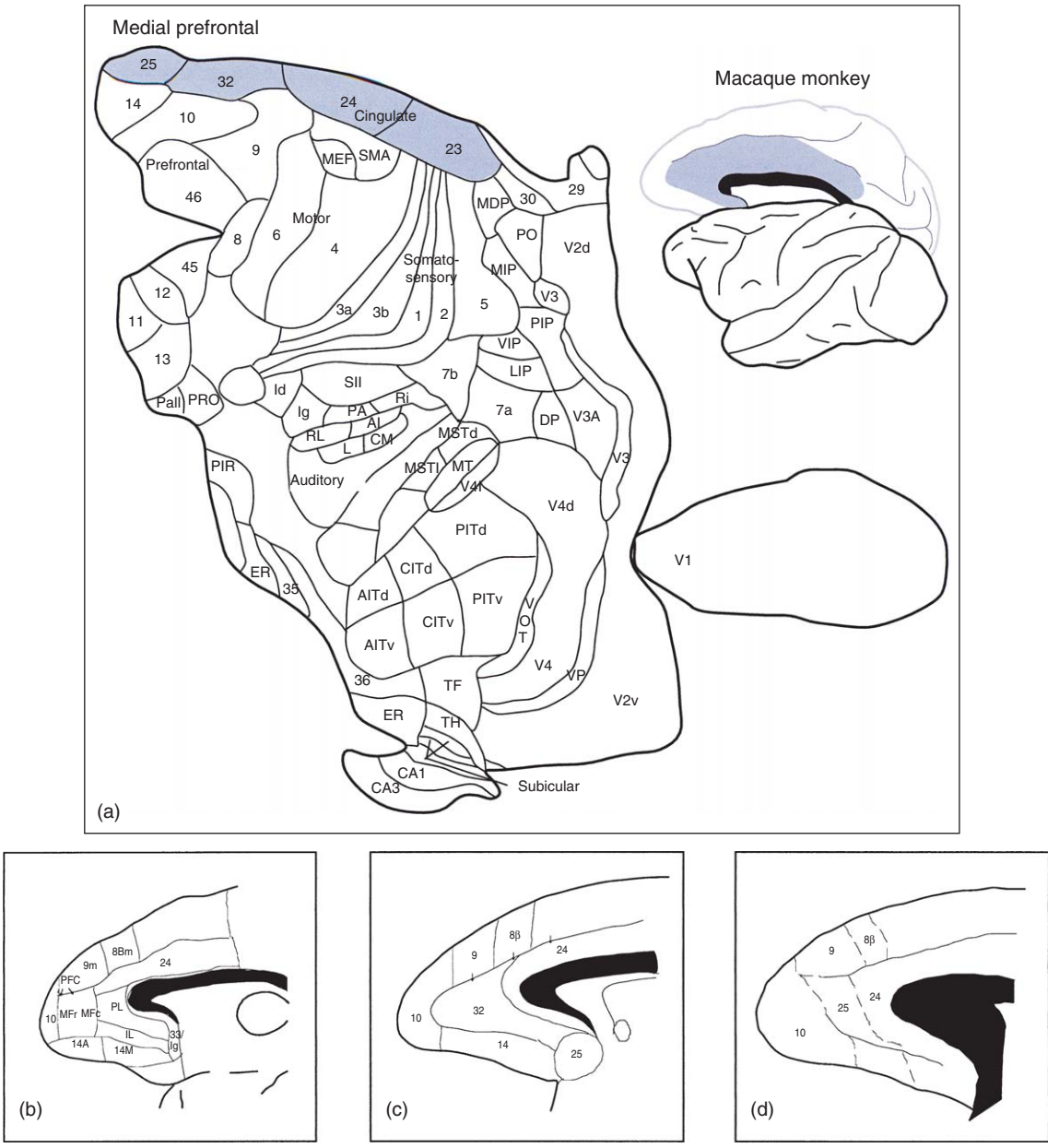

Figure 12 Schematic illustrating the subdivision of cingulate cortex of the macaque according to different authors. Note the differences in number, location, and nomenclature of cortical areas. 1, 2, 3(a, b), 4, 5, 6, 7(a, b), 8(Bm, β), 9(m), 10, 11, 12, 13, 14(A, M), 23, 24, 25, 29, 30, 32, 33, 35, 36, 45, 46, cytoarchitechtonic areas; 1 and 2, somatosensory cortex; 3b, primary somatosensory area (SI); 4, primary motor area (MI); 5, somatosensory association; 6, premotor cortex; 7a, visual association; 7b, somatosensory association; 8, includes the frontal eye field; 9, dorsolateral prefrontal cortex; 10, medial prefrontal cortex; 12 and 46, dorsolateral prefrontal cortex; 13, orbital prefrontal cortex (12 orbital); 23, posterior cingulate cortex; 24, and 32, anterior cingulate cortex; A, auditory; A1, primary auditory; AIT, anterior inferotemporal; c, caudal; CIT, central inferotemporal; CM, caudomedial; d, dorsal; DP, dorsoparietal; ER, entorhinal; Id, insular dysgranular; Ig, insular granular; L, lateral auditory; LIP, lateral intraparietal; M, medial visual area; MDP, mediodorsal parietal area; MEF, medial eye field; MF, medial frontal area; MIP, medial intraparietal area; MST, middle superior temporal area; MSTd, dorsal middle superior temporal; MSTl, lateral middle superior temporal; MT, middle temporal area; PA, periamygdaloid; Pall, periallocortex; PIP, posterior intraparietal area; PIR, piriform; PIT, posterior inferotemporal area; PL, prelimbic; PO, parieto-occipital; PRO, proisocortex; r, rostral; Ri, retroinsular; RL, rostrolateral auditory cortex; SII, second somatosensory area; SMA, supplementary motor area; TF and TH, cytoarchitectonic area; v, ventral; V1, primary visual area; V2, second visual area; V3, third visual area; V4, fourth visual area; VIP, ventral intraparietal; VOT, ventral occipitotemporal; VP, ventroposterior. a, Modified from Felleman, D. J. and Van Essen, D. C. 1991. Distributed hierarchical processing in primate cerebral cortex. *Cereb. Cortex* 1, 1–47. b, Modified from Preuss, T. M. and Goldman-Rakic, P. S. 1991a. Architectonics of the parietal and temporal association cortex in the strepsirhine primate *Galago* compared to the anthropoid *Macaca*. *J. Comp. Neurol.* 310, 475–506. c, Modified from von Bonin, G. and Bailey, P. 1947. The Neocortex of *Macaca mulatta*. University of Illinois. d, Modified from Petrides, M. and Pandya, D. N. 1999. Dorsolateral prefrontal cortex: Comparative cytoarchitectonic analysis in the human and the macaque monkey brain and corticocortical connection patterns. *Eur. J. Neurosci.* 11, 1011–1036.

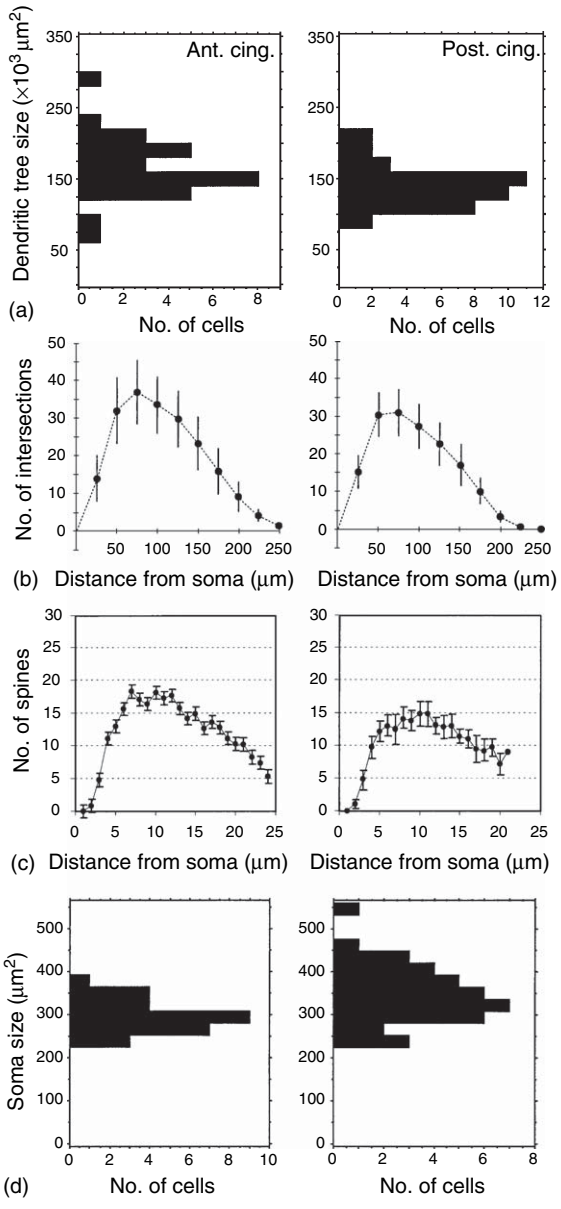

Figure 13 Graphs illustrating the (a), size of; (b), branching complexity in; and (c), spine density along individual dendrites, in the basal dendritic trees of layer III pyramidal cells in the posterior cingulate and anterior cingulate gyrus of the macaque monkey (post. cing. and ant. cing., respectively). d, Frequency histograms of the size of the cell bodies of the same layer III pyramidal cells illustrated in (a–c). Error bars = standard errors. Modified from Elston, G. N., Benavides-Piccione, R., and DeFelipe, J. 2005a. A study of pyramidal cell structure in the cingulate cortex of the macaque monkey with comparative notes on inferotemporal and primary visual cortex. *Cereb. Cortex* 15, 64–73.

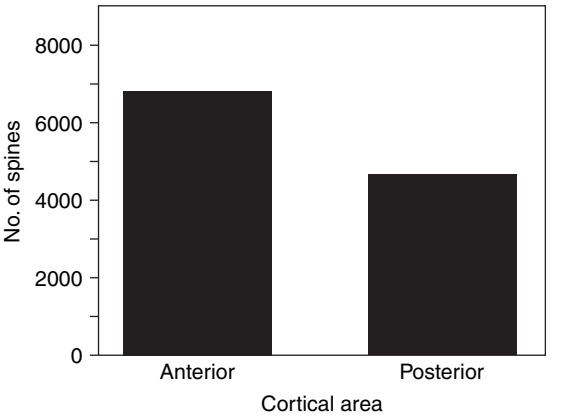

Figure 14 Schematic illustrating differences in the estimates of the total number of spines in the basal dendritic trees of layer III pyramidal cells in the anterior cingulate gyrus (Brodmann's area 24) and the posterior cingulate gyrus (Brodmann's area 23).

4.13.2.2.1 Cellular level Many aspects of dendritic tree structure can be identified that may influence neuronal function (see White, 1989; Koch, 1999; Mel, 1999; Spruston *et al.*, 1999; Magee, 2000; Häusser and Mel, 2003 for reviews). Three of these features – size, branching pattern, and number/distribution of inputs, are considered here. Pyramidal cells characterized by a large dendritic tree extend over a wider region of cortex than cells with smaller tree (Sholl, 1956). In macaque visual cortex, there is a successive increase in both the size of the dendritic tree and the receptive field size for neurons in visual areas V1, V2, V4, and TEO (cf. Hubel and Wiesel, 1974; Dow *et al.*, 1981; Van Essen *et al.*, 1984; Burkhalter and Van Essen, 1986; Felleman and Van Essen, 1987; Schein and Desimone, 1990; Boussaoud *et al.*, 1991; Roe and T'so, 1995; Elston and Rosa, 1998a). Similarly, pyramidal cells have progressively larger dendritic trees through somatosensory areas 3b, 5, and 7, with a parallel increase in their receptive field size (Huntley and Jones, 1991; Pons *et al.*, 1992; Elston and Rockland, 2002). Differences in the size of the dendritic tree of pyramidal cells in motor cortex (area 4) and premotor cortex (area 6) also parallel differences in their motor fields (Mitz and Wise, 1987; Luppino *et al.*, 1991; Donoghue *et al.*, 1992; Elston and Rockland, 2002). In visual cortex, the differences in the size of the dendritic tree, coupled with receptive field map compression, result in more than a 100-fold difference in the region of the visual map sampled by individual pyramidal cells in V1 and cytoarchitectonic area TEO (Elston and Rosa, 1998a). Moreover, the size of the dendritic tree of layer III pyramidal cells and the size of neural receptive fields are significantly correlated in visual areas V1, V2, V4, and TEO (Figure 19). The largest cells reported so far in the macaque monkey cortex are those in the anterior cingulate gyrus. These cells sample a region of cortex, on average, eight times larger than that sampled by cells in V1.

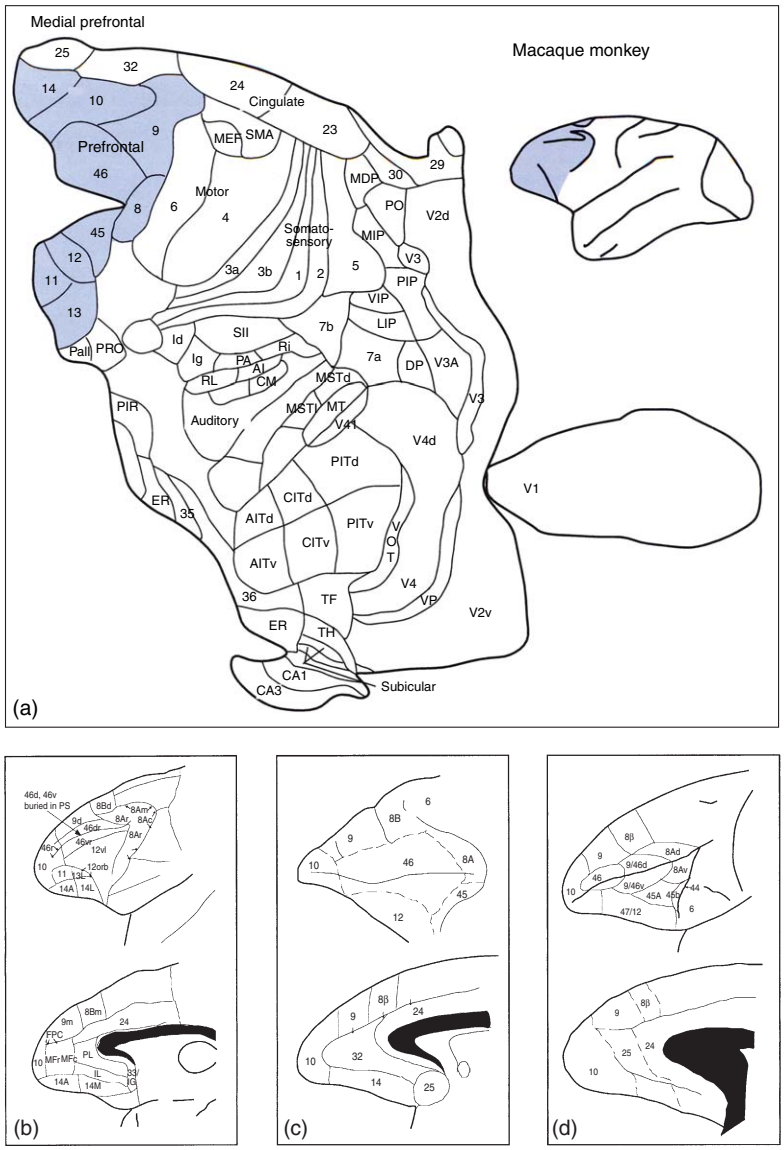

Figure 15 Schematic illustrating the subdivision of PFC of the macaque according to different authors. Note the differences in number, location, and nomenclature of cortical areas as presented by these different authors. 1, 2, 3(a, b), 4, 5, 6, 7(a, b), 8, 9, 10, 11, 12, 13, 14, 23, 24, 25, 29, 30, 32, 33, 35, 36, 45, 46, cytoarchitechtonic areas; 1 and 2, somatosensory cortex; 3b, primary somatosensory area (SI); 4, primary motor area (MI); 5, somatosensory association; 6, premotor cortex; 7a, visual association; 7b, somatosensory association; 8, includes the frontal eye field; 9, dorsolateral prefrontal cortex; 10, medial prefrontal cortex; 12 and 46, dorsolateral prefrontal cortex; 13, orbital prefrontal cortex (12 orbital); 23, posterior cingulate cortex; 24 and 32, anterior cingulate cortex; A, auditory; A1, primary auditory; AIT, anterior inferotemporal; c, caudal; CIT, central inferotemporal; CM, caudomedial; d, dorsal; DP, dorsoparietal; ER, entorhinal; Id, insular dysgranular; Ig, insular granular; L, lateral auditory; LIP, lateral intraparietal; m, medial; M, medial visual area; MDP, mediodorsal parietal area; MEF, medial eye field; MF, medial frontal area; MIP, medial intraparietal area; MST, middle superior temporal area; MSTd, dorsal middle superior temporal; MSTl, lateral middle superior temporal; MT, middle temporal area; PA, periamygdaloid; Pall, periallocortex; PIP, posterior intraparietal area; PIR, piriform; PIT, posterior inferotemporal area; PO, parieto-occipital; PRO, proisocortex; r, rostral; Ri, retroinsular; RL, rostrolateral auditory cortex; SII, second somatosensory area; SMA, supplementary motor area; TF and TH, cytoarchitectonic area; v, ventral; v1, ventrolateral; V1, primary visual area; V2, second visual area; V3, third visual area; V4, fourth visual area; VIP, ventral intraparietal; VOT, ventral occipitotemporal; VP, ventroposterior. a, Modified from Felleman, D. J. and Van Essen, D. C. 1991. Distributed hierarchical processing in primate cerebral cortex. *Cereb. Cortex* 1, 1–47. b, Modified from Preuss, T. M. and Goldman-Rakic, P. S. 1991a. Architectonics of the parietal and temporal association cortex in the strepsirhine primate *Galago* compared to the anthropoid *Macaca*. *J. Comp. Neurol.* 310, 475–506. c, Modified from von Bonin, G. and Bailey, P. 1947. The Neocortex of *Macaca mulatta*. University of Illinois. d, Modified from Petrides, M. and Pandya, D. N. 2001. Comparative cytoarchitectonic analysis of the human and the macaque dorsolateral prefrontal cortex and corticocortical connection patterns in the monkey. *Eur. J. Neurosci.* 16, 291–310.

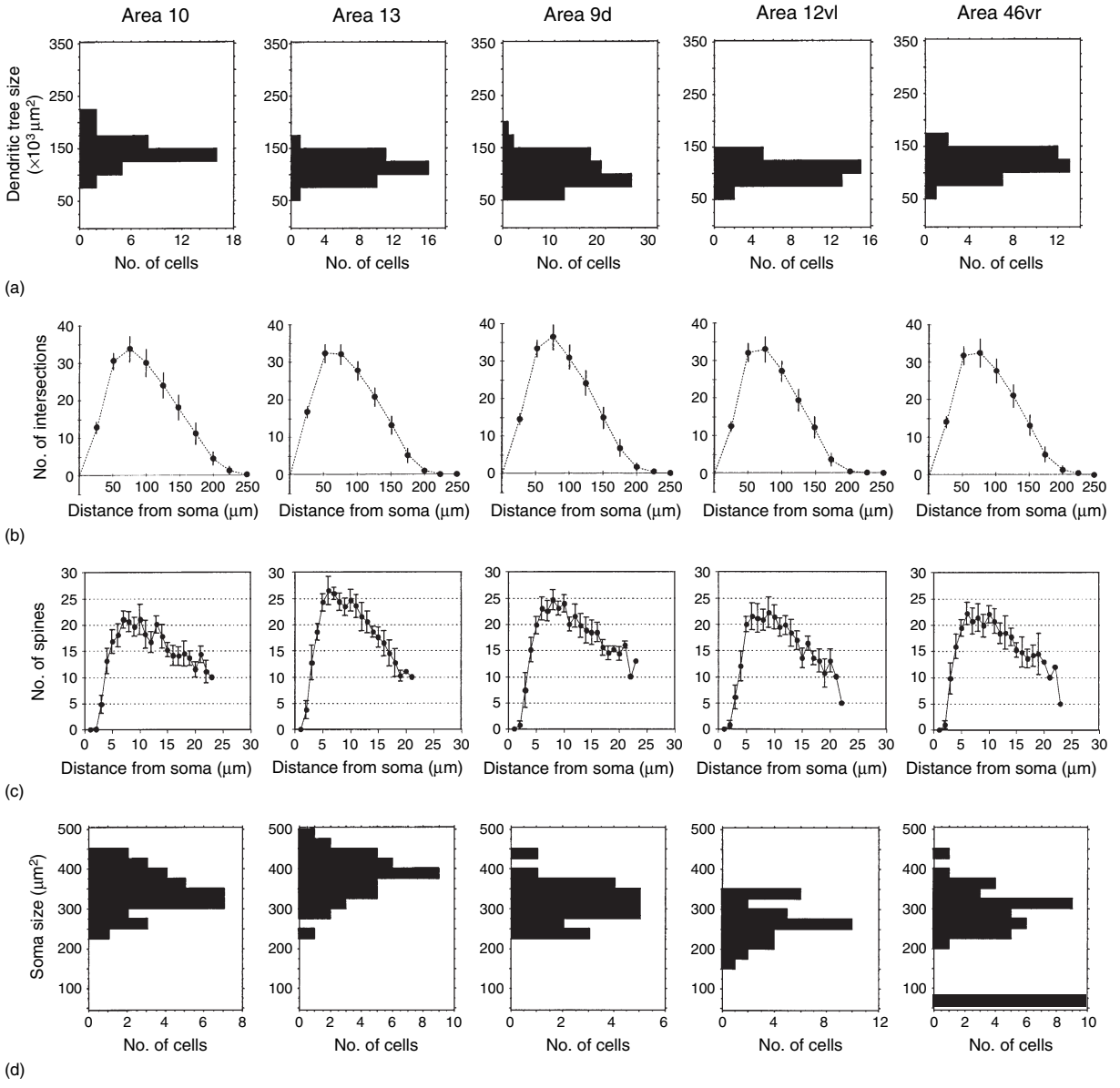

Figure 16 a, Frequency histograms of the size of the basal dendritic trees of layer III pyramidal neurons in cortical areas 9d (dorsal subdivision of cytoarchitectonic area 9), 10, 12vl (ventrolateral subdivision of cytoarchitectonic area 12), 13 (also known as area 12orbital), and 46vr (ventral rostral subdivision of cytoarchitectonic area 46) in gPFCof the macaque monkey. b, Graphs of the results of Sholl analysis of the basal dendritic trees of the same layer III pyramidal neurons. c, Plots of the density of dendritic spines, as a function of distance from the cell body to the distal dendritic tips, in the basal trees of the same layer III pyramidal neurons. d, Frequency histograms of somal areas of pyramidal neurons in areas 9d, 10, 12vl, 13, and 46vr. Error bars = standard errors. Modified from Elston, G. N., Benavides-Piccione, R., Elston, A., Manger, P., and DeFelipe, J., unpublished observations.

In addition to determining the physical area of cortex from which neurons sample inputs, the size of the dendritic tree will influence its geometrical relationship with input axonal arbors. Supragranular pyramidal cells receive inputs from multiple sources (see Rockland, 1997 for a review). Here we focus on the sampling geometry between layer III pyramidal cell dendritic trees and the intrinsic lattice of axons, which arise from supragranular pyramidal cells (Rockland and Lund, 1982, 1983; Rockland *et al.*, 1982; McGuire *et al.*, 1991). In visual cortex, these intrinsic horizontal axonal patches are successively larger in V1, V2, V4, and cytoarchitectonic area TEO (Lund *et al.*, 1993; Fujita and Fujita, 1996), their size paralleling that of the dendritic tree of layer III pyramidal cells (Figure 19). Likewise, the size of these intrinsic horizontal patches increases through the primary somatosensory area

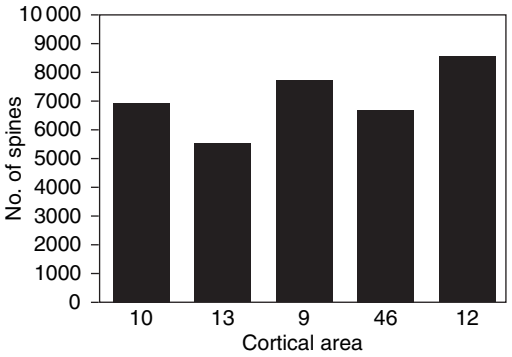

Figure 17 Plot illustrating differences in the estimates of the total number of spines in the basal dendritic trees of layer III pyramidal cells in granular prefrontal areas of the macaque monkey. 9d, dorsal subdivision of cytoarchitectonic area 9; 10, cytoarchitectonic area 10; 12vl, ventrolateral subdivision of cytoarchitectonic area 12; 13, cytoarchitectonic area 13 (also known as area 12 orbital); 46vr, ventral rostral subdivision of cytoarchitectonic area 46. Data taken from Elston, G. N., Benavides-Piccione, R., Elston, A., Manger, P., and DeFelipe, J., unpublished observations.

(area 3b) to areas 5 and 7 (Juliano *et al.*, 1990; Lund *et al.*, 1993) just as the dendritic trees of the cells in these areas become larger (Elston and Rockland, 2002). This parallel between dendritic tree size and intrinsic axonal patch size is also present in motor areas 4 and 6 (Lund *et al.*, 1993; Elston and Rockland, 2002). Studies of the intrinsic axonal patches in IT and dorsolateral gPFC reveal a different pattern. In these cortical regions, the axon patches are more diffuse, extending over much larger regions of cortex (Kritzer and Goldman-Rakic, 1995; Pucak *et al.*, 1996; Melchitzky *et al.*, 1998, 2001; Tanigawa *et al.*, 2005). This geometrical relationship between the size of pyramidal cell dendritic trees and the topography of the intrinsic horizontal lattice of projections reportedly influences the sampling geometry between the intrinsic axons originating from supragranular pyramidal cells and their dendritic trees (Figure 19), cells in cortical areas in which the size of the dendritic trees match the size of the

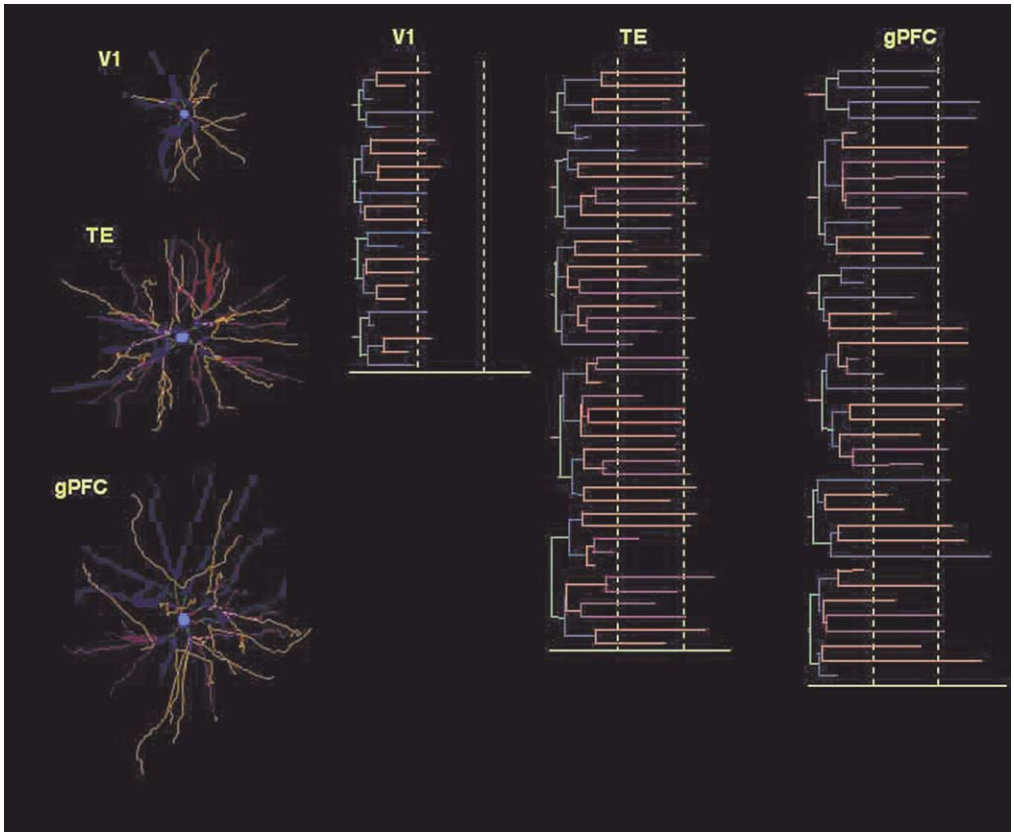

Figure 18 Drawings of pyramidal cells (skeletonized images of the basal dendritic tree in the tangential plane) located in layer III of the primary visual area (V1), IT (cytoarchitectonic area TE), and the dorsolateral gPFC and their respective dendograms. Note the difference in the number of 1° (red), 2° (green), 3° (blue), 4° (orange), and 5° (purple) dendrites, the number of separate compartments supported by the 1° dendrites (three, four, and five in V1, TE, and gPFC, respectively) and differences in the total dendritic length of the illustrated cells. Cells were chosen by virtue of their having a dendritic tree with a size approximating the average of that for each cortical area.

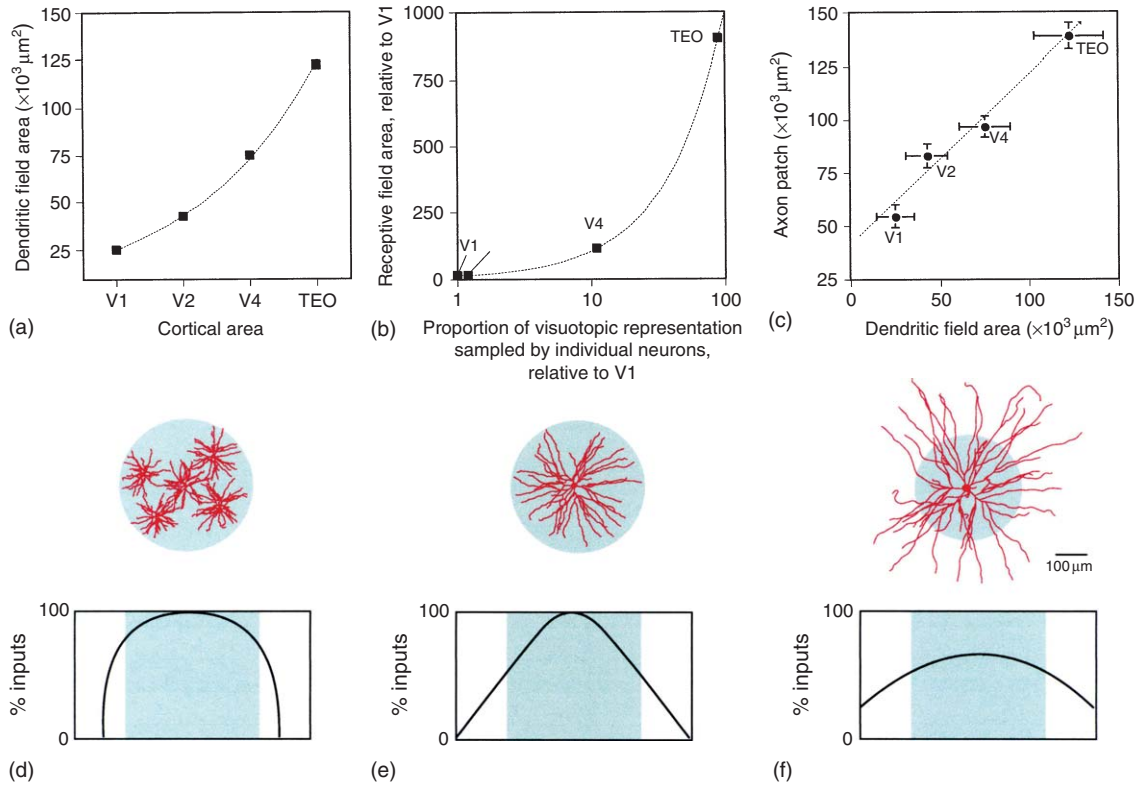

Figure 19 Schematics illustrating (a), the trend for pyramidal cells to have progressively larger dendritic trees through the primary, second, and fourth visual areas (V1, V2, and V4, respectively) and cytoarchitectonic area TEO of the macaque monkey; (b), the relationship between the size of the dendritic trees and the receptive fields of these same cells; (c), the relationship between the size of the basal dendritic trees of these pyramidal cells and the size of the intrinsic axonal patches; and (d, e), different sampling strategies between pyramidal cell basal dendritic trees and the intrinsic axonal patches. In (d), the dendritic trees of pyramidal cells are smaller than the axonal patches from which they receive inputs, meaning that a large population of cells potentially sample from the same source. Maximum connectivity (e) may be achieved if the basal dendritic trees are the same size as the axonal modules. There is greater potential for mixing of inputs (f) when the pyramidal cell dendritic trees are larger than the axonal patches from which they sample. Injected cells, and receptive field sizes selected for comparison, were sampled from the central 5° in the visual field. Data for receptive field sizes taken from Hubel and Wiesel (1974), Dow *et al.* (1981), Van Essen *et al.* (1984), Burkhalter and Van Essen (1986), Felleman and Van Essen (1987), Schein and Desimone (1990), Boussaoud *et al.* (1991), Roe and T'so (1995). Data on the size of the basal dendritic trees taken from Elston and Rosa (1998a), and data on the size of the intrinsic axonal patches taken from Amir *et al.* (1993), Lund *et al.* (1993). The figures related to sampling strategies were modified from Malach (1994).

axonal patches have maximum potential to sample all inputs from a single source, whereas cells that have dendritic trees that are larger than the intrinsic axonal patches are likely to sample more divergent sources (see Malach, 1994; Elston, 2003a for reviews).

Marked differences in the number of dendritic spines, each of which receives at least one asymmetrical synapse (Colonnier, 1968; Jones, 1968; Peters and Kaiserman-Abramof, 1969), the presynaptic terminals of which contain the excitatory neurotransmitter glutamate (DeFelipe *et al.*, 1988; Kharazia and Weinberg, 1993), means that pyramidal cells in different cortical areas integrate different numbers of inputs over the region of cortex from which they extend. For example, in visual cortex, there is a successive doubling in the number of spines (excitatory inputs) in the dendritic trees of pyramidal cells in V1, V2, V4, and TEO. Layer III pyramidal cells in macaque area TE, contain, on average, more than 11 times the spines in their basal dendritic trees than those in macaque V1, while cells in area STP contain, on average, more than 13 times the spines than those in V1 (Elston *et al.*, 1999a). Similar trends are reported in somatosensory areas 3b, 5, and 7, although the extent of the interareal differences is less than that reported in visual cortex. Pyramidal cells in the gPFC have, on average, as many as 20 times more spines than those in V1. These differences cannot be accounted for by the differing size of the dendritic trees of pyramidal

cells, but reflect variations in the density of spines within the dendritic tree. For example, the dendritic trees of cells in the dorsolateral gPFC are smaller than those in IT but they contain more dendritic spines.

The differences in the density of dendritic spines along the dendrites of pyramidal cells may also influence various aspects of the integration of inputs along the dendrites. For example, the peak spine density along dendrites of pyramidal cells in TE is more than three times higher than that found in V1 (Elston *et al.*, 1999a). These differences in spine density are likely to influence local summation of postsynaptic potentials (EPSPs), cooperativity between inputs, and propagation of potentials (Shepherd *et al.*, 1985; Frick *et al.*, 2004). Regional differences in the morphology of spines (Elston *et al.*, 1999a, 1999b; Benavides-Piccione *et al.*, 2003; Konur *et al.*, 2003) may also influence their functional properties (see Harris and Karter, 1994; Shepherd, 1998; Koch, 1999; Tsay and Yuste, 2004 for reviews). For example, the volume of the spine head is reportedly correlated with the postsynaptic density. This correlates with the number of receptors and the potential for current to pass through the membrane, and the number of docked vesicles on the postsynaptic terminal, which reflects both quantity and probability of release of neurotransmitter (Harris and Stevens, 1989; Nusser *et al.*, 1998; Schikorski and Stevens, 2001).

In addition, specialization in the branching structure of pyramidal cells in different cortical regions allows varying degrees of compartmentalization of these inputs within their dendritic trees. Modeling studies have demonstrated how the number of branches in, and the distribution of receptors throughout, the dendritic tree of pyramidal cells may influence its functional capacity: cells with the same number of inputs distributed throughout dendritic trees with different numbers of branches have different functional capacities (Mel, 1992, 1993; Poirazi and Mel, 2000). Cells that sample more inputs in dendritic trees with more complex branching patterns have even greater functional capacities (Figure 20). Thus, in visual cortex, cells in area TEO or TE are likely to have greater functional capacities than those in V1, for example. Similarly, neurons in somatosensory area 7 or premotor cortex are likely to have greater functional capacity than those in areas 3b and 4, respectively. Nowhere is this potential greater than in dorsolateral gPFC, which contains the most branched and spinous of all cells studied. While this remains theoretical (Koch *et al.*, 1982; Rall and Segev, 1987; Shepherd and Brayton, 1987; Koch, 1999), compartmentalization has been demonstrated empirically in the retina (Taylor *et al.*, 2000) and auditory brainstem (Agmon-Snir *et al.*, 1998).

4.13.2.2.2 Systems level Cortical function, whether it be processing of visual information, somatosensation, audition, motor planning, emotion, or executive functions, never occurs solely in a small group of clustered neurons, nor in neurons restricted solely to a single cortical area. Imaging studies in particular have revealed how much of the cerebral cortex may be recruited during a particular sensory stimulus presentation, memory task or cognitive task (see Paulesu *et al.*, 1997; Passingham, 1997; Friston, 2001 for reviews). Ensembles of neurons are recruited across multiple areas for any given task, although, importantly, not all neurons within these different areas are necessarily activated. Neurons located within a particular cortical area can be involved in multiple overlapping circuits, which are activated during specific functions (both within and between cortical areas). Some ways in which specialization in pyramidal cell structure may influence cortical processing at the level of the single cortical area (areal) and/or across multiple cortical areas (multi-areal) are discussed here.

We have already seen in Figure 19 how differences in the size of intrinsic axonal patches result in different sampling strategies for individual neurons in visual, somatosensory, association, and gPFC. In addition, differences in the spacing and lateral extent of these intrinsic patches result in different patterns of connectivity in different cortical areas (Figure 21). For example, the intrinsic patches in V1 are closely spaced and restricted to the immediate surroundings of the injection site, whereas those in visual areas such as TEO and TE are more widely spaced and extend over larger regions of cortex. In topographically organized cortex, this equates to a larger proportion of the topographic representation being sampled by individual neurons. In visual cortex, the differences in the spacing of the intrinsic patches, coupled with compression of the visual map, result in a much larger proportion of the visual scene being sampled by individual neurons in TEO than, for example, in V1. Not surprisingly, these principles also hold true in somatosensory cortex: center-to-center spacing of intrinsic axonal patches increases through areas 3b, 5, and 7, paralleling differences in the receptive field properties of neurons in these areas. In gPFC, the intrinsic axonal 'patches' extend over even larger expanses of cortex. Although the gPFC is not topographically organized in the same way as the visual, somatosensory, and motor cortices, different authors have reported various forms of systematic

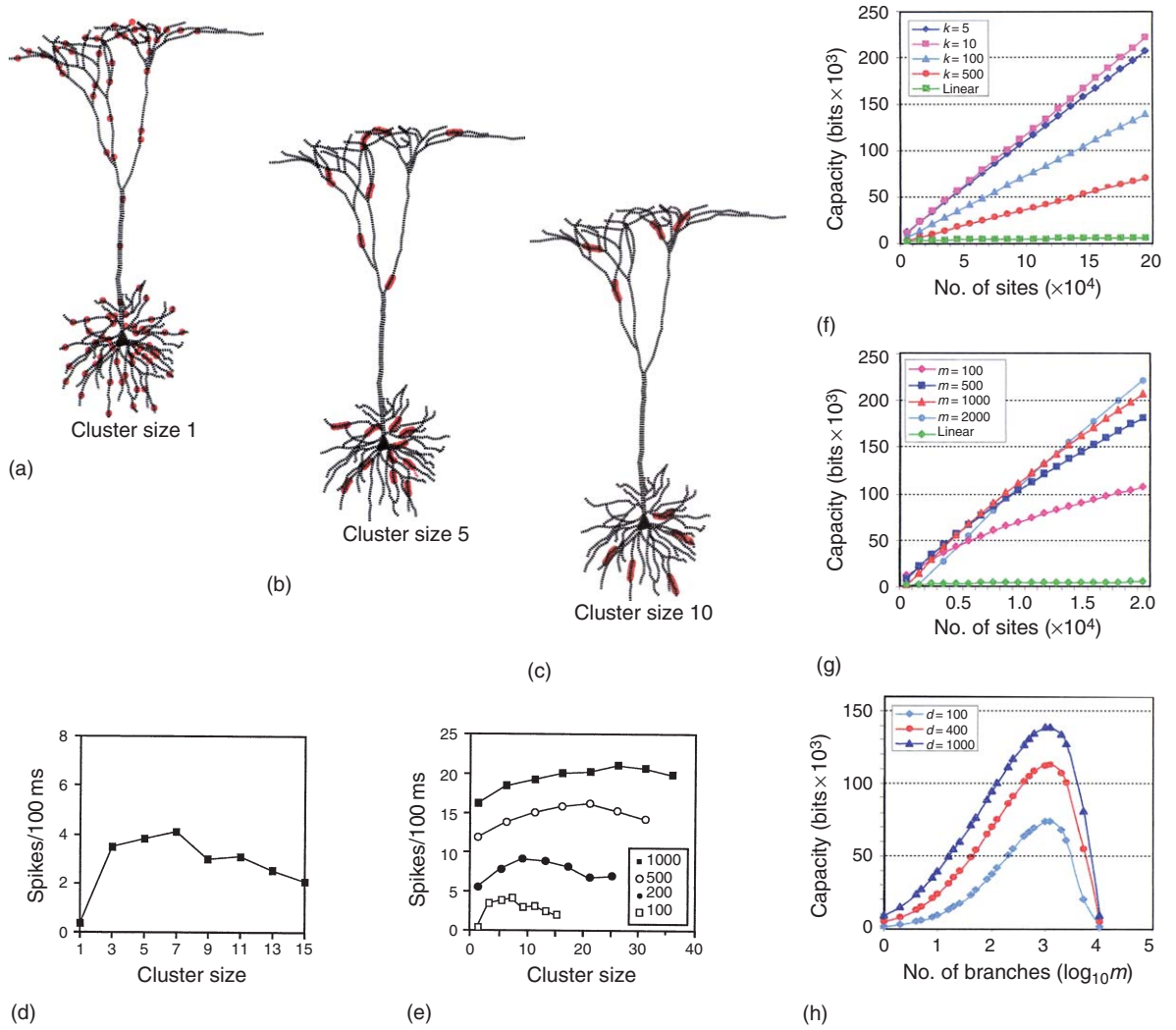

Figure 20 a–c, Drawings of a neocortical pyramidal cell that has a constant number of synchronously active excitatory inputs (red circles) distributed throughout the dendritic tree in different cluster sizes. d, Pyramidal cells receiving different numbers of synchronously active excitatory inputs will be characterized by different saturation levels for cells. e, Increasing the number of inputs (with a set cluster size) also increases the number of spike discharges. The 'capacity' (measured in bits) of a cell of fixed branch length, or total number of branches, increases with an increase in the number of sites (f–h). An increase in either branch length (*k*) or branch count (*m*) results in increasing 'capacity' with an increase in the number of sites ((f) and (g), respectively). The 'capacity' of a cell of fixed size (h), increases to a peak with an increase in the number of branches, before decreasing to values expected by linear models. Adapted from Mel, B. W. 1993. Synaptic integration in an excitable dendritic tree. *J. Neurophysiol.* 70, 1086–1101; Poirazi, P. and Mel, B. 2000. Impact of active dendrites and structural plasticity on the storage capacity of neural tissue. *Neuron* 29, 770–796.

topography across cortex (Funahashi *et al.*, 1989; Wilson *et al.*, 1993; Ó Scalaidhe *et al.*, 1997; Compte *et al.*, 2000). Thus, the more widespread patterns of axon connections in supragranular cortex potentially allow cross-communication between these fields. Unfortunately, studies of the supragranular intrinsic connectivity in cingulate cortex of the macaque monkey are lacking. It will be of great interest to see if any latticework exists and how it compares with that reported in other cortical regions.

As mentioned previously, there are various theories related to multi-areal processing, including hierarchical and distributed models (see Felleman and Van Essen, 1991; Mountcastle, 1995 for reviews). Common to both models is the concept that ensembles of neurons in multiple cortical areas may be co-activated for any given task. How regional differences in pyramidal cell structure could influence processing within these different models has been covered in detail elsewhere (Elston, 2002, 2003b). Briefly, within hierarchical models, the flow of visual inputs along a pathway allows individual neurons within progressively 'higher' areas to process inputs from a

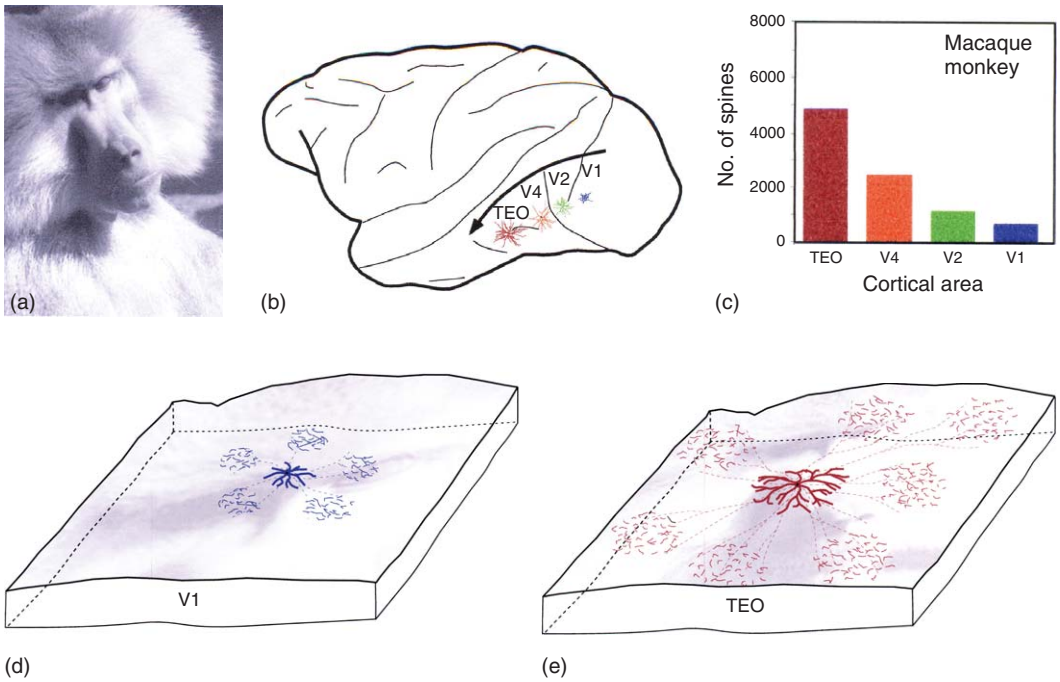

Figure 21 Schematic illustrating various aspects of sampling topography of supragranular pyramidal cells in the primary visual area (V1) and cytoarchitectonic area TEO. A typical visual stimulus is illustrated in (a). Different aspects of the visual stimulus are processed in multiple cortical areas, including the primary, second, and fourth visual areas (V1, V2, and V4, respectively) and cytoarchitectonic area TEO (illustrated in (b)). Cells have progressively larger dendritic trees with anterior progression through these four cortical areas. They also become progressively more branched and spinous, resulting in dramatic differences in the number of spines (putative excitatory inputs) in their dendritic trees (c). In addition to the increase in size, branching complexity, and number of spines in their basal dendritic trees, there is a progressive increase in the size and center-to-center spacing of intrinsic horizontal axonal clusters in the supragranular layers of these cortical areas (c and d). In V1 (d), the intrinsic axonal clusters are relatively small and are tightly grouped, whereas in cytoarchitectonic area TEO (e), these axonal clusters are less uniform, generally larger, and extend over a larger region of cortex (in absolute size). These features, coupled with the visuotopic compression in these cortical areas (e.g., the relative absolute size of the baboon face in (d) and (e)), result in patterns of connectivity that encompass markedly different proportions of the visual scene.

proportionately larger region of the visuotopic map. The potential advantage of such 'serial reconstruction' of the visual scene is conceptually straightforward in the occipitotemporal (OT) pathway where neurons in successively higher visual areas distinguish more complex features (see Felleman and Van Essen, 1991; Gross *et al.*, 1993; Tanaka, 1996; Elston, 2002; Fujita, 2003 for reviews). Within a distributed system, co-activation of ensembles of highly spinous pyramidal cells in association areas may be important for binding sensory perceptions (see Singer and Gray, 1995; Llinás and Paré, 1996 for reviews). In addition, there is a greater potential for re-entrant sampling in circuits composed of neurons that integrate large numbers of excitatory inputs and are highly interconnected than in circuits composed of sparsely interconnected neurons that sample relatively few inputs (see Wang, 2001 for a review). Thus, cooperativity of ensembles of pyramidal cells of varying phenotype in different cortical areas potentially leads to a richness in diversity of, and functional cohesiveness in, cortical function not attainable in cortex composed of the same basic repeated, or canonical, circuit.

4.13.2.2.3 Circuits and memory formation Multiple converging criteria, including cortical damage (both, that inflicted by foreign objects and that which results from internal insult such as calcification or hemorrhage), experimental ablation studies, electrophysiological recording, imaging, and theoretical studies, reveal that memories are stored across large expanses of cortex, including but not exclusive to neocortex (see Fuster, 1995 for a review). While this remains a highly controversial field of investigation, there is widespread agreement that the hypothesis proposed by Hebb (1949) is fundamental to the storage of memory – that is, put simply, synapses are strengthened through use. Considerable advances have been made in our understanding of the molecular mechanisms involved at the synaptic level (e.g., see Kandel, 2001 for a review). Here we focus on the implications of synaptic reinforcement at the neural/circuit level.

During the last few years, there has been a resurgence of interest in Hebbian-type reinforcement at the circuit level (see Mel, 2002; Chklovskii *et al.*, 2004 for reviews). Of particular interest here is the distinction made between 'weight-based' learning and 'wiring-based' learning. Put simply, weight-based learning refers to the synaptic reinforcement of a pre-existing synapse (in series), whereas wiring-based learning refers to the establishment of a new synaptic contact by association (in parallel) (see Chklovskii *et al.*, 2004). How this bears relevance to the regional difference in pyramidal cell structure reported here becomes clear when we focus on the word 'association'. In particular, are cortical circuits composed of neurons with quantifiably different associative potential characterized by differing memory capacities? Intuitively, the answer is yes but let us consider how.

Typically, the associative potential of a neuron is measured by the number of inputs it can sample. As we have seen, pyramidal cells in V1 contain, on average, ~600 spines (putative excitatory inputs) in the basal dendritic trees. Those in gPFC contain, on average, more than 10 000 spines in their basal dendritic trees. Based on what is known of intrinsic connectivity in V1 and the gPFC (e.g., McGuire *et al.*, 1991; Melchitzky *et al.*, 1998, 2001), 20–30% of inputs received by pyramidal cells in both these cortical regions originate from neighboring pyramidal cells (excitatory). By way of example, lets assume that cells in V1 are connected with 200 local pyramidal cells, whereas those in the gPFC are connected with 3000 neighboring pyramidal cells. Application of these numbers to a wiring-based model makes it clear that there is a greater potential for association in the gPFC. Extension of this logic to include multiple synapses across three or more levels reveals how the associative potential of a circuit is ramped up, particularly in the gPFC. The exponential increase in the associative potential in a multi-neuron circuit in the gPFC soon eclipses that in V1 as successive synaptic steps are added to the circuit (eqn [1]). Applying this logic, based on what is known of the structure of neurons, reveals intermediate levels of association in IT and parietal association cortex, as well as cingulate cortex:

$$\begin{aligned}\text{V1: } & 200 \times 200 \times 200 \times 200 \times 200 \times \ 200 \ldots n \\ \text{gPFC: } & 10\,000 \times 10\,000 \times 10\,000 \times 10\,000 \\ & \times 10\,000 \times 10\,000 \ldots n.\end{aligned} \qquad [1]$$

Equation [1], as simple as it is, serves to illustrate several points. First, note the different associative potentials of neurons interspersed throughout a cortical circuit. In this equation, we have illustrated the magnitude of this difference through a series of six neurons, up to n. Even in this crude example of a cortical circuit, there is a 1.5×10^{10}-fold difference in associative potential in those first six steps even when assuming a strict serial order. However, as we know, parallel and reciprocal connections are common in the cerebral cortex. Thus, this simple equation also serves to illustrate, or prompt, the question, how many neurons are included in a memory circuit in the neocortex? The answer is simple, we do not know – certainly, more than two but less than 10 billion. We leave it to the reader to calculate the difference in associative potential in circuits composed of 100 or more cells in V1 and the gPFC.

Of course, this is a grossly oversimplified representation of memory. Memories are not coded entirely within a single cortical area. Imaging studies have revealed that neurons may be active in many widely distributed cortical areas during memory recall. Thus, another level of complexity in trying to determine the associative potential in a memory circuit is conspicuous by its absence in eqn [1]. How many neurons of different associative potentials (from different cortical areas) are incorporated in a given memory engram? The scope of this discussion falls outside this review; however, we would like to point out one other aspect of neuron structure that adds further complexity to the above.

As outlined above (Section 4.13.2.2.1), the functional capacity of individual neurons is influenced by their branching structure. In turn, the branching structure of, and the distribution of ion channels throughout, the dendritic trees of neurons will influence the association potential of the circuits they comprise. More specifically, the structure of the dendritic tree determines both the number of computational subunits, and their geometric interface with other neurons (Williams and Stuart, 2002; Krapp and Gabbiani, 2005; Nolan *et al.*, 2004). Differences in the distribution of inputs throughout the dendritic trees of pyramidal cells, as exemplified by their different branching structure, may result in a difference of up to two orders in magnitude in the memory storage capacity (Poirazi and Mel, 2000).

4.13.2.2.4 More on the structure/function relationship Many investigators have made a concerted effort over the years to detect possible structural abnormalities in the cerebral cortex associated with specific pathologies. Although the scope of this body of work is beyond the thrust of this review, it is worth drawing the readers attention to

some examples where a parallel has been demonstrated between cortical microstructure and psychopathogy. This growing body of evidence suggests that structural modifications to pyramidal cell structure observed in senescence, mental disorder, and disease are cause rather than effect. For example, the decrease in dendritic branching and spine loss with aging is paralleled by a decline in cognitive ability (Scheibel *et al.*, 1975, 1976; Huttenlocher, 1979; Jacobs and Scheibel, 1993; Jacobs *et al.*, 1997; de Brabander *et al.*, 1998). The impoverished structure of pyramidal cells in the brains of individuals with Down syndrome and Fragile X reflect their diminished cognitive abilities (Marin-Padilla, 1976; Suetsugu and Mehraein, 1980; Takashima *et al.*, 1981; Hinton *et al.*, 1991). Decreased branching structure and number of dendritic spines have been reported in schizophrenia, which are thought to be responsible for altered mental functions (Garey *et al.*, 1998; Glantz and Lewis, 2000; Kalus *et al.*, 2000; Black *et al.*, 2004; Cullen *et al.*, 2006). Dendritic atrophy has also been reported in Rett syndrome (Cornford *et al.*, 1994). In addition, manipulation of sensory inputs to the cerebral cortex results in plastic changes in microcircuitry – new dendrites grow, dendritic spines form, and synapses are established, which are not present in the intact animal (e.g., Valverde, 1967, 1968). Neuronal and circuit function is consequently changed. Such dendritic growth, spinogenesis, and synaptogenesis observed in the adult (e.g., Elston and DeFelipe, 2002; Churchill *et al.*, 2004; Tailby *et al.*, 2005) is thought to consolidate immediate functional changes observed in the cerebral cortex following damage to sensory inputs (e.g., Merzenich *et al.*, 1983a, 1983b; Calford and Tweedale, 1988; Kaas *et al.*, 1990; Pons *et al.*, 1991; Gilbert and Wiesel, 1992). Moreover, pyramidal cells in experimental animals reared in an enriched environment have a more complex structure (Figure 22) and the animals perform better on behavioral tasks, than animals that have been reared in a nonenriched environment (Volkmar and Greenough, 1972; Globus *et al.*, 1973; Greenough *et al.*, 1973; Floeter and Greenhough, 1979; Withers and Greenough, 1989; Dierssen *et al.*, 2002) (Figure 22). For in-depth reviews the reader is referred to articles by Huttenlocher (1991), Harris (1999), Kintsova and Greenough (1999), Wooley (1999), Irwin *et al.* (2000), Jones (2000), Kaufmann and Moser (2000), Calford (2002), Elston and DeFelipe (2002), Dierssen *et al.* (2003), and Kaas *et al.*

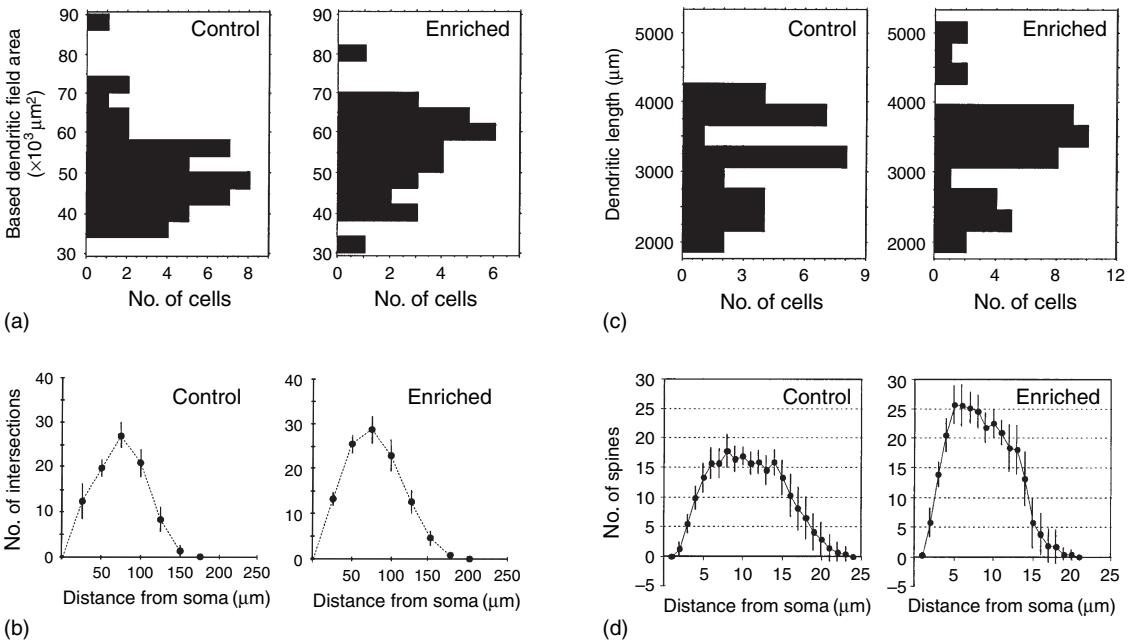

Figure 22 a, Frequency histograms of the size of the basal dendritic trees of layer III pyramidal neurons sampled in the second motor area (M2) in adult mice that were raised in nonenriched (control) and enriched environments. b, Graphs of the results of Sholl analysis of the basal dendritic trees of the same layer III pyramidal neurons. c, Frequency histograms of the total dendritic length in the basal trees of layer III pyramidal neurons in mice that were raised in control and enriched environments. d, Plots of the density of dendritic spines, as a function of distance from the cell body to the distal dendritic tips, in the basal trees of the same layer III pyramidal neurons. Error bars = standard errors. Modified from Dierssen, M., Benavides-Piccione, R., Martínez-Cué, C., *et al.* 2002. Alterations of neocortical pyramidal cell phenotype in the TS65Dn mouse model of Down syndrome: Effects of environmental enrichment. *Cereb. Cortex* 13, 758–764.

(2003). See also DeFelipe and Jones (1988) for a translation of Cajal's works (1894a, 1894b).

4.13.2.2.5 Summary of the structure/function relationship In summary, systematic areal specializations in cortical circuitry are likely to influence various aspects of visual processing. The small pyramidal cells in V1, and the circuits they form, allow high-fidelity sampling of the visual scene, the relatively small number of inputs they integrate, and their patterns of connectivity, being instrumental in determining their phasic discharge properties. V1 circuit structure is specialized to subserve rapid processing of a constantly changing visual scene, allowing quick reset time for processing saccadic inputs. In other words, V1 circuitry is specialized to fire and flush. The integrative ability and patterns of connectivity of the larger, more branched, and more spinous pyramidal cells in visual areas in IT provide an anatomical substrate for the global integration of the visual scene. Each neuron's ability to sample a large number of excitatory inputs is central to the sustained tonic activity reported in these cells, which is widely believed to be important for visual memory. The more branched, more spinous pyramidal cells in the gPFC sample, the larger the number of excitatory inputs in a smaller volume of cortex than those in IT, potentially facilitating greater recurrent excitation necessary to sustain tonic discharge even when presented with distractors.

4.13.3 Comparative Data on the Pyramidal Cell Phenotype in the Primate Brain

In a bid to begin to understand something of the evolution of cortical microcircuitry in the primate brain, pyramidal cell structure has been quantified in multiple cortical areas in a diverse range of species. More specifically, pyramidal cell structure has been studied in visual, sensorimotor, cingulate, and gPFC of human and nonhuman, New World and Old World, simian and prosimian primates. Where possible, we have attempted to study pyramidal cells in homologous cortical areas. Where there is uncertainty regarding homology, analogous or corresponding cortical regions have been selected for study. These investigations have always been focused on pyramidal cells in a homologous cortical layer (layer III, unless otherwise stated). In a bid to minimize sampling error, studies have concentrated on the same hemisphere (left) of brains taken from individuals of the same sex (male) of similar developmental age (all sexually mature but not aged). In addition, the cell injection techniques and the methods of quantification have been standardized across all studies. In nearly all species, data have been sampled from two or more cases.

4.13.3.1 Visual Cortex

Visual cortex has been mapped extensively in a number of primate species. Data from the laboratories of Allman, Kaas, Rosa, and Zeki, in particular, reveal the presence of V2, MT, and a number of other extrastriate visual areas in the temporal and parietal lobes of the cerebral cortex of primates (Zeki, 1969a, 1969b, 1971, 1977, 1978, 1980; Allman and Kaas, 1971a, 1971b, 1975, 1976; Kaas *et al.*, 1972; Allman *et al.*, 1973, 1979; Kaas and Lin, 1977; Sesma *et al.*, 1984; Weller *et al.*, 1984; Zeki and Shipp, 1989; Rosa *et al.*, 1993, 1997; Rosa and Schmid, 1995; Rosa and Elston, 1998; Rosa and Tweedale, 2000; Lyon and Kaas, 2002; Lyon *et al.*, 2002). These studies have paved the way for cell injection studies of pyramidal cells in a number of different visual areas in species other than the macaque monkey. In particular, studies of pyramidal cell structure have been focused on occipitotemporal (OT) visual areas. As a basis for comparison, pyramidal cells have been studied in well-documented visual areas in the galago (bush baby), marmoset, and owl monkey. In addition, the study of pyramidal cells has been extended to the OT cortex of the baboon and vervet monkey (savannah guenon), for which detailed maps do not exist but data are available regarding the size of V1. Comparison of these data with those obtained from the OT cortex of the macaque monkey reveals several interesting trends. In all species included for investigation, both simian and prosimian, New World and Old World, diurnal and nocturnal, there was a trend for pyramidal cells with progressively larger, more spinous-basal dendritic trees with anterior progression through the OT cortex (Figure 23). Moreover, the relative extent of the difference among cell structure in OT areas differs between species.

These species differences in the extent of the trend for pyramidal cells with progressively larger, more spinous basal dendritic trees through OT visual areas do not result from a random increase in cell complexity in a given cortical area. Nor do they occur as a result of scaling of the cerebrum: cells in the primary visual areas have remarkably similar structure in all species despite up to a fivefold difference in the size/cortical surface area of V1 (Table 2). Instead, the

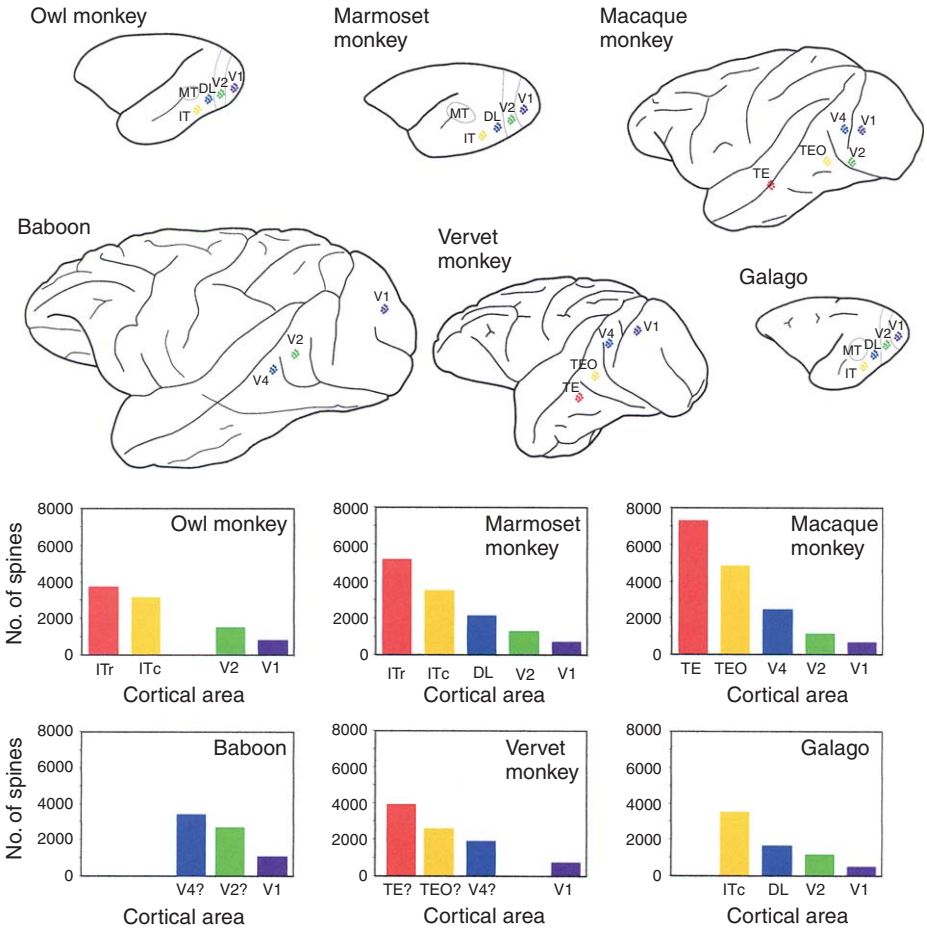

Figure 23 Schematic illustrating the location from which neurons were sampled (colored dots) in the OT cortex of the left hemisphere of the galago, owl monkey, marmoset monkey, macaque monkey, vervet monkey, and baboon, and the corresponding bar graphs of the estimates of the total number of spines in the basal dendritic trees of layer III pyramidal cells in cortical visual areas. Although we have not sampled from all cortical areas in OT cortex of all six species, and there is confusion regarding the homology of cortical areas in the temporal lobe, there is a clear and consistent trend for more spinous cells with anterior progression through OT cortex. In addition, the relative extent in the number of dendritic spines (putative excitatory inputs) in the basal dendritic trees of these cells differs between species. Despite large variations in the size of the primary visual area (V1) between species, there is remarkable consistency in the number of spines in their basal dendritic trees. The same is not true, however, for cortical areas in the temporal lobe. There is a parallel between the size of the temporal lobe (the degree to which it has expanded) and the extent of the increase in the number of spines in the dendritic trees of cells. Schematics of the different brains are not drawn to scale. DL, dorsolateral area; IT, inferotemporal cortex; MT, middle temporal area; TE and TEO, cytoarchitectonic area; V1, primary visual area; V2, second visual area; V4, fourth visual area. Data taken from Elston and Rosa (1998a), Elston *et al.* (1999b, 2005b, 2005e, 2005j), and Elston (2003c).

species differences are primarily manifest in IT. The extent to which pyramidal cells become larger and more spinous with anterior progression through OT cortex appears to reflect the expansion of the temporal lobe. A plot of the estimates of the total number of spines in the basal dendritic trees of layer III pyramidal cells in OT visual areas reveals a progressive increase in the number of spines as a function of increasing distance from the occipital pole (Figure 24).

4.13.3.2 Sensorimotor Cortex

Like visual cortex, sensorimotor cortex has been studied extensively in a number of primate species. Based on Brodmann's observations, motor cortex has expanded to different degrees in different primate species. While still controversial, it has been proposed that new cortical areas have differentiated in premotor cortex during its expansion. For example, Watanabe-Sawaguchi *et al.* (1991) identified a new cortical area, area 6aγ, in premotor cortex of the baboon, which they claim is not present in the macaque or vervet monkeys. Area 6aγ reportedly has cytoarchitectonic characteristics intermediate between the more posterior 'area 6' and anterior area 8. However, studies in the prosimian galago suggest that it shares a similar complement of motor areas with monkeys (Wu *et al.*, 2000), despite the fact that the lineages that led to these present-day

Table 2 Relative proportion of cortical volume occupied by granular prefrontal and precentral cortex in a number of different species

	Species	*Total cortical volume (mm^3)*	*Granular prefrontal cortex (gPFC)*	*Agranular precentral cortex*	*gPFC[a] agranular precentral cortex (frontal lobe)*	*Primary visual area*
Strepsirhini	Human	108 221	30 254 = 27.9	6117 = 5.7	36 371 = 33.6	3495 = 2.9
	Human	135 470	39 287 = 29.0	9833 = 7.3	49 120 = 36.3	
	Chimpanzee	39 572	6 719 = 16.9	5389 = 13.6	12 108 = 30.5	3212 = 8.1
	Gibbon (*Hylobates*)	16 301	1 839 = 11.3	1651 = 10.1	3 490 = 20.4	1899 = 11.6
	Mandrill (*Cynocephalus*)	31 321	2186 = 10.1	2194 = 10.3	4 362 = 20.4	3537 = 16.6
	Baboon (*P. hamadryas*)	20 594	1967 = 9.5	2898 = 14.1	4 865 = 23.6	2130 = 10.4
	Baboon (*P. cynocephalus*)	20 376	2 111 = 10.3	2200 = 10.8	4 311 = 21.1	2559 = 12.5
	Macaque	15 308	1 733 = 11.3	1817 = 11.9	3 550 = 23.2	1866 = 12.2
	Guenon (*Cercopithecus*)	14 641	1 625 = 11.1	1976 = 13.5	3 601 = 24.6	
	Capuchin	13 682	1 260 = 9.2	1822 = 13.3	3 082 = 22.5	1786 = 13.1
	Marmoset	1649	148 = 8.9	167 = 10.1	315 = 19.0	341 = 20.8
Haplorhini	Black Lemur	4 054	337 = 8.3	453 = 11.2	790 = 19.5	440 = 10.8
	Microcebus (*Cheirogaleus*)	921	70 = 7.2	94 = 10.2	164 = 17.8	
	Flying Fox[a]	1097	26 = 2.3	73 = 6.6	99 = 8.9	163 = 14.9
Carnivores	Dog (*Canis*)	9 527	657 = 6.9	1283 = 13.4	1 940 = 20.3	1061 = 10.7
	Cat (*Felis*)	4 474	152 = 3.4	412 = 9.2	564 = 12.6	522 = 11.6
Lagomorpha[b]	Rabbit (*Lepus*)	1627	36 = 2.2	148 = 9.1	184 = 11.3	140 = 8.6
Insectivore	Hedgehog (*Erinaceus*)	575	0 = 0	24 = 4.0	24 = 4.0	23 = 4.0
Edentate	Armadillo (*Dasypus*)	2 010	0 = 0	93 = 4.6	93 = 4.6	126 = 6.2
Marsupial	Opossum (*Didelphys*)	804	0 = 0	51 = 6.3	51 = 6.3	55 = 6.8

[a]Included as a primate in the original table (see Pettigrew *et al.*, 1989 for a review).
[b]Labeled as a rodent in the original table.
Modified from Brodmann, K. 1913. Neue Forschungsergebnisse der Grosshirnrindenanatomie mit besonderer Berücksichtigung anthropologischer Fragen. *Gesselsch. Deuts. Naturf. Artze.* 85, 200–240.

species have been separated for a longer period of time than those leading to present-day baboons and macaque monkeys (see Figure 4).

As in visual cortex, investigations of the sensorimotor cortex revealed consistent trends for pyramidal cell specialization, but the extent of these specializations varies among species. For example, comparison of the dendritic trees of layer III pyramidal cells in sensorimotor cortex of the macaque monkey, the vervet monkey, and the baboon reveal a common trend for increasing cell complexity with posterior progression from the central sulcus through somatosensory areas and with anterior progression from the primary motor area to premotor cortex. As in visual cortex, these estimates reveal dramatic differences in the total number of spines in the dendritic trees of pyramidal cells in sensorimotor areas, with up to a fivefold difference reported in the vervet monkey (Figure 25).

What is also clear from Figure 25 is that pyramidal cells in sensorimotor cortex of the macaque monkey are considerably more spinous than those in corresponding cortical areas in either the baboon or vervet monkey. For example, based on estimates of the total number of spines in the basal dendritic tree of pyramidal cells in area 6, cells in the macaque monkey are approximately twice as spinous as those in the other two species. However, several caveats need to be considered when interpreting these data. First, neurons in the macaque monkey were sampled from the forelimb/hand representation, whereas those in the vervet monkey and baboon were sampled more dorsally, probably in the trunk representation (cf. Jones *et al.*, 1978; Matsumura and Kubota, 1979; Waters *et al.*, 1990; Huntley and Jones, 1991; Manger *et al.*, 1996, 1997). Sampling cells from these different topographic representations is likely to influence the data, particularly in view of forelimb dexterity in primates. Second, the age of some of the animals included in these investigations was unknown: differences in age may influence both the branching structure

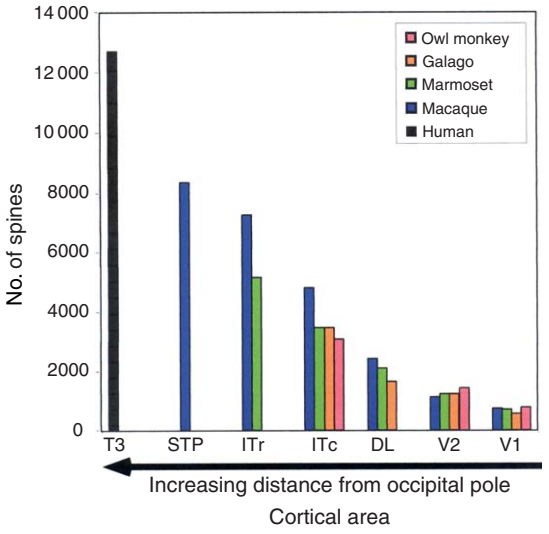

Figure 24 Plots of the estimates of the total number of spines in the basal dendritic tree of the 'average' pyramidal cell in OT cortical areas in the galago, the owl monkey, the marmoset monkey, the macaque monkey, and human. The number of spines in the basal dendritic tree of the 'average' pyramidal cell is calculated by summing the product of the mean spine density and mean number of dendritic branches over successive 25 μm annuli across the entire dendritic tree of all neurons sampled in a given cortical area. Note the general trend toward an increase in the number of dendritic spines in the basal dendritic trees of pyramidal cells in species with progressively larger temporal lobes. These data, however, should not be interpreted as suggesting that pyramidal cells become increasingly more complex in cortical areas anterior to those illustrated. Our unpublished observations suggest that there is a decrease in the number of spines in the basal dendritic trees of pyramidal cells in close proximity to the temporal pole, much the same as reported in the frontal pole (Elston *et al.*, 2001). Data taken from Elston and Rosa (1998a), Elston *et al.* (1999b, 2005b, 2005e, 2005j), and Elston (2003c).

and the number of dendritic spines. Third, some of the animals included for study were raised in animal colonies (macaque and vervet monkeys), whereas others (baboons) lived in the wild. All of these variables have been shown to influence pyramidal cell structure (Greenough *et al.*, 1973; Scheibel *et al.*, 1975, 1976; Huttenlocher, 1979; Cupp and Uemura, 1980; Huttenlocher *et al.*, 1982; Nakamura *et al.*, 1985; Huttenlocher and de Courten, 1987; Anderson and Rutledge, 1996; Huttenlocher and Dabholkar, 1997; Jacobs *et al.*, 1997; Dierssen *et al.*, 2002). Nonetheless, studies in the cingulate cortex of these same animals (see below) reveal similar numbers of spines in the dendritic trees of pyramidal cells in corresponding cortical areas, suggesting that the differences reported here in sensorimotor cortex (macaque monkey versus baboon and vervet monkey) are likely to be attributable to the body-part representations from which the data were sampled, rather than any other reason.

4.13.3.3 Cingulate Cortex

Although cingulate cortex is often considered to be a primitive structure (Sanides, 1970; MacLean, 1989), comparison of this region of cortex across different primate species reveals dramatic variation in its size. For example, the cingulate gyrus in the Chacma baboon is at least five times longer than that in the marmoset monkey, and more than double the width, while that in humans may be more than 10 times longer than that in the marmoset. There are remarkably few studies that have explored the functional implications of these differences, even at the gross level (see Allman *et al.*, 2001 for a review). Although the data are relatively limited, a picture is beginning to emerge with respect to specialization in pyramidal cell structure in the cingulate gyrus in primates (Figure 26). For example, there is a consistent trend for larger, more branched, and more spinous pyramidal cells in the anterior cingulate gyrus than in the posterior cingulate gyrus (Brodmann's areas 24 and 23, respectively). Estimates of the total number of spines in the basal dendritic tree of pyramidal cells in area 24 are remarkably similar between the Chacma baboon and the macaque and vervet monkeys, as are those for cells in area 23. In addition, in all three species, pyramidal cells in the anterior cingulate gyrus are considerably larger, more branched, and more spinous than those in other visual, somatosensory, and motor cortical areas sampled from the same cases (cf. Figures 23, 25, and 26). In future studies, it will be of interest to study pyramidal cell structure in the cingulate cortex of great apes, particularly in view of the presence of specialized spindle cells reported in humans and chimpanzees (Nimchinsky *et al.*, 1999; Hayashi *et al.*, 2001; see Role of Spindle Cells in the Social Cognition of Apes and Humans).

4.13.3.4 Prefrontal Cortex

The gPFC has become greatly expanded in some primates, particularly in humans (Table 2, Figure 27). Because of this relatively recent and rapid expansion, many researchers have concluded that the gPFC is important in executive functions such as planning, prioritizing, and conceptualizing. This view, however, is not without its critics. For example, Semendeferi and colleagues suggest, based on imaging data, that there is no relative difference in the size of the frontal lobe in humans and great apes (Semendeferi *et al.*, 2002). However, more extensive studies in which gPFC was identified and quantified reveal differences in both the absolute size of gPFC in different primate species and its size relative to the frontal lobe. In addition, contrary to the claims of the above authors,

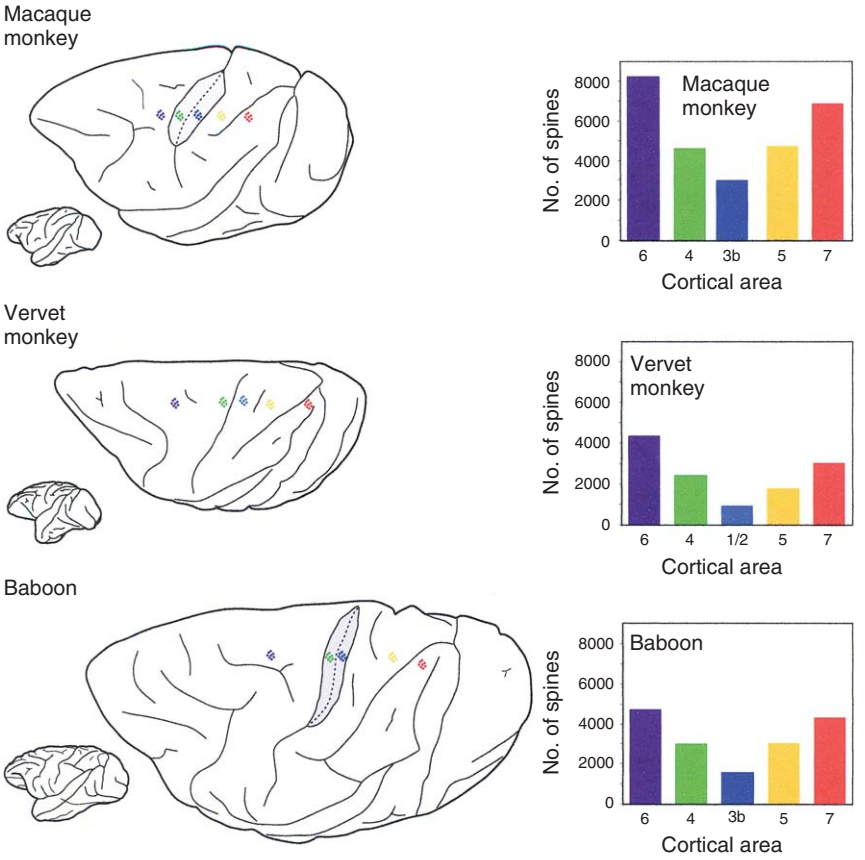

Figure 25 Schematic illustrating the location from which neurons were sampled (colored dots) in the sensorimotor cortex of the left hemisphere of the macaque monkey, vervet monkey, and baboon, and the corresponding bar graphs of the estimates of the total number of spines in the basal dendritic trees of layer III pyramidal cells in these areas. Note the consistent trend toward more spinous cells with anterior progression from the primary motor area (area 4) to premotor cortex (area 6) in all three species. Note also the consistent trend for progressively more spinous cells with posterior progression through somatosensory areas from the central sulcus to the angular gyrus. As seen from the graphs, the estimate of the number of dendritic spines (putative excitatory inputs) was higher for cells in the macaque monkey than in either the baboon or vervet monkey (across all cortical areas studied). These differences are probably due to the different body-part representations that were sampled in the different species: those in the macaque monkey were likely to have been sampled in the forelimb/hand representation whereas those in the other species were likely to have been sampled in the trunk/hindlimb representations (see Elston *et al.*, 2005g, for a discussion). Schematics of the different brains are not drawn to scale. Data taken from Elston and Rockland (2002) and Elston *et al.* (2005d, 2005g).

gPFC has been identified and quantified in the brains of different great apes: gPFC occupies 52.7% of the frontal lobe in the chimpanzee and 83.2% in humans (corresponding to 11.3% and 29% of the total cerebral surface area, respectively; Brodmann, 1913). Schoenemann *et al.* (2000) have argued that in humans there is no correlation between the size of the brain and cognitive abilities in different individuals of the same family.

Studies of pyramidal cell structure in the gPFC of different primate species are beginning to provide new insights into how microcircuitry in this region has evolved in different species, and how it may vary within a species (Elston *et al.*, 2006a). While there is still the greatest confusion and controversy regarding the number and locations of prefrontal cortical areas in different species, and any potential homology between these areas, the study of neuronal structure in corresponding cortical regions of different species (Figure 28) is beginning to reveal some common trends between species, as well as some interesting differences (see Do All Mammals Have a Prefrontal Cortex?). In all three species in which pyramidal cell structure has been quantified across multiple cortical areas in the gPFC (Chacma baboon and macaque and vervet monkeys), marked regional variation in the branching of, and number of spines in, the basal dendritic trees was observed. Unlike in visual, somatosensory, motor, and cingulate cortex, regional differences in pyramidal cell structure between cortical areas within the gPFC varied between cases 'for each species'. For example, the consistency in the trends for progressively more branched, spinous neurons through a selection of

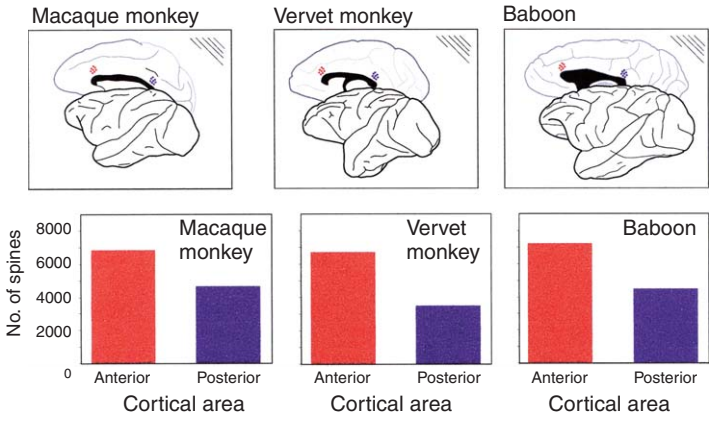

Figure 26 Schematics illustrating regions of cingulate gyrus from which neurons were sampled (colored dots) in the macaque monkey, vervet monkey, and baboon, and bar graphs of the estimates of the number of spines in the basal dendritic trees of pyramidal cells located in these areas. In all three species, cells were injected in the caudal region of the cingulate gyrus, dorsal to the splenium and in the rostral cingulate gyrus, caudal to the genu (Brodmann's areas 23 and 24, respectively). In all three species there was a consistent trend toward more spinous pyramidal cells in the anterior cingulate gyrus than in the posterior cingulate gyrus. Cells in the anterior cingulate gyrus of the baboon were slightly more spinous than those in the corresponding region in the macaque monkey, which, in turn, were more spinous than those in the vervet monkey. Schematics of the different brains not drawn to scale. Data taken from Elston *et al.* (2005a, 2005c, 2005f).

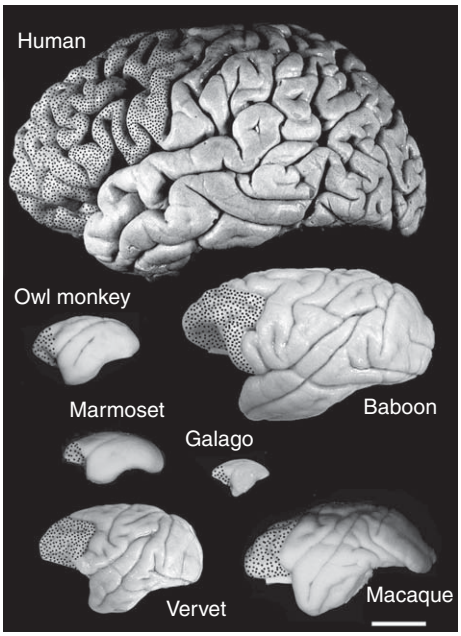

Figure 27 Photomicrographs of the lateral aspect of the left cerebral hemisphere of the human, baboon, macaque monkey, vervet monkey, owl monkey, marmoset monkey, and the galago illustrating the relative differences in the extent of gPFC (stipple). According to Brodmann (1913), gPFC occupies 29% of the total cortex in human, considerably more than in the baboon (10.3%), macaque (11.3%), guenon or vervet monkey (9.2%), and marmoset (8.9%). While Brodmann did not include the galago and owl monkey in his studies, data published by others (Preuss and Goldman-Rakic, 1991c; Preuss *et al.*, 1996) indicate that the galago has the smallest gPFC of all species included here; that of the owl monkey is intermediate between the marmoset monkey and the vervet monkey. Scale bar: 2 cm.

cortical areas (e.g., V1, V2, V4, and TEO in visual cortex, areas 3b, 5, and 7 in somatosensory cortex, or areas 23 and 24 in cingulate cortex) were not always observed in the gPFC. Nonetheless, despite this inter-individual variation, cells in gPFC were characterized by a relatively complex structure (i.e., the gPFC contained among the most complex of all cells studied in the cerebral cortex, but their location differed between species and between individuals).

These data suggest that the functional characteristics of neurons, and the computational abilities of the circuits they form, differ between the medial, orbital, and dorsolateral gPFC within a given species. In addition, the comparative data suggest that corresponding regions of the gPFC across species are characterized by different functional abilities. In the following section, regional and species differences in the pyramidal cell phenotype are related to cortical volume/surface area.

4.13.4 Specialization of the Pyramidal Cell during Cortical Evolution

We have already seen how pyramidal cell structure may vary considerably in corresponding, if not homologous, cortical areas in different primate species. However, we have not yet compared these data with the size of the different cortical areas, which are known to differ between the various primate species (Figures 29 and 30). This is clearly the next logical step in establishing any trends for specialization of

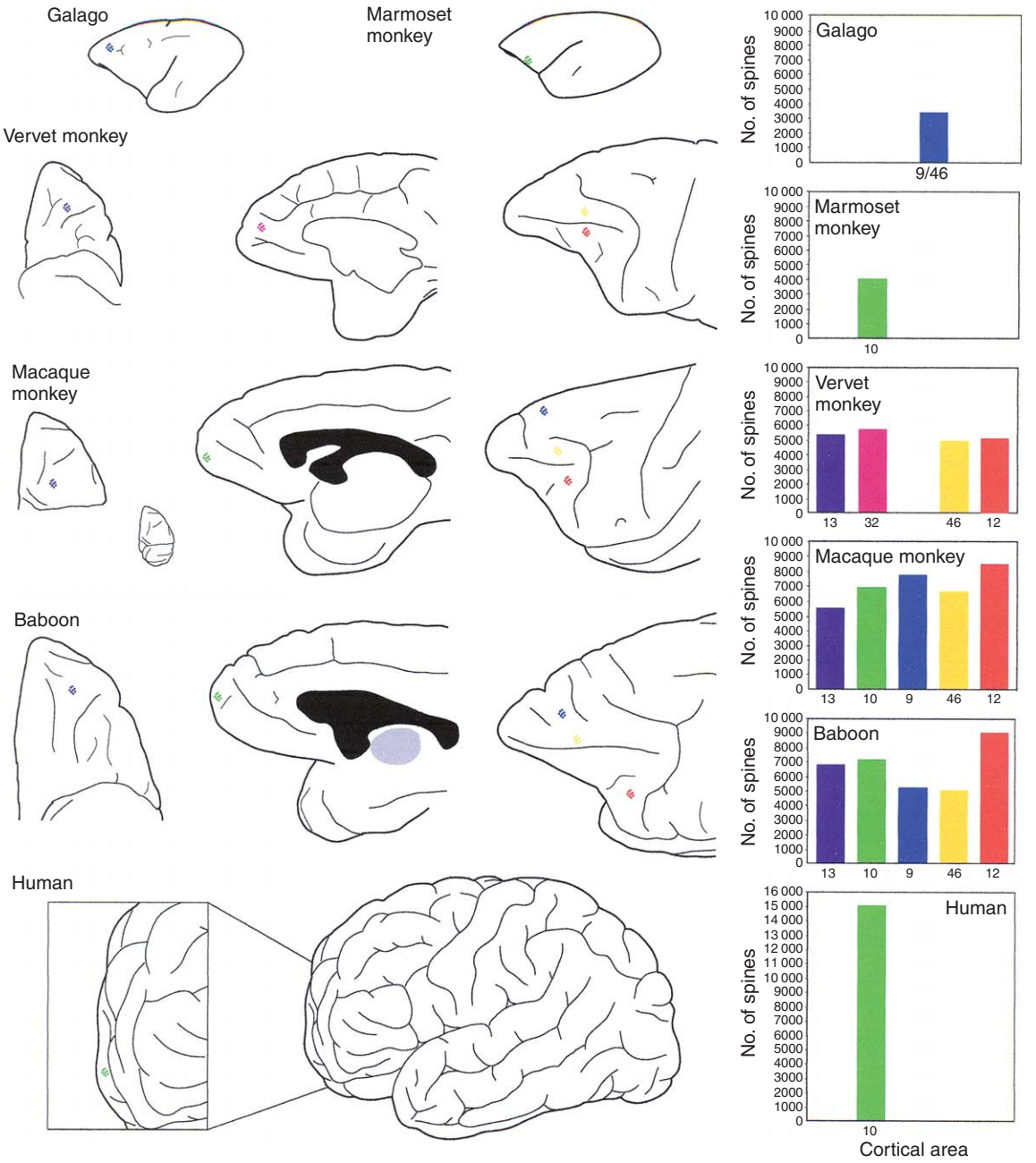

Figure 28 Schematic illustrating the location from which neurons were sampled (colored dots) in the gPFC of the left hemisphere of the marmoset, macaque and vervet monkeys, as well as the galago and the baboon. Despite the greatest of confusion with regard to the number, location, and homology of cortical areas in the gPFC in primates, we have attempted to inject cells in specific cortical regions to allow meaningful comparisons across species. While we have been unable to inject cells in the same cortical regions in all species, we have sampled large numbers of cells in gPFC of at least three species (macaque monkey, vervet monkey, and the baboon). These data are compared with those sampled from the galago and the marmoset monkey. Cells were injected in the dorsolateral, orbital, and medial prefrontal cortex in the macaque monkey, vervet monkey, and the baboon. They were sampled from the dorsolateral gPFC in the galago, and orbital gPFC in the marmoset monkey. Cells injected in corresponding regions are color coded. Bar graphs of the estimates of the total number of spines in the basal dendritic trees of layer III pyramidal cells in gPFC of these different species are illustrated on the left. While the picture is by no means complete, several trends can be gleaned from these data. Note, in particular, the lack of consistency in the trends reported here, as compared with those in visual, somatosensory, motor, and cingulate cortex. Also note the relative species differences for particular cortical areas, for example, areas 12 and 46 in the macaque monkey, vervet monkey, and baboon. Data taken from Elston *et al.* (2001, 2005i, 2006a, unpublished observations).

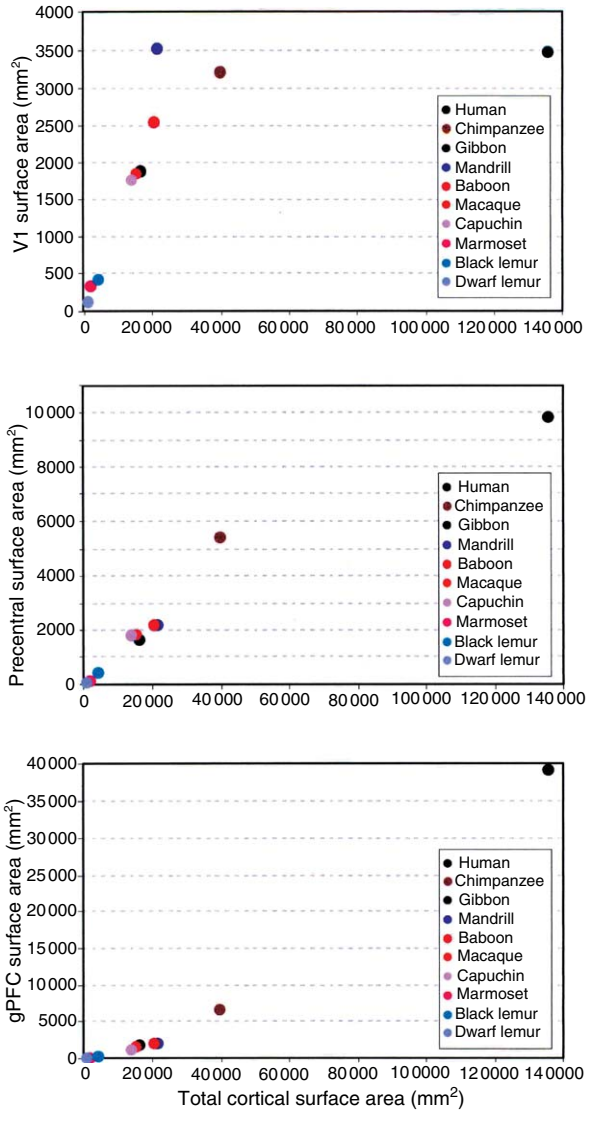

Figure 29 Plots of Brodmann's (1913) data on the cortical surface area of the primary visual area (V1), motor cortex (precentral agranular cortex), and the gPFC in the human, chimpanzee, gibbon, mandrill, baboon, macaque monkey, capuchin monkey, marmoset monkey, black lemur, and dwarf lemur. Note the relative differences in the cortical surface area of V1, precentral (motor), and gPFC in the different species, particularly in the nonhuman species. Note also that V1, precentral (motor) cortex, and gPFC are disproportionately large in the human.

the pyramidal cell phenotype associated with the volumetric increase in the cortical mantle during the evolution of the cerebral cortex. This is of particular importance as it might provide insights into the evolution of specialized cortical functions in different species.

At least three principles may act in concert to determine the pyramidal cell phenotype (and regional specializations thereof) in the mammalian cerebral cortex: laws of form, phylogenetic constraints, and species adaptations (see Gould, 2002; Manger, 2005b for reviews). It remains to be determined how each of these principles influences the pyramidal cell phenotype and to what extent they may interact in different mammalian orders. If we restrict the scope of this discussion to primates, interspecies differences in pyramidal cell structure may parallel the relative degree of expansion of particular cortical areas, lobes, the cortical mantle, or the entire brain. Alternatively, patterns of connectivity may dictate pyramidal cell structure, irrespective of volumetric differences in brain size. Both of these possibilities relate to laws of form (see also Section 4.13.4). Another possibility is that variation in pyramidal cell structure may reflect species-specific specializations that occur irrespective of size and patterns of connectivity. Any or all of these possibilities may vary between different mammalian orders. Indeed, preliminary findings in rats and mice suggest that trends observed in primates are not necessarily observed in rodents (e.g., Benavides-Piccione *et al.*, 2006; Ballesteros-Yáñez *et al.*, 2006).

We are now exploring these issues systematically among rodent species (e.g., Elston *et al.*, 2006b). Establishing which of these possibilities has occurred is essential if we are to better understand how phylogeny may predetermine specialized cortical function. Importantly, regional and species differences in pyramidal cell structure contradict one of the central assumptions of the prevailing dogma related to brain size and intelligence: the bigger the brain the more intelligent the species/individual (cf. Jerison, 1973). Clearly, any such relationship between brain size and functional ability fails if the different brains are composed of circuits with different functional capabilities.

In a bid to study the brain size/pyramidal cell phenotype issue further, it is necessary to study pyramidal cell structure in homologous cortical areas of multiple species with different-sized brains. We immediately run into a problem; that is, with the exception of the primary sensory areas and the primary motor area, there is little agreement in the literature regarding the homology of different cortical areas – particularly when comparing among mammalian orders. This is not an inconsequential problem, nor is it an insurmountable problem. One solution immediately presents itself – study all cortical areas at least in a handful of species. This, perhaps, is not as ridiculous a suggestion as it may seem. A team of investigators numbering less than usually observed in a molecular biology laboratory could sample $>10\,000$ cells in the space of a few days from a single brain, at 50 or so cells per cortical area, which is a lot of cortical areas, enough to more than satisfy even the most complex human brain

maps thus far presented. For the present, I, and more recently with the help of Ruth Benavides-Piccione, have been sampling as many cells as possible in the cerebral cortex of a selection of primate species. Although the data are by no means complete, we have sampled sufficient numbers of cells to make some cross-species comparisons.

Here we focus on data obtained from V1, V2, and gPFC of the human, baboon, macaque monkey, vervet monkey, owl monkey, marmoset monkey, and galago (Figure 31). We focused on these areas because there is widespread agreement about the size and location of V1 and V2 in these primate species. Moreover, Brodmann (1913) published a monograph in which he included quantitative data on the size of the gPFC of these species. While the means by which Brodmann determined the size of these cortical regions probably lead to inaccuracies, any sources of error are likely to be standardized across the species he investigated. Thus, while the absolute sizes of these different cortical regions as reported by Brodmann may be erroneous, the relative difference in their sizes should be reasonably accurate – arguably more accurate than that obtained by drawing data from multiple laboratories using different methodologies.

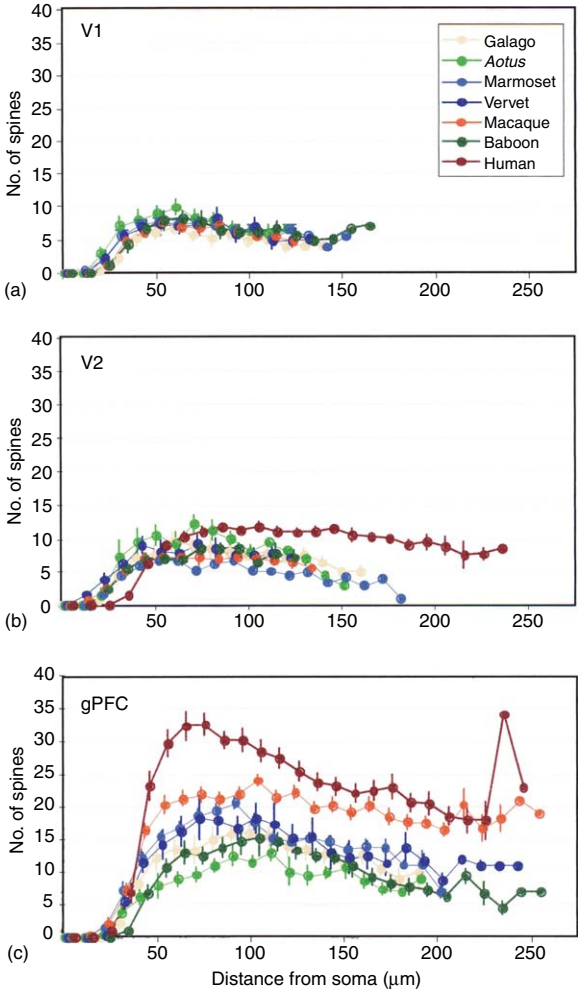

Figure 30 Plots of the spine density along randomly selected dendrites in the basal dendritic trees, as a function of distance from the cell body, of layer III pyramidal cells sampled in (a), the primary visual area (V1); (b), the second visual area (V2); and (c), the gPFC in the human, baboon, macaque monkey, vervet monkey, marmoset monkey, owl monkey (*Aotus*), and galago. Note the differences in the profiles in the different cortical regions. Spine densities along the basal dendrites of pyramidal cells in V1 and V2 are tightly grouped, with those in V2 being, on average, higher than those in V1. In gPFC, however, there are impressive differences among the species. Data taken from Elston *et al.* (2001, 2005c, 2005d, 2005e, 2005i).

Comparison of the size of the basal dendritic trees of pyramidal cells with the cortical surface area occupied by areas V1, V2, and gPFC reveals a trend for larger cells with absolute increase in the size of the cortex (Figure 31a). Based on these data, it might be tempting to conclude that dendritic tree size scales with the absolute size of the cortical area. However, notably, the data also reveal that dendritic trees in a given cortical area, such as V1 or V2, may be larger in a species in which this cortical area occupies a smaller absolute size than another species. Moreover, the size of the dendritic trees of pyramidal cells in the human gPFC does not reflect the dramatic difference in the absolute size of this region (note the difference of two orders of magnitude in the absolute size of human gPFC). To further explore this issue, we compared our estimates of the total number of spines in the basal dendritic tree of the 'average' neuron in each cortical area with the absolute size of the cortical area. As each dendritic spine represents one putative excitatory input, different trends observed in such a plot would suggest different degrees of connectivity among populations of pyramidal cells. Indeed, these plots reveal marked differences in the relationship number of putative excitatory inputs received by pyramidal cells and the absolute size of the cortical area between visual cortex and gPFC (Figure 31b). Of particular note, the slope of the linear regression for the gPFC data is considerably steeper than that for either V1 or V2. Not only do these data suggest that pyramidal cells in these different regions sample different numbers of excitatory inputs, but the rate of spine acquisition during the evolutionary expansion of gPFC far exceeds that in visual cortex. To test whether this effect could be attributed to the increasing size of the dendritic trees of the neurons, the total number of spines in the dendritic tree was plotted against tree size (Figure 31c). These plots reveal progressive increase in the slopes of the linear regression lines from V1 to V2 to gPFC. In addition, there is an extraordinary amount of variance in the gPFC data,

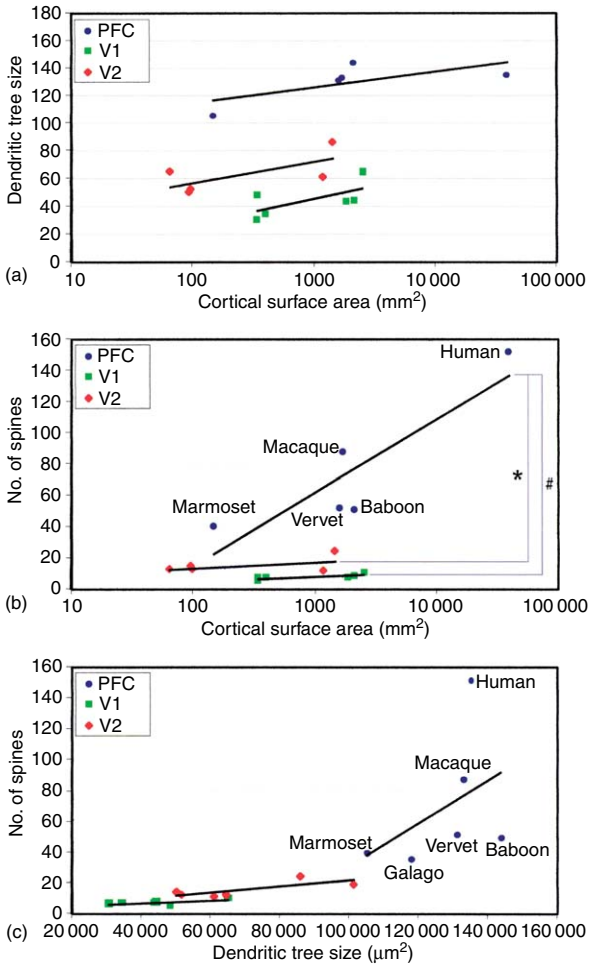

Figure 31 Plots of (a), the size; and (b), the number of spines in the basal dendritic trees of pyramidal cells in the gPFC, primary (V1), and second (V2) visual areas versus the surface area of each cortical area. c, Plot of the number of spines versus the size of the basal dendritic trees of pyramidal cells in the gPFC, V1, and V2. Moderated multiple regression revealed a significant difference between the slopes of regression lines of gPFC and V2 for comparisons between total number of spines in the dendritic trees of pyramidal cells versus cortical surface area ($F_{change}(1,6) = 6.19$, $p < 0.05$). Significance was approached for the comparison between the total number of spines in the dendritic trees of pyramidal cells versus cortical surface area for gPFC and V1 ($F_{change}(1,7) = 5.20$, $p < 0.057$). Data taken from Elston *et al.* (1999a, 1999b, 2001, 2005b, 2005e, 2005i, 2005j, 2006a) and Elston (2003c, 2003d).

which is not present in either V1 or V2 (see also Table 3).

Although only restricted to three cortical areas/regions, these data reveal that the extent to which different aspects of pyramidal cell structure (size, branching pattern, and number of spines) vary in different species depends on the cortical region studied. Note that the trend observed for gPFC differs from that for V1 and V2 for plots of the total number of spines in their dendritic trees and either the size of their basal dendritic trees or the size of the cortical area (Figures 31b and 31c). It has been suggested that these relative differences in pyramidal cell structure in the gPFC of different species endow the neurons, and the circuits they comprise, with different functional capabilities (i.e., compartmentalization within their dendritic trees, the number and diversity of inputs sampled, the potential for recurrent connectivity, and differences in memory storage capacity). Moreover, it has been suggested that species differences in executive functions of the gPFC such as conceptualizing, planning, and prioritizing result from differences in the pyramidal cell phenotype (Elston *et al.*, 2006a; see also Cajal, 1894a).

4.13.5 How Complex Can Pyramidal Cells Become?

Based on these data, one might conclude that pyramidal cells could evolve increasingly more complex branching structures as the cortex continues to expand. The obvious question, then, is how complex can pyramidal cell structure become? In theory, at least, the dendritic trees could contain so many branches that they almost form a solid sphere. However, considering principles of connectivity, this is unlikely. For example, if the dendrites became too densely packed, there would be a decrease in the amount of space available for axons to pass through the dendritic tree. Presumably, then there is an optimal space-filling characteristic for the dendritic tree to maximize both the number of inputs they can receive and the number of inputs that can pass in close proximity to form synapses (see Ringo, 1991 for a discussion).

In order to investigate the space-filling characteristics of the dendritic trees of pyramidal cells in different cortical areas/species, a systematic study of their fractal values has been initiated (Elston and Jelinek, 2001; Jelinek and Elston, 2001, 2003; Zietsch and Elston, 2005). Fractal analyses (Figure 32) allow the objective determination of the complexity, or space-filling capacity, of dendritic trees (see Jelinek *et al.*, 2005 for a review). The entire dendritic tree is defined by a single number, the fractal value, which can be correlated with other anatomical variables (e.g., the size of, or estimates of the asymmetry or the total number of spines in, the dendritic tree, or the size of the cortical area in which they are located). Fractal analyses were used to quantify the dendritic trees of pyramidal cells in V2, inferotemporal cortex (ITr or TE) and gPFC of human, baboon, macaque monkey, vervet monkey, owl monkey, marmoset monkey, and galago (Figure 33). These

Table 3 Data related to the size of the primary (V1) and second (V2) visual areas and gPFC in the prosimian galago (bush baby), simian monkeys (marmoset, owl, vervet, and macaque), baboon, and human, and the morphology of neurons contained therein

		Species						
Cortex	*Variable*	*Galago*	*Marmoset monkey*	*Owl monkey*	*Vervet monkey*	*Macaque monkey*	*Chacma baboon*	*Human*
V1								
	CSA (mm^2)	343	341	400	2156	1866	2559	2826
	BDFA (μm^2)	48 362	30 600	34 400	44 301	43 570	65 019	
	DF	1.2897	1.2710	1.32	1.3323	1.320 9	1.3614	
	TNS	556	699	773	795	734.75	1077	
V2								
	CSA (mm^2)	65	98	95		1203		1454
	BDFA (μm^2)	6474	51 700	50 400	61 246	43 900	101 210	86 000
	DF	1.36	1.39	1.38	1.39	1.326	1.446	1.36
	TNS	1216	1240	1459	1776	1139	1962	2417
gPFC								
	CSA (mm^2)		148		1625	1733	2111	39 287
	BDFA (μm^2)	118 002	105 022	104 000	130 986	133 000	143 672	135 000
	DF	1.396	1.37		1.42	1.43	1.475	1.48
	TNS	3579	3983		5152	8766	5009	15 138

BDFA, basal dendritic field area; CSA, cortical surface area; DF, fractal value; TNS, total number of spines in the basal dendritic tree. Reproduced from Elston, G. N., Benavides-Piccione, R., Elston, A., *et al.* 2006a. Specializations of the granular prefrontal cortex of primates: Implications for cognitive processing. *Anat. Rec.* 288A, 26–35, with permission from John Wiley & Sons, Inc.

plots reveal several interesting features related to areal and species specializations in the branching pattern of these cells. First, there is a degree of heterogeneity in the branching structure of pyramidal cells in all three cortical regions. Second, space filling is greater for cells in IT than in V2 in all species except the baboon. Third, between-species variability in the space filling of the dendritic trees is most notable in gPFC. Fourth, in V2 and IT there is no systematic correlation between the space-filling capacity of the dendritic trees of pyramidal cells and volumetric differences in the cortical area in the cerebrum of the different species. Finally, in gPFC there is a systematic increase in the space-filling characteristics of the dendritic trees of pyramidal cells that parallels the increase in the absolute size of this region in different species. These data suggest that optimal space-filling characteristics of pyramidal cells may differ between cortical areas and possibly between species for a given cortical area. These findings were confirmed dramatically by comparing the fractal value of cells with the size of the cortical area in which they were located (Figure 34). The trend for increasing space filling during expansion of the cortical area, which was present in V1 and gPFC, was clearly not present in V2. Instead, cells in V2 appear to occupy a progressively smaller volume of the neuropil within their dendritic tree in species in which this cortical area occupies a progressively larger absolute size (Figure 34a). In addition, it is clear from Figure 34b that there has been a disproportionate increase in the number of spines contained within the dendritic trees of pyramidal cells in the gPFC as these neurons have become more branched.

Based on the data presented in Figures 33 and 34, it might be reasonable to conclude that pyramidal cells in the gPFC in Old World anthropoids (humans and macaques in particular) will become increasingly more branched if this region continues to expand. Replotting the data on the fractal dimensions of cells reported in Figure 33 reveals a trend for increasingly more complex branched structure with increasing size of the dendritic trees (Figure 35). However, the trends in the data plotted in Figure 35 suggest that pyramidal cells, particularly those in the gPFC of humans, are reaching an upper limit of branching complexity (i.e., the regression slope reaches a plateau somewhere between 1.5 and 1.6). What this means in terms of prefrontal function in future humans is obviously highly speculative. It could be argued, for example, that increasing branching complexity would result in increased connectivity and, thus, greater functional capabilities (see Section 4.13.2.2). Alternatively, it is possible that the space-filling relationship between axonal input and recipient dendritic trees in human gPFC has already reached stasis at an optimal point and increasing the branching (space filling) of dendritic trees could only occur by decreasing the volume of neuropil occupied by other processes (and/or cells), resulting in decreased functional complexity. What can be said with some degree of

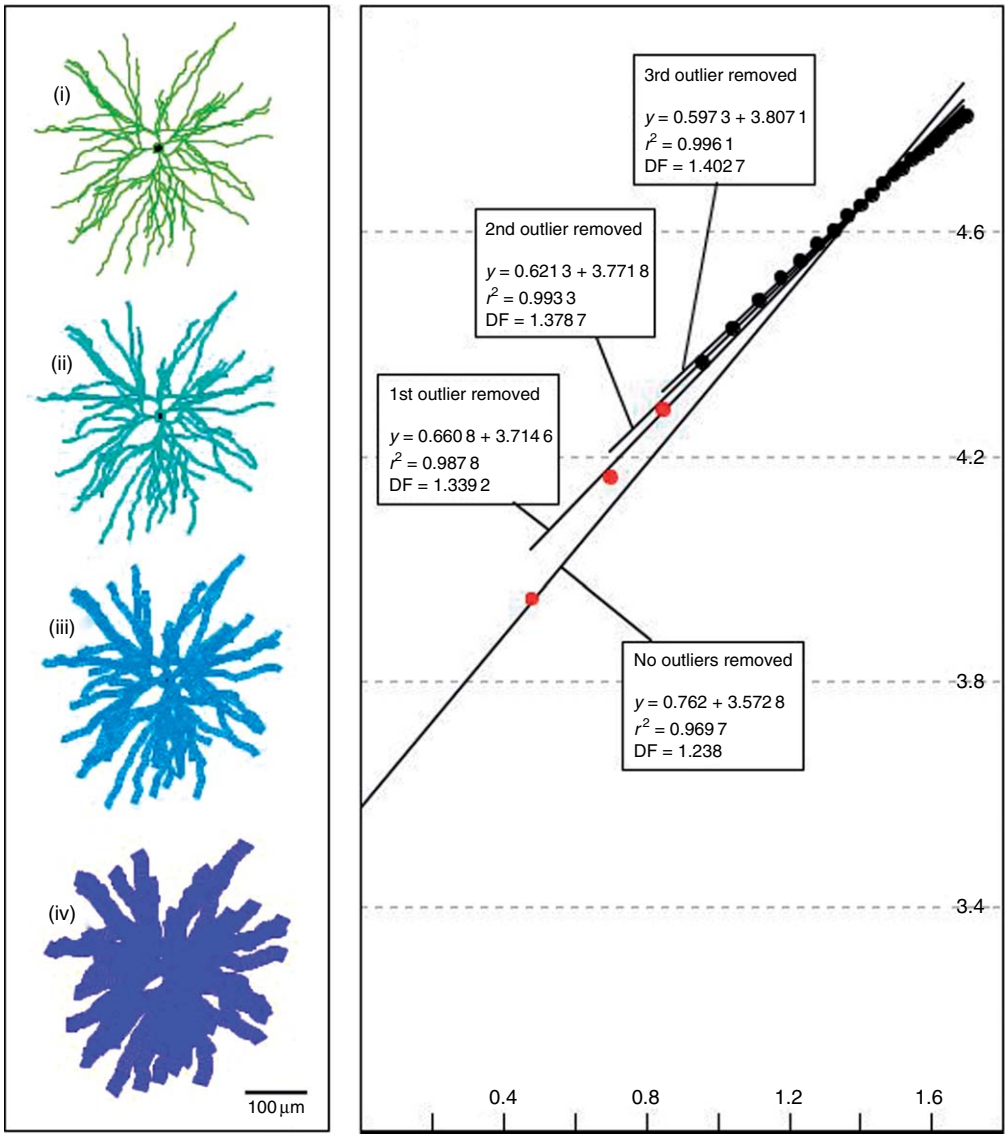

Figure 32 Schematic illustration of the methodology used in determining the fractal values (DFs) of the dendritic trees of pyramidal cells. Drawings of cells are digitized and the dendrites reduced to a thickness of one pixel (a(i)). Circles of increasing diameter are drawn over each pixel along the skeletonized dendrites (a(ii–iv)) and the percentage coverage within the dendritic tree calculated. The process is repeated 26 times and the data plotted (b). Outliers are then removed from the data set (colored dots) to reach a significance >0.95 and the fractal value calculated. Data taken from Elston, G. N. and Rosa, M. G. P. 1998b. Complex dendritic fields of pyramidal cells in the frontal eye field of the macaque monkey: Comparison with parietal areas 7a and LIP. *Neuroreport* 9, 127–131.

certainty is that differences in density and distribution of cells/neurites in the neuropil in different cortical areas/species makes it likely that optimal connectivity will be achieved by cells with dendritic trees characterized by different space-filling characteristics. Although untested, the data suggest that such an optimal connectivity is likely to vary appreciably between cortical areas. If true, this is a radical departure from present thinking on the ubiquity and uniformity of column structure in the cerebral cortex (see Mountcastle, 1997).

4.13.6 A New Model for Circuit Specialization during Cortical Evolution

The cerebral cortex differs in volume by more than 10 000-fold in extant mammals (see Stephan *et al.*, 1981; Hofman, 1985, 1988; Kaas, 1989; Krubitzer, 2000; Kaas and Collins, 2001; Northcutt, 2002 for reviews). These differences in volume do not adhere strictly to any cladistic variable, as dramatic differences in the volume of the cerebral cortex can be found within any given order or family, even between

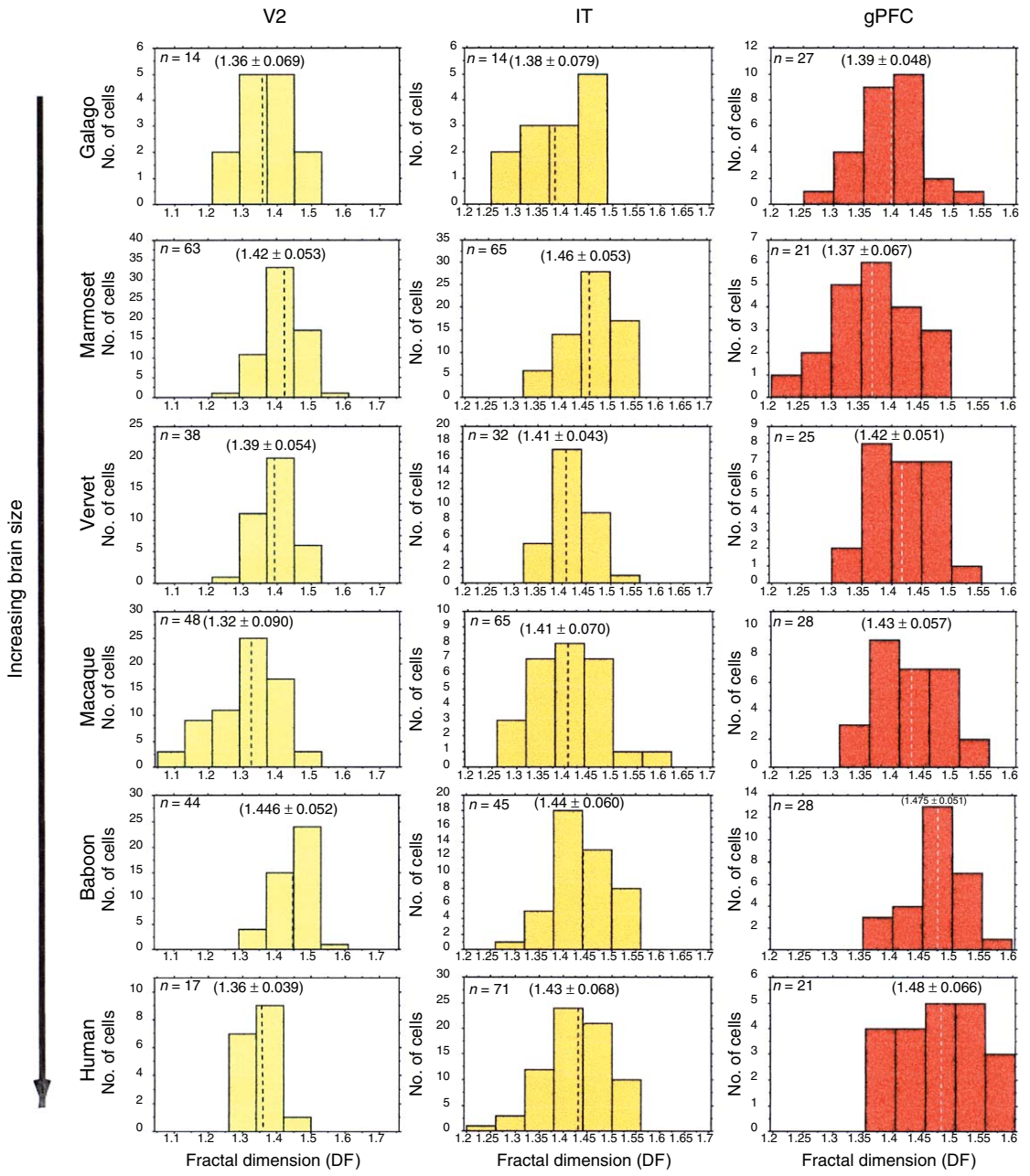

Figure 33 Frequency histograms of the fractal values (*DFs*) of the basal dendritic trees of layer III pyramidal cells in the second visual area (V2), inferotemporal cortex (IT), and granular prefrontal cortex (gPFC) of the marmoset monkey, galago, vervet monkey, macaque monkey, baboon, and human. Note there is no consistent change in the average *DF* (dashed line) with respect to brain size in either V2 or IT. There is, however, a trend for increasing *DF* (branching complexity) with increasing size of the gPFC. Mean ± SD illustrated in brackets. Modified from Elston, G. N. and Zietsch, B. 2005. Fractal analysis as a tool for studying specialization in neuronal circuitry: The study of the evolution of primate cerebral cortex and human intellect. *Adv. Compl. Syst.* 8, 217–227.

subspecies (e.g., pygmy marmoset and common marmoset, greater and lesser galagos). Notwithstanding this variability, comparisons of the brain volume of large numbers of individuals suggest that the cerebrum has expanded in some species, remained relatively constant in others, and possibly shrunk in yet other species. Narrowing the scope to primates, it is generally accepted that a common ancestor of extant primates had a relatively small cerebral cortex, which has become larger in different species. If you have read this far you may have already come to the realization that I do not believe that there is a common or unifying principle regarding specialization in pyramidal cell structure and brain size/cerebral volume.

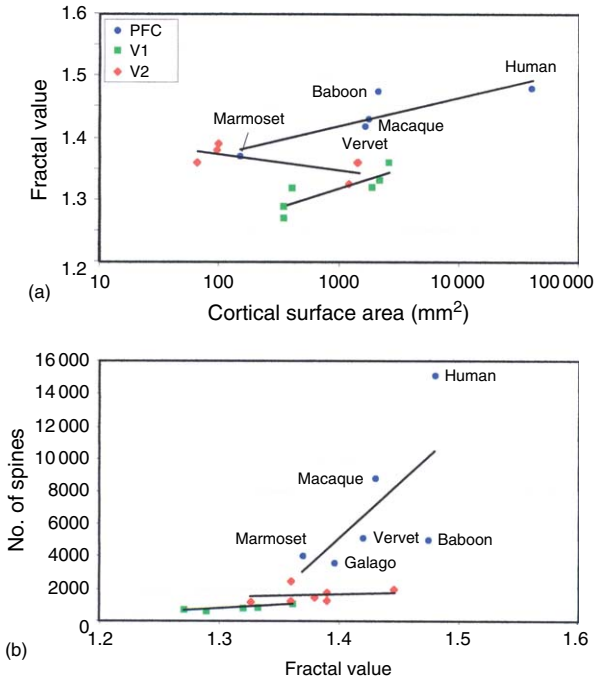

Figure 34 Plots of (a), the fractal value of the basal dendritic trees of pyramidal cells in the gPFC, primary (V1), and second (V2) visual areas versus the size of each cortical area; and (b), the number of spines versus the fractal value of the basal dendritic trees of pyramidal cells in gPFC, V1, and V2. Moderated multiple regression revealed a significant difference between the slopes of regression lines of gPFC and V2 for comparisons between the fractal values versus cortical surface area ($F_{change}(1,6) = 8.71$, $p < 0.05$). Note in (a) the negative slope generated by regression analysis, suggests that as V2 has undergone expansion in these species there has been a decrease in the space-filling capacity of the basal dendritic trees. Note also the radical increase in the number of spines in gPFC as cells have increased their space-filling capacity. Reproduced with permission from Elston, G. N. and Zietsch, B. Advances in Complex Systems, Vol. 8, Nos. 2–3, (2005) 217–227 © World Scientific Publishing Company.

Instead, the data on pyramidal cell structure suggest that evolutionary and developmental features that act in concert to shape the mature neuronal phenotype are likely to vary in different cortical regions and species. In the previous sections, the discussion was focused, in particular, on V1, V2, and gPFC, for which there are systematic and standardized data. Here we extend the discussion to include other regions of the cerebral cortex, and review data from the archontan tree shrew (a close relative of primates).

Based on data obtained from the occipital, parietal, temporal, and frontal lobes of primates, two different outcomes were proposed during evolutionary expansion of cortex: (1) expansion whereby mature cortex contains pyramidal neurons of similar structure or (2) expansion whereby mature cortex contains pyramidal neurons of increasingly complex structure (Elston *et al.*, 2005j). Evidence can be gleaned for both outcomes. For example, the total cortical volume occupied by V1 varies between primates, with as much as a fivefold difference reported between macaques and marmosets (Brodmann, 1913), yet layer III pyramidal cells have a remarkably similar number of spines in these species. Evidence for expansion of cortex by the addition of progressively more complex pyramidal cells comes from the temporal lobe: there is a consistent and dramatic increase in the number of spines in the dendritic trees of pyramidal cells as a function of absolute distance from the occipital pole (Figure 24). Comparison of the gPFC data in the galago, marmoset monkey, vervet monkey, macaque monkey, baboon, and human suggests a general trend for increasingly more spinous pyramidal cells as this region as a whole has undergone cortical expansion (Figure 36).

Quantification of pyramidal cell structure in the tree shrew (Elston *et al.*, 2005h) has revealed at least three other possibilities with respect to specialization in the pyramidal cell phenotype during the evolution of cortex: (1) an increase in complexity in the structure of pyramidal cells with little or no cortical expansion; (2) an increase in complexity in the structure of pyramidal cells during reduction in cortical size; and (3) a decrease in complexity in the structure of pyramidal cells during cortical expansion (Figure 37). These possibilities arise from the V1 data, but are not exclusive to striate cortex. In the first scenario, pyramidal cells in V1 have become more structurally complex even though the cortical area has undergone little or no expansion. In the second scenario, pyramidal cells in V1 have become more structurally complex and V1 has reduced in size. These outcomes would require that the structurally simple pyramidal cell phenotype in primate V1 is the apomorphic state while that seen in tree shrew V1 is a specialization. Such an interpretation requires that V1 in a common ancestor of tree shrews and monkeys was of a size similar to that in the tree shrew in the first scenario, or larger than that in the tree shrew in the second scenario. The third possibility is that the relatively complex pyramidal cell phenotype reported in tree shrew V1 could be the apormorphic state and that reported in primates a plesiomorph. That is to say, the less complex pyramidal cell structure is a specialization in primate V1. It remains unclear which of these scenarios may have occurred. On the one hand, it could be argued that relatively simple pyramidal cell phenotype is a primitive feature, as suggested by Cajal's observations in reptiles, amphibians, birds, and mammals (Cajal, 1894a, 1894b), and that observed in tree shrews is a plesiomorphy. On the

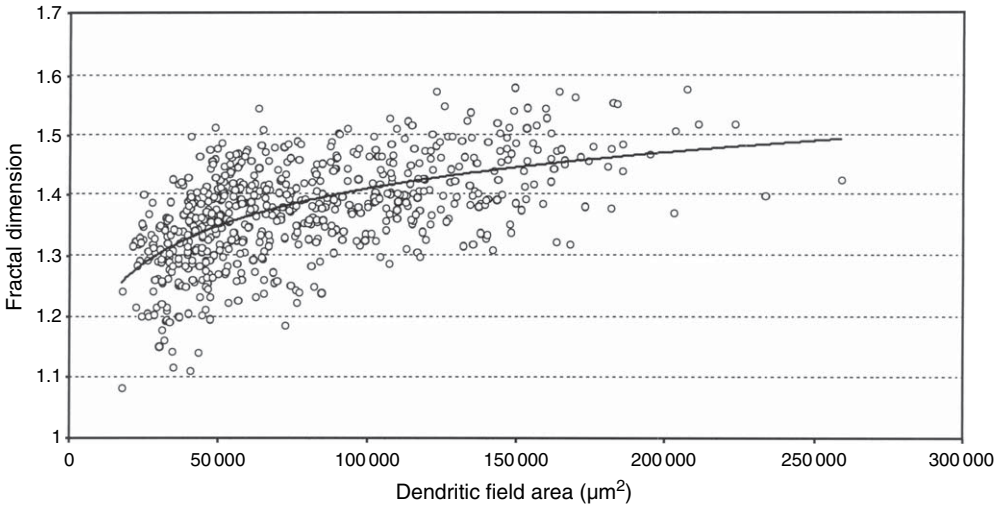

Figure 35 Plot of the fractal value (dilation method) versus the size of the dendritic tree of over 600 layer III pyramidal cells sampled from the primary, second, fourth, dorsolateral inferotemporal (caudal and rostral subdivisions), cytoarchitectonic areas TEO and TE, and gPFC of human, baboon, macaque monkey, vervet monkey, owl monkey, marmoset monkey, and galago. Note the lack of linear correspondence between the size and branching complexity of pyramidal cells. Instead, there is a plateau in the fractal value (branching complexity) of pyramidal cells with increasing size. The average fractal value of neurons varies between different cortical areas, suggesting that of their space-filling characteristics (and constraints) differ. We have proposed that the space filling of the dendritic trees of pyramidal cells is influenced during evolution, maturation, and adult experience, and varies according to the functional demands placed upon the cells. Data taken from Elston, G. N. and Zietsch, B. 2005. Fractal analysis as a tool for studying specialization in neuronal circuitry: The study of the evolution of primate cerebral cortex and human intellect. *Adv. Compl. Syst.* 8, 217–227.

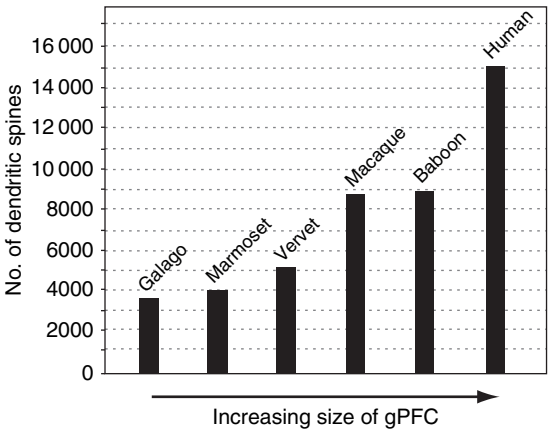

Figure 36 Estimates of the total number of spines in the basal dendritic tree of the 'average' pyramidal cell in gPFC in the young adult galago, marmoset monkey, vervet monkey, macaque monkey, Chacma baboon, and human. Note that there is a trend for a systematic increase in the total number of spines in the basal dendritic tree of pyramidal cells with increasing size of gPFC.

other hand, it could be argued that as Scandentia radiated prior to the emergence of the galago and New World and Old World monkeys, complex cell structure is likely to be a primitive feature. Clearly, further comparative studies are required, particularly if we are to determine whether any of these trends may be phylogenetically constrained.

4.13.7 A Note on Possible False Positives through Sampling Bias

It could be argued that interareal heterogeneity of cell structure reported in the cell injection studies could be attributed to us having sampled different subpopulations of pyramidal cells in the different cortical areas. For example, even within a given cortical layer, there may exist different populations of pyramidal cells of markedly different structure. Perhaps the best example of such intralaminar variation in pyramidal cell structure is that reported for cells that have different types of axonal projections. For example, pyramidal cells that form intrinsic projections have a notably different structure from those that make corticocortical projections, which in turn differ from those that send their axons to subcortical structures (Klein *et al.*, 1986; Schofield *et al.*, 1987; Hallman *et al.*, 1988; Hübener and Bolz, 1988; Hübener *et al.*, 1990; Vogt Weisenhorn *et al.*, 1995; Matsubara *et al.*, 1996). To address this issue, V1-projecting and MT-projecting neurons in different cortical areas of the marmoset monkey were specifically targeted for intracellular injection and their structures compared (Elston and Rosa, 2000, 2006). In particular, the fluorescent tracer Fluoro-emerald (FE) was injected into V1 or MT in different animals, and FE-labeled cells were then injected intracellularly with Lucifer

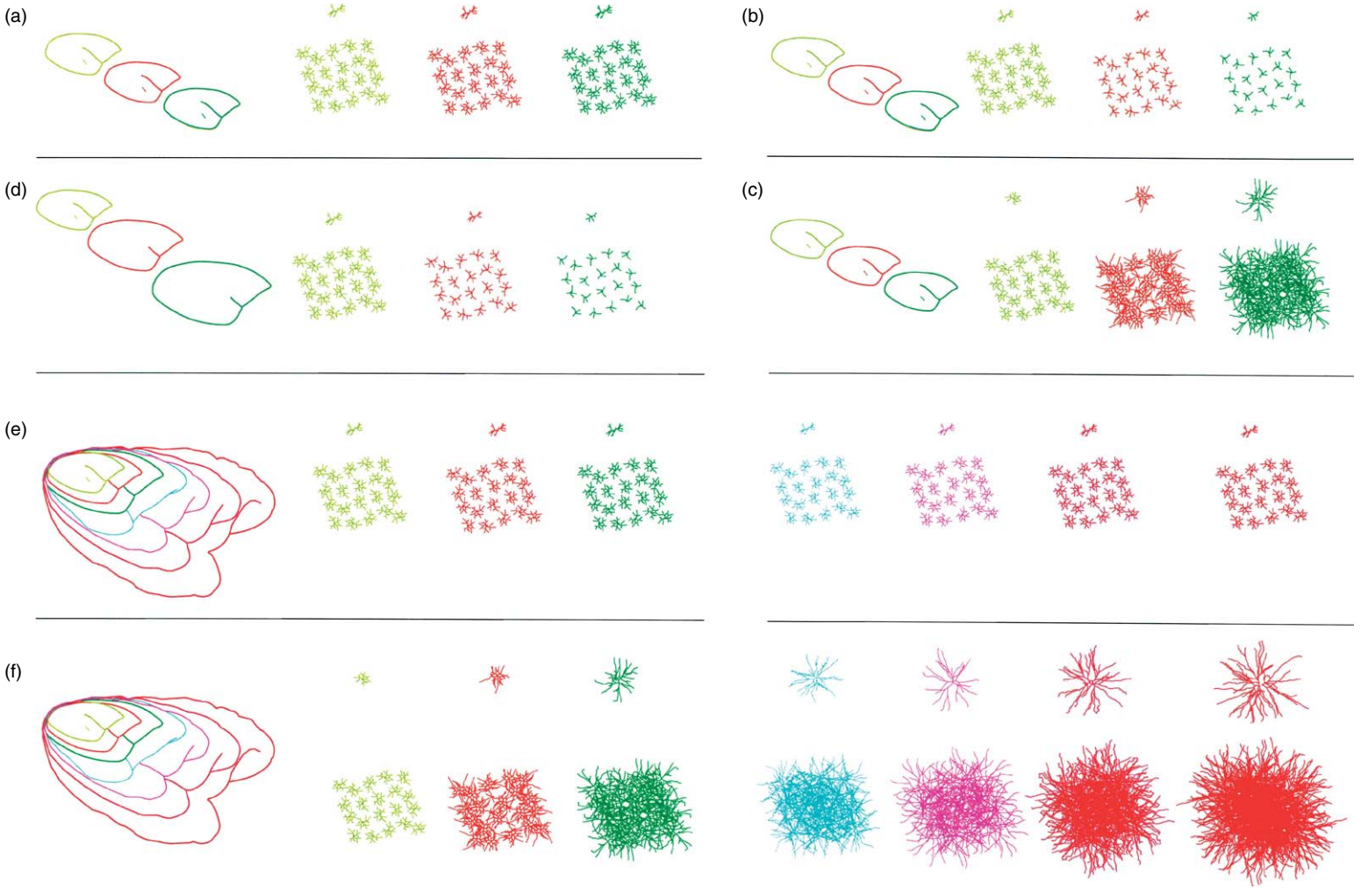

Figure 37 Schematic illustrating some possible specializations in pyramidal cell structure during the evolution of the cerebrum. In (a–c) there is no volumetric increase in the cerebrum but neurons either (a), retain the same structure; (b), become less complex; or (c), become more complex. In (d–f) the cerebrum has undergone a volumetric increase during its evolution and neurons either become less complex (d); retain the same structure (e); or become more complex (f). Note that pyramidal cell density is constant in all illustrated scenarios. Differences in dendritic coverage, especially visible in (f), are attributed solely to increasing size and branching complexity of the basal dendritic tree. Differences in cell density that may occur during the evolution of cortex result in three possible outcomes for each scenario illustrated here (increasing, decreasing, or constant neuron density).

Yellow. Thus, two distinct populations of neurons were studied, feed-forward and feedback, in multiple visual areas. In total, 242 feed-forward and 124 feedback cells from five animals were investigated (Figure 38).

We found that the morphology of both feed-forward and feedback extrastriate neurons varied systematically between cortical areas, paralleling previous findings for randomly labeled cells (Elston *et al.*, 1996, 1999b). For example, MT-projecting cells in V2 were smaller and less spinous than those in dorsolateral cortex (DL), which, in turn, were smaller and less spinous than those in IT. V1-projecting cells in V2 were smaller and less spinous than those in DL, which, in turn, were smaller and less spinous than those in IT (Figure 39). MT-projecting cells in V1 were the exception to the rule; however, these cells are located in a specialized sublamina in V1 (layer IIIC of Hassler, 1966) (Spatz, 1977; Tigges *et al.*, 1981; Diamond *et al.*, 1985; Shipp and Zeki, 1989; Elston and Rosa, 1997) not present in extrastriate cortical areas. In a similar series of experiments on the cat, Vercelli and Innocenti (1993) reported systematic variation in callosally projecting pyramidal cells in different visual areas. They concluded that the structure of pyramidal cells is determined by the cortical area in which they are located rather than the type of projections they

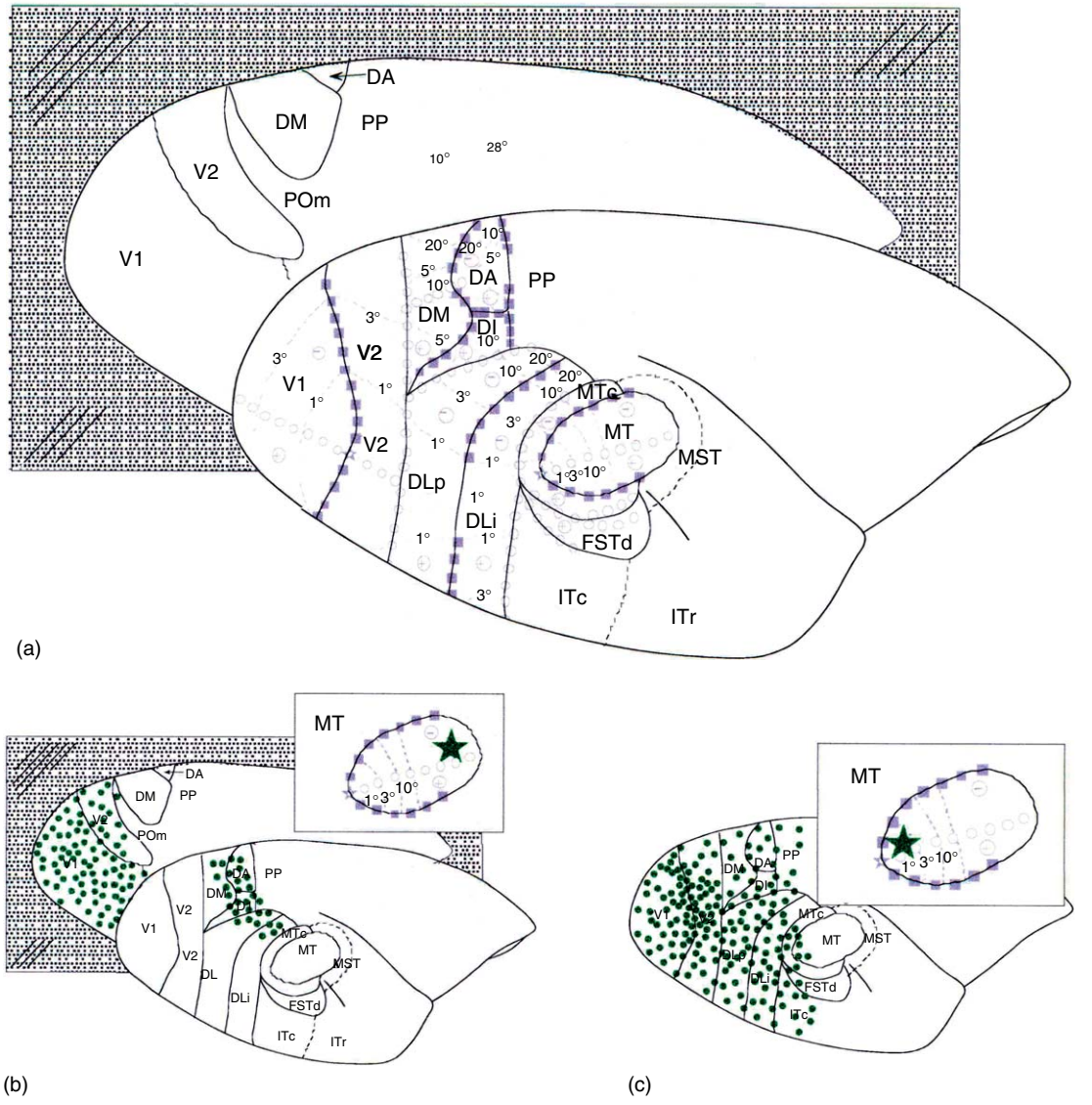

Figure 38 Schematics illustrating placement of injections (green star) of Fluoro-emerald into the area MT in two different cases. MT-projecting neurons (green dots) were labeled in the first (V1), second (V2), inferotermporal (IT) cortex, dorsolateral (DL), dorsomedial (DM), dorsointermediate (DI), dorsoanterior (DA), and the posterior parietal (PP) areas, as well as in the middle temporal crescent (MTc), the fundus of the superior temporal area (FST), and IT.

form. Thus, regional variation in pyramidal cell structure appears not to be attributed to sampling different subpopulations of these cells in different cortical areas, but rather reflects fundamental differences in cortical circuitry. This finding adds further support to the view that pyramidal cell structure in any given cortical area is specialized for its local functional requirements.

In addition, these experiments revealed that the size, branching complexity, spine density, and cell body size of cortical pyramidal cells is not necessarily correlated with the length of their axonal projection. More specifically, it was found that the cells making the longest feed-forward projection to area MT had the smallest, least branched, and least spinous dendritic trees, and the smallest cell bodies. Moreover, cells in V2, which made the second-longest projections, had only a slightly more complex structure than those in V1, but less complex structure than those in DL that projected a shorter distance (Figure 40). These findings challenge classic ideas regarding the relationship between cell body size and length of

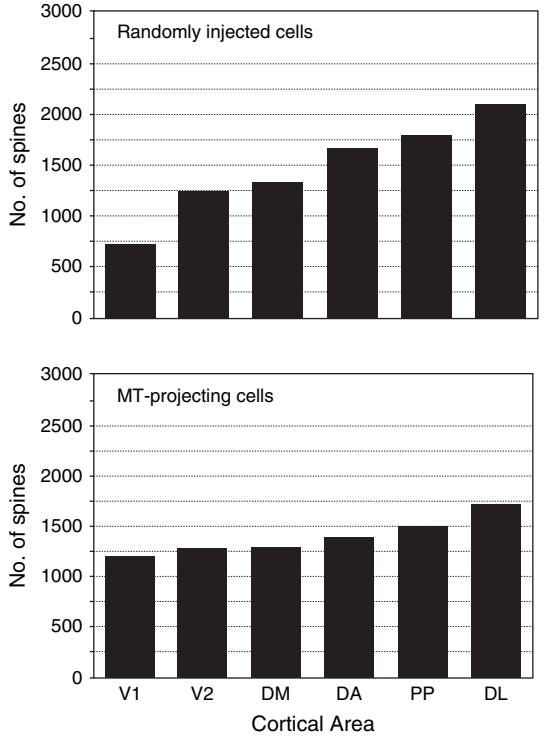

Figure 39 Plots of estimates of the total number of spines in the basal dendritic tree of the 'average' randomly injected (upper) and corticocortical projection (lower) layer III pyramidal cells in the primary, second, dorsomedial, dorsoanterior, posterior parietal, and dorsolateral visual areas (V1, V2, DM, DA, PP, and DL, respectively) in the marmoset monkey. Data for both randomly injected and corticocortical projecting cells were restricted to those in the central visual representations of the different cortical areas ($<5^\circ$ eccentricity). Note the trend for progressively more spinous cells through the same complement of cortical areas (note that visual areas are arranged in the same order along the *x*-axis in both graphs). Note also the difference in the V1 data: it has been well documented that the subpopulation of cells in V1 that project to MT are larger and more branched than non-MT-projecting cells in the same sublamina. The overall similarities in the estimates of the total number of spines in the basal dendritic trees of layer III pyramidal cells yielded by the different methods (random injection and retrogradely labeled cells), notwithstanding the difference in V1, is quite remarkable given that the calculations are influenced by the size and branching structure of the dendritic trees as well as the spine density along individual dendrites (see Elston, 2001). Differences in any one of these three variables can easily result in a doubling or tripling in the estimates of the total number of spines.

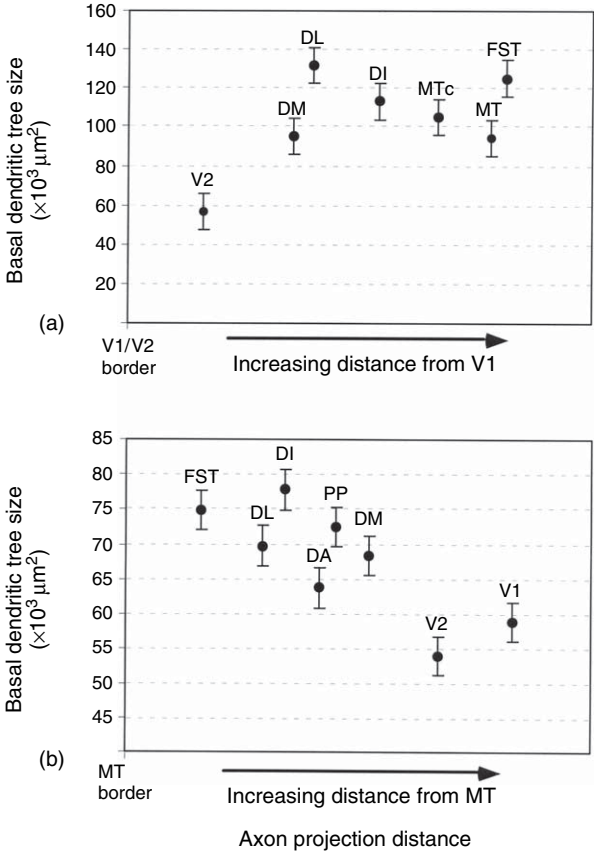

Figure 40 Plots of the estimates of the total number of spines in the basal dendritic tree of the average projection neuron in the first (V1), second (V2), third (V3), dorsolateral (DL), dorsomedial (DM), dorsointermediate (DI), and dorsoanterior (DA) areas, as well as in the middle temporal crescent (MTc), the fundus of the superior temporal area (FST), inferotemporal (IT) and the posterior parietal area (PP). Data are illustrated for the V1-projecting (a) and MT-projecting (b) cells, as a function of distance from projection site. Note that while it could be argued from the V1-feedback projecting data (a) that there is a rough correspondence between the size of the dendritic trees and the length of the axons they project, this is clearly not the case for MT-projecting cells. These data provide further evidence that regional differences in pyramidal cell structure are determined according to the functional requirements of the cells therein, over any other variable.

axonal projection, and dendritic tree size and length of axonal projection. For corticocortically projecting pyramidal cells, this appears not to be the case. Our unpublished data in the giraffe also reveal that these traditional theories related to cell body/dendritic tree size and length of axonal projection do not apply to neocortical pyramidal cells that form subcortical projections. For example, pyramidal cells in layer V of the primary motor area of the giraffe have a similar structure to those in layer III in its primary visual area (Elston, Elston, Badlangana, and Manger, unpublished observations).

4.13.8 The Paradox in the Frontal Lobe

Based on the data presented in visual cortex and somatosensory cortex, it may be tempting to assume that there is a correlation between complexity in pyramidal cell structure and a hierarchy in cortical pathways (see Section 4.13.2.2). However, data from the macaque monkey, the vervet monkey, and the baboon reveal that cells in area 4 (the primary motor area), which is generally considered to be the end of a cortical motor decision–execution pathway (Jones, 1986; Geyer *et al.*, 2000), are smaller and less branched than those in area 6. Moreover, cells in area 6 are less branched, and less spinous than those in dorsolateral gPFC, which are involved in purposive motor movements. Thus, in this proposed pathway, pyramidal cells become smaller, less branched, and less spinous with progression through successive levels involved in purposive motor movements (Figure 41). Of course, it could be argued that pyramidal cell structure reflects the functional complexity of neurons in these cortical areas (i.e., cells in gPFC are involved in the planning and initiation of purposive movements based on incoming sensory information and juxtaposing this with motivation and necessity to actuate a particular movement, whereas those in area 4 'merely' execute the movement).

One possible explanation can perhaps best be presented with an analogy from the physical world – nuclear power. We have seen how neurons in gPFC have a complex structure and, in dorsolateral gPFC, these cells are characterized by tonic discharge sustained over long periods of time. If these cells were to project directly out of the cerebral cortex to initiate motor movements, the muscles that they stimulate would likely be in a state of tonic spastic paralysis and would quickly fail. Even if these neurons were to project from gPFC to the efferent cells (area 4), they would be likely to cause tonic spastic paralysis, as cells in area 4 would be constantly stimulated. However,

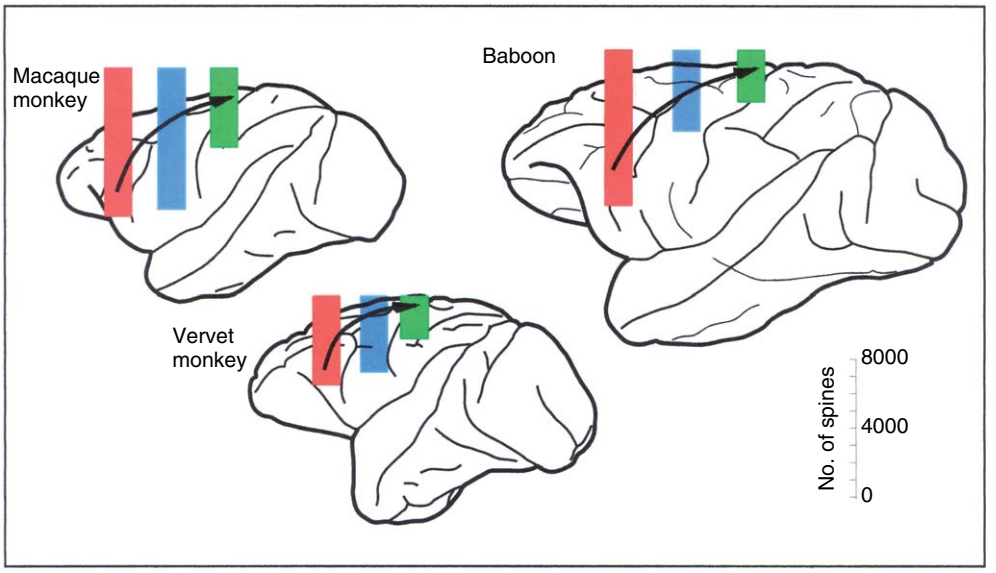

Figure 41 Schematic illustration highlighting phenotypic variation in the structure of pyramidal cells involved in voluntary motor movements in the vervet monkey, the macaque monkey, and the baboon. Cells in dorsolateral prefrontal cortex (area 12, red), which are involved in the initiation of purposive motor movements, are larger, more branched, and more spinous than those in premotor cortex (area 6, blue), which are larger, more branched, and more spinous than the effector cells in the primary motor area (area 4, green). Cortical areas included here represent only a few areas involved in purposive motor planning. In future studies, it will be worthwhile to quantify pyramidal cell structure in other cortical areas such as the supplementary motor area and motor areas on the medial wall.

if these highly active neurons in gPFC project through a series of cortical areas, each composed of cortical circuitry with less recurrent feedback and, thus, a decreased ability to sustain tonic activity, there may be more control over the efferent signals. In terms of the nuclear power analogy, cells in gPFC are like hot rods, while circuitry in the supplementary/premotor and the primary motor cortex act as progressively cooler water baths, reducing the self-sustaining activity to controllable levels before the activation of motor movement (efferent signal or output current). Differences in the threshold of neurons between motor areas (e.g., Wu *et al.*, 2000) provide evidence for their different 'energy states'. As pyramidal cells in gPFC have become more complex, as in the baboon and human, their patterns of connectivity have become more complex, resulting in more recurrent excitation and more powerful sustained activity – hence the expansion of motor cortex and the inclusion of more synaptic steps between area 4 and gPFC (i.e., premotor cortical areas) to modulate purposive motor movements. Clearly, more remains to be discovered regarding the implications of regional variation in cell structure and processing within cortical pathways involved in purposive motor movements, particularly in view of the results of studies that suggest distributed cortical processing (see Jones, 1986; Kaas, 1990; Paulesu, *et al.*, 1997; Calvert, 2001 for reviews).

4.13.9 Summary

Marked differences in pyramidal cell structure in different cortical areas of the primate cerebral cortex, and between corresponding cortical regions in different primate species, are likely to result from different evolutionary trends, development, and adult experience. Behavioral advantages endowed by specialization in pyramidal cell structure, by influencing the functional capacity of individual neurons and the computational power of the circuits they comprise, are likely to act as important selective pressures during the evolution of species. There is no unifying principle that determines phenotypic specialization in pyramidal cell structure in the primate cerebral cortex. Different evolutionary pressures appear to have resulted in different trends in the occipital, parietal, temporal, and frontal lobes among species.

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4.14 The Development and Evolutionary Expansion of the Cerebral Cortex in Primates

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Glossary

anthropoid primates The group of primates that includes monkeys, apes, and humans.

bromo-deoxyuridine Also called BrdU; an analogue of thymidine, one of the four bases of DNA. When applied to dividing cells, BrdU is incorporated into newly replicating DNA during the S-phase of the cell cycle. Thus labeled, dividing cells and their progeny can be identified using immunohistochemical procedures. See 'tritiated thymidine'.

corticogenesis The process by which the cerebral cortex grows and develops.

cytoarchitectonic areas Regions of cerebral cortex distinguished by differences in the relative prominence of particular cellular layers or the size or packing density of the resident cells.

GABAergic neurons Neurons that synthesize and release GABA as a neurotransmitter.

ganglionic eminence A mound of dividing neural precursors situated on the floor of the lateral ventricles in the embryonic forebrain.

GFAP Glial fibrillary acidic protein; a cytoplasmic protein that can be used to identify radial glial cells in the developing primate brain.

gliogenesis The process by which new glial cells are generated from dividing cells.

gliophilic cells Migrating cells that preferentially use glial cells as a migratory substrate. Compare with 'neurophilic cells'.

gyrencephalic Characterized by the presence of convolutions. Compare with 'lissencephalic'.

isocortex The part of the cerebral cortex characterized by having six horizontal layers of neurons.

lissencephalic Characterized by the absence of convolutions, e.g., a relatively smooth cortex. Compare with 'gyrencephalic'.

local circuit neurons Neurons with relatively short axonal projections that predominantly use GABA as an inhibitory neurotransmitter.

neocortex The part of the cerebral cortex characterized by having six horizontal layers of neurons. The prefix 'neo' confers the idea that this was the part of the cerebral cortex to appear most recently during cortical evolution.

neurogenesis The process by which new neurons are generated from dividing neural precursor cells.

neurophilic cells Cells that migrate preferentially along other neurons and their axons, in particular. Compare with 'gliophilic cells'.

telencephalon The part of the embryonic brain that will develop into the cerebral cortex, striatum, and olfactory bulbs of the adult forebrain.

tritiated thymidine A radioactive form of thymidine, one of the four bases of DNA. When applied to dividing cells, tritiated thymidine is incorporated into newly replicating DNA during the S-phase of the cell cycle. Thus labeled, dividing cells and their progeny can be identified using autoradiographic procedures. See 'bromo-deoxyuridine'.

4.14.1 Introduction

The mechanisms underlying the evolutionary expansion of the cerebral cortex are central to our understanding of the potential and limits of our mental capacity. Since the time of its origin in a mammalian ancestor, perhaps 250 Mya, the neocortex has undergone expansion in both relative and absolute size independently in several mammalian radiations. This expansion is particularly apparent in anthropoid primates, including humans, in which the neocortex comprises up to 80% of the brain mass. The expansion occurs primarily in surface area rather than in thickness. Further, the neocortex is parcellated into different cytoarchitectonic areas, which have increased in number, size, and complexity during cortical evolution.

Our insights into the evolution of the neocortex have traditionally come from physical anthropology and comparative anatomy (e.g., Ariëns Kappers *et al.*, 1936; Herrick, 1948; Nauta and Karten, 1970; Armstrong and Falk, 1982; Kaas, 1988; Jerison, 1991; Preuss, 1993; Butler, 1994; Northcutt and Kaas, 1995). More recently, genetic, molecular, and cellular mechanisms by which the cerebral cortex might have evolved have begun to be scientifically explored (e.g., Simeone, 1998; Smith Fernandez *et al.*, 1998; Caroll, 2003; Pruess *et al.*, 2004). The present article is an attempt to interpret some of these recent advances in our understanding of cortical development within the context of neocortical evolution. Examining the embryonic development of living species for clues about possible mechanisms underlying evolution is a well-established approach in evolutionary biology (e.g., Haeckel, 1879; Gould, 1977; Gerhart and Kirschner, 1997; Richardson *et al.*, 1997; Striedter, 1997, 1998). Recent advances in developmental neurobiology provide new insights into possible genetic and cellular mechanisms at a level of analysis that was previously unattainable.

The most notable feature of the cerebral cortex in all species, particularly in primates, is its parcellation into distinct laminar, radial, and areal domains (Rakic and Singer, 1988; Mountcastle, 1997) (see The Role of Transient, Exuberant Axonal Structures in the Evolution of Cerebral Cortex, Cerebral Cortical Folding Patterns in Primates: Why They Vary and What They Signify). The neocortex consists of six basic cellular layers, each having distinct neuronal organization and connections: layers 5 and 6 contain neurons that project to subcortical structures, layer 4 contains local circuit neurons, and layers 2 and 3 are composed mostly of neurons that project to other cortical areas, both ipsilaterally and contralaterally. The neocortex is also organized into radial (vertical) groups of neurons that are linked synaptically in the vertical dimension and share common functional properties. This radial arrangement of cortical neurons is unquestionably a fundamental principle of cortical organization that reflects its unique developmental history (Rakic and Singer, 1988; Mountcastle, 1997). Furthermore, the neocortex is parcellated into structurally and functionally distinct cytoarchitectonic areas, which increased in number, size, and proportion during the evolution of bigger brains (Brodmann, 1909). Recent re-examination of cortical parcellation by modern hodological, cytochemical, and physiological methods indicates that the definition as well as the border assignments of areas in the cortex may be more complex than previously thought by Brodmann and others. Nonetheless the principle of functional localization remains the same (Felleman and Van Essen, 1991).

The origin and evolution of the cerebral cortex is among the most fundamental questions in biology. Answers to this question may in turn provide long-sought answers to the questions of who we are, where we come from, and where we might be going. This subject can be approached from the standpoint of (1) its origin from phylogenetically older structures, (2) the expansion of its size following its emergence, and (3) the parcellation and elaboration of the neocortex into functionally and structurally specialized domains, or cytoarchitectonic maps. We will interpret various aspects of cortical development within the context of cortical evolution. In particular, we will focus on those cellular events that most likely underwent evolutionary modifications to build a larger and more parcellated cortex.

4.14.2 Neocortical Origins

The origin of the neocortex in vertebrate evolution has been a focus of interest to evolutionary biologists for more than a century (Darwin, 1871). Although the neocortex is recognized as a neural structure unique to mammals, its possible evolutionary antecedents and its homologues in the brains of other vertebrates are still disputed (see reviews by Northcutt, 1981; Butler, 1994; Northcutt and Kaas, 1995; Karten, 1997; Striedter, 1997; Smith Fernandez *et al.*, 1998). Perhaps the most frequently stated hypothesis is that the mammalian neocortex developed as an enlargement of the reptilian dorsal pallium (e.g., Herrick, 1948; Northcutt, 1981). Another frequently mentioned concept, although no longer tenable, is that the neocortex is an elaboration of the olfactory system (Ariëns Kappers *et al.*, 1936). One attractive hypothesis, proposed

in the early 1970s, was based on the comparative anatomy of neuronal organization, and suggested that the neocortex may have originated from two sources of cells from lower parts of the neuraxis (Nauta and Karten, 1970; Karten, 1997). The basic idea is based on comparative anatomical studies that suggest that the lateral pallium, or general cortex, of amphibians, birds, and reptiles may be homologous to the six-layered mammalian neocortex (reviewed in Northcutt, 1981). The basic tenet of this dual origin hypothesis is that during evolution, the expansion of the lateral pallium was associated with displacement of the archicortex (hippocampal formation) and the paleocortex (piriform cortex) toward the medial wall of the telencephalon (Figure 1). Furthermore, it was suggested that the lateral pallium was simultaneously transformed from a simple neural structure into a more complex six-layered mammalian cortex.

It is not clear how the predecessors of the neurons that eventually form the neocortex arose during evolution. One possibility is that during evolution, the neuroblasts of the dorsal ventricular ridge (DVR) of ancient reptiles shifted into the lateral pallium and provided additional cells for the six-layered neocortex (Nauta and Karten, 1970). Not everyone is convinced that the DVR has a homologue in the mammalian telencephalon or that the isocortex originates in topographically different pallial compartments (e.g., Aboitiz, 1999). Nevertheless, according to Nauta and Karten's model, the mammalian neocortex is formed by contributions from two distinct populations of founder cells. Results from studies of chimeric mice give implicit support for the dual origin hypothesis, and in addition indicate that the two phylogenetically discrete populations remain segregated during ontogeny as the separate laminar and radial clones within the mammalian cerebral cortex (Kuan *et al.*, 1997). This example illustrates how experimental methods in developmental biology may provide new insights into evolutionary questions. As elaborated below, neuroembryology provides not only new evidence to evaluate existing hypotheses, but can also generate new ideas of how the cortex may have evolved. An instructive example is the radial unit hypothesis of cortical expansion (see below).

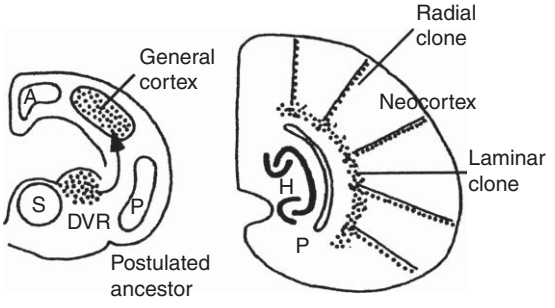

Figure 1 Comparative studies suggest that during evolution the general cortex of a mammalian ancestor expanded and propelled the archicortex (A; hippocampus, H) and the paleocortex (P; pyriform cortex, P) toward the medial wall of the telencephalon. It has also been postulated that neuroblasts of the dorsal ventricular ridge (DVR) located in the dorsal striatum (S) migrated and incorporated into the general cortex in ancestral mammals to result in the modern six-layered isocortex (Nauta and Karten, 1970). One possible explanation of the coexistence of radial and laminar clones in the mouse cerebral cortex is that these two phylogenetically distinct populations of cells remain ontogenetically segregated and are allocated differently. From Kuan *et al.* (1997), modified after Nauta and Karten (1970).

4.14.3 Key Events in Cortical Development

Because the embryonic development of living species can provide clues about possible mechanisms underlying its evolution, we will first briefly review major developmental events that lead to the formation of the cerebral cortex. We will describe primarily the timing and sequence of cellular events that occur in the macaque monkey, since the focus of this review is on cortical evolution in primates.

At the beginning of the second month of gestation in rhesus monkeys and humans, the process of differential cell proliferation causes the anterior-most end of the embryonic neural tube to balloon outward, forming a pair of telencephalic vesicles, which will become the future cerebral hemispheres (Sidman and Rakic, 1982). It is within the dorsal walls of these vesicles that the neocortex forms. It was initially suspected from observations in human embryos, by using classical methods, and later confirmed by the tritiated thymidine autoradiographic technique, that the neurons of the mammalian neocortex are generated only during a restricted period of early development, the period of neurogenesis. Unlike most species that have been examined, in which cortical neurogenesis lasts until birth or shortly thereafter, primates, including humans, acquire their full complement of cortical neurons before the last trimester (Rakic and Sidman, 1968; Sidman and Rakic, 1973; Rakic, 1974, 1988b). Some recent studies using bromo-deoxyuridine labeling and immunocytochemistry reported that new neurons continue to be generated and incorporated into the neocortex of macaque monkeys and rodents well into adulthood (Gould *et al.* 1999, 2001). However, subsequent studies by other groups of investigators using the same experimental methods have been unable to replicate these findings. Despite finding new neurons

in other areas known to be neurogenic in adulthood, i.e., the hippocampal dentate gyrus and lateral subventricular zone, convincing evidence for neurogenesis in adult neocortex was not observed (Magavi *et al.*, 2000; Kornack and Rakic, 2001; Rakic, 2002; Keketsu *et al.*, 2003; Ehninger and Kempermann, 2003). Thus, it appears that in all mammals investigated, neocortical neurogenesis is normally restricted to early developmental periods.

The initial concept that cortical neurons migrate from the place of their origin near the cerebral ventricle to their final destinations in the overlying cortex was discovered about a century ago, based on the exquisite observations and ingenious interpretation of human embryonic tissue sections stained with the classical histological methods. The critical finding was that mitotic figures (which signify cell division) are situated among neuroepithelial cells along the lumen of the cerebral ventricles (i.e., the ventricular zone, at the time called germinal layer or matrix) and are virtually absent in the overlying developing cortical plate, situated below the pial surface (His, 1874). The use of the autoradiographic method confirmed the fact that cortical neurons are not generated within the cortex itself, but rather are generated deep in the brain, near the surface of the cerebral ventricle (Angevine and Sidman, 1961; Sidman and Rakic, 1973; reviewed in Rakic, 1974, 1982, 1988b, 2006) (Figure 2). His' proposal regarding the noncortical site of dividing precursor cells in developing human cortex was eventually proven by using tritiated thymidine in one of the first applications of live tissue slice preparations (*in vitro*) in developmental biology (Rakic and Sidman, 1968). The majority of the supravitally labeled cells in the wall of the human fetal cerebrum were situated close to the ventricular surface, although some could also be found in the intermediate zone (IZ, prospective white matter) and marginal zone (MZ, prospective layer 1). Recent immunohistochemical analysis of the human telencephalon at early embryonic stages (30–37 postconceptual days) revealed that some abventricular mitosis occurs even before the onset of neurogenesis in the local ventricular zone (Bystron *et al.*, 2006). However, the developing cortical plate itself was found to be basically devoid of divisions until the beginning of gliogenesis (Rakic and Sidman, 1968). In primates, as in other mammalian species, progenitors of cortical neurons are confined to the narrow ventricular zone, where they form a transient pseudostratified epithelium, i.e., the ventricular zone (Rakic, 1972). Dividing cells in the ventricular zone are attached to each other at the ventricular surface by their endfeet and have radial

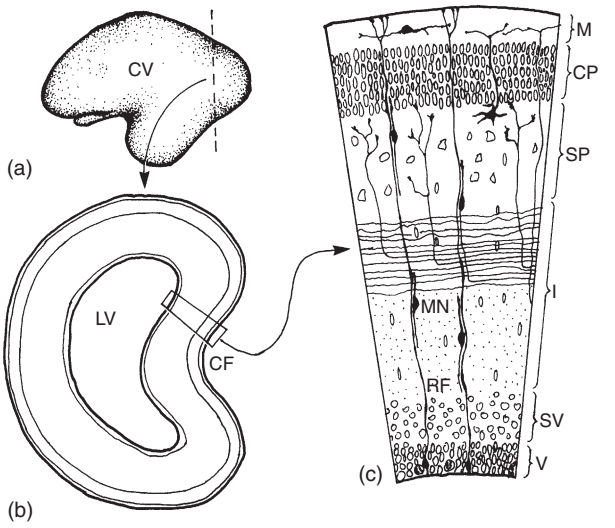

Figure 2 The cytological organization of the primate cerebral wall during the first half of gestation. a, The cerebral vesicle (CV) of a 60- to 65-day-old monkey fetus is still smooth and lacks the characteristic convolutions that will emerge in the second half of gestation. b, A coronal section across the occipital lobe at the level indicated by a vertical dashed line in (a). The lateral ventricle (LV) at this age is still relatively large and only the identification of the incipient calcarine fissure (CF) marks the position of the prospective visual cortex. c, A block of the tissue dissected from the upper bank of the calcarine fissure. At this early stage, one can recognize six embryonic layers from the ventricular surface (bottom) to the pial surface (top): ventricular zone (V); subventricular zone (SV); intermediate zone (I); subplate zone (SP); cortical plate (CP); and marginal zone (M). Note the presence of migrating neurons (MN) moving along radial glial fibers (RF), which span the full thickness of the cortex. The early afferents from the brainstem, thalamus, and other cortical areas invade the cerebral wall and accumulate in the subplate zone, where they make transient synapses before entering the cortical plate (Rakic, 1990).

processes that protrude toward the pial surface and into the outer cell-free marginal zone (Figure 2c). At later stages, particularly in primates, increasingly larger proportions of neurons are produced in the subventricular zone. Although in rodents virtually all interneurons of the neocortex are imported from ganglionic eminence within the basal telencephalon (reviewed in Marin and Rubenstein, 2001), in the human, most cortical interneurons originate in the subventricular zone of the dorsal telencephalon within a given area (Letinic *et al.*, 2002). This is particularly evident in the primary visual cortex (area 17), which in Old World primates, such as macaque and human, contains a significantly larger number of interneurons than the adjacent areas (Rakic, 1976; Smart *et al.*, 2002; Lukaszewicz *et al.*, 2005). The cells originating from the subventricular zone population in humans comprise about two-thirds of the neocortical GABAergic neurons; the remaining third originate from the ganglionic eminence, similar to rodents.

Besides harboring progenitors, the ventricular and subventricular zones contain a population of elongated radial glial cells that span the entire embryonic cerebral wall, from the ventricular to the pial surface (Rakic, 1972; Schmechel and Rakic, 1979a; Levitt *et al.*, 1981). This class of non-neuronal cells is present transiently in all mammals, but is particularly prominent in the large primate telencephalon (Schmechel and Rakic, 1979b; deAzevedo *et al.*, 2003; Zecevic, 2004; reviewed in Rakic, 2003). The radial glial fibers, which connect ventricular and pial surfaces, elongate as the cerebral wall widens (Schmechel and Rakic, 1979a). Postmitotic neurons produced in succession within the proliferative zones migrate outward along radial glial fascicles and traverse the intermediate and subplate zones before entering the developing cortical plate (Figure 2c). Radial glial scaffolding is considered to be essential for the preservation of positional information among cells within the protomap of the ventricular zone after they migrate to the suprajacent cortical plate, which, in primate brain, shifts as the hemispheric surface becomes more convoluted (Figure 3; Rakic, 1978). In addition to their role in providing a scaffold for radial neuronal migration, recent evidence indicates that radial glia can also directly produce

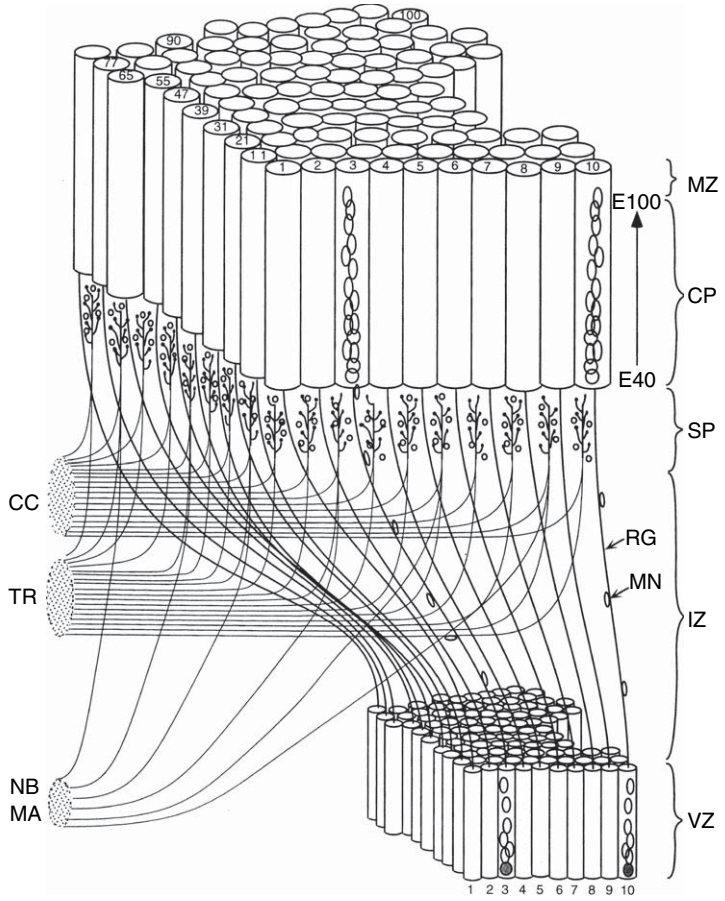

Figure 3 A three-dimensional illustration of the basic developmental events and types of cell–cell interactions occurring during early stages of corticogenesis, before formation of the final pattern of cortical connections (Rakic, 1988b). This drawing emphasizes radial migration, a predominant mode of neuronal movement that, in primates, underlies the elaborate columnar organization of neocortex. After their last division, cohorts of migrating neurons (MN) first traverse the intermediate zone (IZ) and then the subplate zone (SP) where they have an opportunity to interact with waiting afferents arriving sequentially from the nucleus basalis (NB) and monoamine subcortical centers (MA), from the thalamic radiation (TR), and from several ipsilateral and contralateral corticocortical bundles (CC). After newly generated neurons bypass the earlier generated ones, situated in the deep cortical layers, they settle at the interface between the developing cortical plate (CP) and marginal zone (MZ) and eventually form a radial stack of cells that share a common site of origin but are generated at different times. For example, neurons produced between embryonic day E40 and E100 in radial unit no. 3 follow the same radial glial fascicle and form ontogenetic column no. 3. Although some cells, presumably neurophilic, may detach from the cohort and move laterally, guided by an axonal bundle, most are gliophilic, i.e., have an affinity for the glial surface, and strictly obey constraints imposed by transient radial glial cell scaffolding. This cellular arrangement preserves relationships between proliferative mosaic of the ventricular zone (VZ) and the corresponding protomap within the subplate zone and cortical plate, even though the cortical surface in primates shifts considerably during the massive cerebral growth during mid-gestation. (For details see Rakic, 1972, 1988b.)

postmitotic neurons (Noctor *et al.*, 2001; Rakic, 2003; Gal *et al.*, 2006). The relative number of $GFAP^+$ and $GFAP^-$ precursors changes systematically over the course of cortical neurogenesis (Levitt *et al.*, 1983).

Upon entering the cortical plate, most neurons settle in an orderly inside-out temporospatial gradient, such that the earliest-born neurons form the deepest layer and successively later-born cells make progressively more superficial layers (Rakic, 1974). This inside-out gradient of laminar formation was initially discovered in rodents using tritiated thymidine autoradiography. Subsequent exposure of a series of macaque embryos to tritiated thymidine at different embryonic stages *in vivo* that were sacrificed postnatally, indicated that the inside-to-outside sequence is even more pronounced in the larger, gyrencephalic, and more slowly developing monkey neocortex, which is similar in size, shape, and cellular composition to the human (Rakic, 1974, 1988b). The reason for this pattern is not clear, but one hypothesis is that earlier generated neurons provide some information to the later generations, since genetic and/or environmental interference with sequential development has serious behavioral consequences (reviewed in Caviness and Rakic, 1978; Rakic, 1988a).

Although some postmitotic neurons do not obey radial constraints (Rakic *et al.*, 1974; Schmechel and Rakic, 1979b; Misson *et al.*, 1991; Rubenstein *et al.*, 1994; Ware *et al.*, 1999) and a selective population undergoes tangential, neurophilic modes of movement (Rakic, 1990; O'Rourke *et al.*, 1992; Ware *et al.*, 1999), the majority of neurons in a given column of the cortex are generated in the underlying sectors of the ventricular zone and migrate radially by gliophilic interactions (Rakic, 1978; Luskin *et al.*, 1988; Nakatsuji *et al.*, 1991; Parnavelas *et al.*, 1991; Tan and Breen, 1993; Rakic, 1995a; Tan *et al.*, 1998). Early divergence of basic cell types has been confirmed using the retroviral gene transfer method, which enables the study of cell lineages in the developing mammalian telencephalon, including primates (Luskin *et al.*, 1988; Kornack and Rakic, 1995). The use of the retroviral gene transfer method to trace cell lineages in the fetal monkey shows that even in the large convoluted primate cerebrum, clones of neurons in the cortex remain in strict radial alignment (Kornack and Rakic, 1995). Neural stem cells can also be tagged by the retroviral gene transfer method and their phenotype and migratory pattern followed in slices of postmortem human fetal tissue (Letinic *et al.*, 2002). Postmitotic neurons that are confined to a radial pathway exhibit a strong affinity for the radial glial cells (Rakic, 1972, 1978, 1988a; Gadisseux *et al.*, 1990). The class of migrating cells that use a glial substrate are termed gliophilic, in contrast to neurophilic cells, which move preferentially along axonal pathways (Rakic, 1990). The identity of surface molecules that provide differential adhesion between migrating neurons and glial fibers is being actively investigated (reviewed in Hatten and Mason, 1990; Rakic *et al.*, 1994; Rakic, 1997). In the context of the present article, it is sufficient to state that this cellular system may be an essential prerequisite for building a cerebral cortex as a sheet of radial columns intersected by horizontal layers of isochronously generated neurons.

4.14.4 The Radial Unit Hypothesis

The first step in cortical evolution is the increase in the number of its constituent cells. Since this increase precedes the formation of connections, it is the primary and essential change that has occurred during evolution. In addition, the manner by which a larger number of postmitotic cells migrates from proliferative ventricular zone to become deployed in the cortical plate as a sheet rather than a lump (as they do, for example, in the neostriatum) is crucial for understanding cortical expansion during evolution (Rakic, 1995b). The interpretation of the data on kinetics of cell production and their allocation in the embryonic telencephalon has led to the postulate of the radial unit hypothesis of cortical development and evolution (Rakic, 1988b).

The radial unit hypothesis postulates that the developing cortical plate basically consists of arrays of ontogenetic columns. The dynamic cellular events that underlie the development of this organization in the embryonic cerebral wall are presented schematically in Figure 3. Autoradiographic studies of neuronal origin indicate that neurons within a given radial column originate from several clones (polyclones) that share the same birthplace, migrate along a common pathway, and settle on top of each other within the same ontogenetic column (Rakic, 1988b). This has been confirmed by retroviral lineage tracing experiments in primates (Kornack and Rakic, 1995), rodents (Reid *et al.*, 1995) and in chimeric and transgenic animals (Nakatsuji *et al.*, 1991; Tan and Breen, 1993; Soriano *et al.*, 1995). Progenitor cells within the individual radial units that generate neurons destined for the cortical plate are coupled by specialized junctions that allow for intercellular communication (Bittman *et al.*, 1997). These radial units may form the developmental basis of

functional columns or modules that are observed in the adult cerebral cortex (Szenthágothai, 1978; Eccles, 1984; Mountcastle, 1997). However, the relation of ontogenetic columns to functional columns of the adult cortex remains to be defined.

According to the radial unit hypothesis, tangential (horizontal) coordinates of cortical neurons are determined by the relative position of their precursor cells in the ventricular zone, whereas their radial (vertical) position is determined by the time of their origin (Rakic, 1988b). Moreover, the number of the ontogenetic columns determines the size of the cortical surface, whereas the number of cells within the columns determines the thickness. Therefore, questions about the evolution of cortical size and thickness become translated into questions about developmental regulation of total cell number, and how this regulation was modified during evolution.

4.14.5 Cortical Expansion

Even cursory comparison of cerebral hemispheres among different mammalian species reveals dramatic differences in the number of cortical neurons and overall size of cortical surface. For example, surface area of the neocortex in mouse, macaque monkey, and human has an approximate ratio of 1:100:1000, respectively, whereas the thickness barely varies by factor of two (Blinkov and Glezer, 1968). The difference in the size of the neocortex between human and macaque monkey is particularly instructive as their basic cytoarchitectonic and hodological organization are rather similar (Polyak, 1957; Shkol'nik-Yarros, 1971). Thus, it appears that in the 23 Mya since macaque monkeys and humans diverged from a common ancestor (Fleagle, 1988), the cerebral cortex of these two Old World primate species has undergone different rates of expansion in cell number and cortical surface without a significant increase in the thickness of the cortical mantle.

According to the radial unit hypothesis, the increase in the cortical surface without a comparable increase in its thickness during mammalian evolution can be accounted for by changes in the proliferation kinetics of founder cells in the ventricular zone that increase the number of radial units without significantly changing the number of neurons within each unit (Rakic, 1995b). How was this expansion initiated and, once established, how was it preserved? How does one explain that approximately 15-fold more postmitotic cells in humans (compared to macaques) are generated and deployed to form a thin, regular sheet? This question can be further extended to the approximately 100-fold difference between the surface of the mouse and monkey neocortex.

4.14.5.1 Determinants of Neuron Number

Theoretically, total neuronal number in the cortex is determined by five basic parameters:

1. the number of founder cells;
2. the duration of the cell-division cycle;
3. the number of successive cell cycles during the period of neurogenesis;
4. the modes of cell division; and
5. selective cell death.

Recent progress in understanding the relevance of these parameters and their genetic control to cortical expansion is described below.

4.14.5.1.1 Kinetics of cell division The length of the cell cycle is a major determinant of the number of cells produced because it determines the total number of successive cell divisions that elapse over the entire period of neurogenesis. Accordingly, to gain insight into which parameters were modified during the evolution of larger cortices, we measured the length of the cell cycle of progenitor cells that generate neurons of the primary visual cortex in fetal macaque monkeys and compared it to cell cycle measurements in the smaller-brained mouse. Surprisingly, we found that the duration of the cell cycle in monkeys is as much as five times longer than that in the mouse neocortex (Kornack and Rakic, 1998). Nevertheless, due to the greatly extended duration of cortical neurogenesis in primates (60 days in monkeys compared to 6 days in mice), substantially more successive rounds of cell division elapse during neurogenesis in monkeys than in mice. Specifically, the generation of cortical cells in monkeys requires at least 28 successive rounds of cell division in the ventricular zone, in contrast to the much smaller cortex of mice, which is produced by only 11 mitotic cycles. Moreover, in contrast to the progressive slowing of the cycle described in rodents (Caviness *et al.*, 1995), the rate of cell division accelerates during neurogenesis of the enlarged cortical layers in monkeys (Kornack and Rakic, 1998). This evolutionary amplification in the number of successive cell-division cycles that generate cortical cells could account for the expansion of monkey cortical surface and the hypercellularity of upper layers in the monkey visual cortex (Kornack, 2000). One question is whether the difference in the laminar width between cortical regions, such as observed between striate (area 17) and extrastriate

(area 18) in primates, is a result of an increased production of cells destined for these areas in the subjacent proliferative zones or is due to an increase in cell death of postmigratory neurons. This difference could be predicted from previous studies showing that area 17 develops some of its specific cytological properties even if deprived from the thalamic input (Rakic, 1988a; Rakic *et al.*, 1991). It has also been shown that cell cycle duration in the ventricular zone subjacent to areas 17 and 18 is different at the time when the supragranular layers (i.e., layers 2 and 3) are being generated (Kornack and Rakic, 1998; Lukaszewicz *et al.* 2005). Thus, evolutionary modification of the duration and number of progenitor cell divisions has contributed to both the expansion and laminar elaboration of the primate neocortex. Uncovering the genes and cellular signals that control the length of cell cycle and duration of neurogenesis in ontogeny may provide clues to how these changes have occurred during evolution. Progress has already been made in identifying particular genes and signaling molecules that influence proliferation during cortical development in the mouse. For example, deleting the gene for the winged helix transcription factor, *BF-1*, decreases the proliferation of progenitor cells in the forebrain and results in greatly reduced cerebral hemispheres (Xuan *et al.*, 1995), whereas overexpression of *p27* can increase the number of cortical cells generated (Lukaszewicz *et al.*, 2005; Tarui *et al.*, 2005).

4.14.5.1.2 Modes of cell division In addition to the length of the cell cycle and the total duration of neurogenesis, the mode of cell division that produces (1) two equal progenitors (symmetric, nonterminal mode), (2) one progenitor and one postmitotic cell (stem or asymmetrical mode), or (3) two postmitotic cells (symmetric, terminal mode) can also significantly influence the total output of the proliferative ventricular zone (Kornack and Rakic, 1995; Rakic, 1995b). Since, as reviewed above, the entire cortical plate is created by neurons generated in the ventricular zone, the way they are generated may have profound consequences on their allocation in the cortex. In the macaque monkey, which has a 165-day gestation, before the 40th embryonic day (E40), all cells in the ventricular zone are dividing symmetrically: each progenitor produces two additional progenitor cells during each mitotic cycle (Rakic, 1988b). With each extra round of symmetric divisions, the number of founder cells in this phase doubles the number of progenitor cells, resulting in an exponential increase in the size of ventricular zone (Figure 5a). Conceivably, a slight prolongation of this phase of telencephalic development of proliferation could be indirectly responsible for the large surface enlargement of the cerebral cortex (Rakic, 1995b).

After E40 in macaque monkeys and approximately E42 in humans, some progenitor cells begin to produce postmitotic neurons that leave the ventricular zone, differentiate into neurons, and never divide again (Rakic, 1974, 1985). Autoradiographic and retroviral analyses of the patterns of cell division indicate that, after E40, many precursors begin to divide asymmetrically (Rakic, 1988b; Kornack and Rakic, 1995) (Figures 4a and 5b). This mode of division, also known as stem cell division, produces one daughter cell that is permanently postmitotic, and the other, which continues to divide or dies. The postmitotic cell that will become a neuron detaches from the ventricular surface and begins to migrate toward the pial surface, eventually settling in the cortical plate (Figures 4a and 5b). The other daughter cell remains attached to the ventricular surface by the endfoot and usually continues to divide, producing an additional pair of unequal cells: one progenitor and one postmitotic neuron that departs for the cortical plate. This pattern of

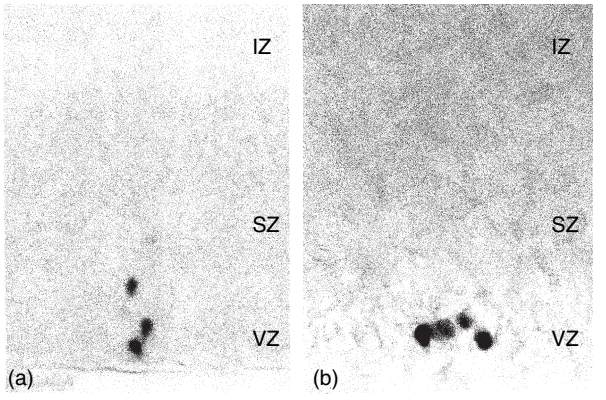

Figure 4 The distribution of retrovirally labeled cell clones in the inner cerebral wall of a fetal monkey near the end of corticogenesis, 3 weeks after an intraventricular injection of retroviral vectors carrying the histological marker gene, *lac z*. These distribution patterns of labeled cells provide evidence for the coexistence of two distinct modes of cell division in the proliferative ventricular zone (VZ), i.e., asymmetric and symmetric. a, The radial alignment of these three clonally related cells suggests that they are the offspring of an asymmetrically dividing progenitor. The innermost cell, near the ventricular surface, is most likely the progenitor that produced, in sequence, the outer two sibling cells, which are migrating outward toward the developing cortical plate. b, Clonally related cell clusters that remain in the VZ are most likely progenitor cell cousins that are the offspring of a symmetrically dividing progenitor. Note the lateral displacement of the resultant progenitors. SZ, subventricular zone; IZ, intermediate zone, Modified from Kornack, D. R. and Rakic, P. 1995. Radial and horizontal deployment of clonally related cells in the primate neocortex: Relationship to distinct mitotic lineages. *Neuron* 15, 311–321.

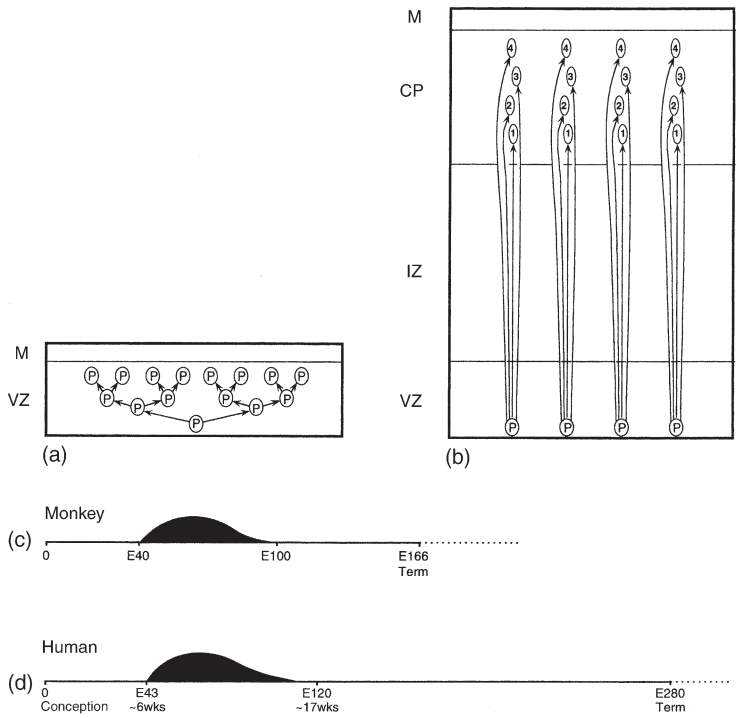

Figure 5 a, A schematic model of symmetric cell divisions, which predominate before the 40th embryonic day (E40). At this early embryonic age, the cerebral wall consists of only the ventricular zone (VZ), where all cells proliferate and the marginal zone (M), where some of them extend their radial processes. Symmetric division produces two progenitors (P) during each cycle and causes rapid horizontal lateral spread. b, A model of asymmetric or stem division, which becomes predominant in the monkey embryo after E40. During each asymmetric division, a progenitor (P) produces one postmitotic neuron that leaves the ventricular zone and another progenitor that remains within the proliferative zone and continues to divide. Postmitotic neurons migrate rapidly across the intermediate zone (IZ) and become arranged vertically in the cortical plate (CP) in reverse order of their arrival (1, 2, 3, 4). c, A diagrammatic representation of the time of neuron origin in macaque monkey. The data are obtained from the ^{3}H-thymidine autoradiographic analyses (Rakic, 1974). d, An estimate of the time of neuron origin in the human neocortex based on the number of mitotic figures within the ventricular zone, supravital DNA synthesis in slice preparations of fetal tissue and the presence of migrating neurons in the intermediate zone of the human fetal cerebrum. Based on Rakic (1978) and Rakic and Sidman (1968).

cell division in the monkey fetus proceeds during the next 30–60 days depending on the cortical area (Rakic, 1974, 1976, 1982). The number of cells contributing to each column depends on the duration of the period of corticogenesis and length of the cell cycle.

After the onset of cortical neurogenesis in primates (Kornack and Rakic, 1995) as well as other mammals (Caviness *et al.*, 1995; Chenn and McConnell, 1995; Mione *et al.*, 1997; Gall *et al.*, 2006), many precursors continue to divide symmetrically (Figure 4b). During the course of neurogenesis, this mode appears to be gradually supplanted by the asymmetric mode until the ventricular zone becomes exhausted as a source of cortical neurons and neurogenesis ceases (Rakic, 1988b; Caviness *et al.*, 1995; Rakic, 1995b; Kornack and Rakic, 1998; Kornack, 2000). However, the retrovirus gene transfer method of labeling clonally related cells in both rodent and primate embryos supports the hypothesis that neurons produced by a single asymmetrically dividing stem cell migrate radially and settle in the same ontogenetic column (Luskin *et al.*, 1988; Misson *et al.*, 1991; Kornack and Rakic, 1995; Reid *et al.*, 1995). It should be emphasized that even neurons produced from symmetrical division that become distributed over several columns, after their terminal division, nevertheless appear to migrate radially and settle within the same layer (Kornack and Rakic, 1995; Rakic, 1995a). The elimination of the isochronously dividing cells by low doses of ionizing radiation in monkey embryos at different stages of development supports this concept (Algan and Rakic, 1997). Specifically, irradiation of monkey embryos before E40 results in a decrease in cortical surface with little effect on its thickness, whereas irradiation after E40 deletes individual layers and reduces cortical thickness without an overall decrease in total surface.

To summarize, the kinetics of mitotic activity in the macaque ventricular zone can be divided into

two broad phases: (1) the phase before E40, when the most of the founder cells of the prospective cerebral cortex are formed, and (2) the phase of ontogenetic column formation, which proceeds mainly by asymmetric and terminal symmetric divisions that begin after E40 and continue until the completion of corticogenesis in a given region (Rakic, 1988b). The duration of the first phase, and of the cell cycle, determines the number of radial units and, indirectly, the size of cortical areas. The duration of the second phase regulates the number of neurons within each ontogenetic column. It is also during this second phase that the laminar phenotype of generated neurons is determined (reviewed in McConnell, 1995). It was proposed that the switch between the two phases of cortical development may be triggered by the activation of putative regulatory genes that control the mode of mitotic division in the ventricular zone (Rakic, 1995b). This activation is initiated within the telencephalon prior to the arrival input from the periphery.

Several lines of evidence from experimental manipulation as well as the pathogenesis of particular cortical malformations in humans suggest that these two phases can be separately affected: a deficit occurring during the first phase produces a cortex with a small surface area but normal or enlarged thickness (lissencephaly), whereas a defect during the phase of ontogenetic column formation produces a thin cortex with a relatively normal or larger surface size (polymicrogyria). This has been put to the test in mice in which neuronal production has been increased through an acceleration in the production of proliferative units (Chenn and Walsh, 2003).

4.14.5.1.3 Programmed cell death An additional, though frequently neglected, mechanism that may be involved in the control of cell production in the ventricular zone is the extent of apoptosis, or programmed cell death (PCD). Several lines of evidence support the notion that PCD is an active, inherently regulated phenomenon of selective cell elimination that is clearly distinct from cell necrosis, which occurs in response to harmful mechanical or chemical injuries (Kerr *et al.*, 1972). Although PCD has been considered a major factor contributing to the formation of the vertebrate brain (Glucksman, 1951), current research has focused on histogenetic cell death occurring at late developmental stages where it is primarily involved in eliminating incorrect axonal connections (Cowan *et al.*, 1984; Rakic, 1986; Oppenheim, 1991). Relatively little attention has been paid to the reports documenting sporadic cell death in the ventricular zone (e.g., Rakic, 1972) or more massive elimination of cells that fail to migrate away from the proliferative centers, prior to formation of any connections (Rakic and Sidman, 1973).

Recently, the use of methods that identify dying cells by labeling the exposed ends of their fragmented nuclear DNA suggests that early apoptosis may be much more widespread than generally assumed (Blaschke *et al.*, 1996). The possibility of studying this issue in mammals at the cellular/molecular level has dramatically increased in the past few years with the identification of regulatory genes that regulate PCD (Ellis and Horvitz, 1986). Because biologically important molecules tend to be conserved during evolution, we can use the same genes that were identified, for example, in *Drosophila* or the nematode, *Caenorhabditis. elegans*, and examine their function in mice. We can, for example, delete, overexpress, underexpress, or differentially express genes as nerve cells are produced or allocated. This can be illustrated by our recent work on genes controlling apoptosis in the mouse (Kuida *et al.*, 1996, 1998; Haydar *et al.*, 1999). We have reduced apoptosis by inactivating genes for caspase 3 and caspase 9, which must be expressed for a cell to die. In mice lacking both copies of the gene, apoptosis is reduced in the cerebral ventricular zone at early stages, during production of the founder progenitor cells. The main finding, relevant to the expansion of cortical size, is that in accord with the radial unit hypothesis, a larger-than-normal number of founder cells resulted in a cortex with an increased surface area and the formation of convolutions. By this single gene mutation, a lissencephalic mouse cortex was transformed into a gyrencephalic cerebrum, which is usually a hallmark of larger brains, as if there was a recapitulation of evolution. In this instance, the mutation resulting in more cortical neurons was not good for the homozygous mice; most of the mice died before birth. However, during evolution over millions of years, numerous mutations that increase the number of founder cells by either changes in the kinetics of proliferation or cell death could occur, and at some point supernumerary cells may have formed functionally useful connections that have helped in the survival of the species. Although the developmental mechanisms underlying the natural occurrence and patterning of cortical gyri in other species remain largely unknown, several theories have been proposed, which are beyond the scope of this review (Welker, 1990; Armstrong *et al.*, 1995; Van Essen, 1997). This experiment with apoptotic genes addresses this issue and illustrates the remarkable power of molecular and developmental neurobiology: here we used a gene

identified in a nematode worm that may help us understand principles of cortical development that could be extrapolated to primate evolution. In summary, the size of the cortex results from interactions of genes involved in progenitor proliferation and genes that cause the death of some of those progenitors.

4.14.5.2 Implications of the Radial Unit Hypothesis for Cortical Expansion

The radial unit hypothesis of cortical development provides a useful framework for understanding cortical expansion during evolution: the larger the number of radial units in a given species, the larger the surface of the neocortex (e.g., Figure 3). The radial unit hypothesis predicts that the difference in cortical surface area between two species depends on the difference in the number of founder cells generated during the first phase of unit formation (Rakic, 1995b). As is evident from Figure 5, during this phase, each round of symmetric cell divisions could double the number of founder cells, whereas, during the second phase, when asymmetrical divisions begin to predominate, each additional mitotic cycle adds only a single neuron to a given ontogenetic column. Indeed, in the monkey, the second phase begins 4 weeks later than it does in the mouse (E40 and E11, respectively). Thus, although the length of the cell cycle at the onset of neuron production is about twice as long in primates as that in mice (Kornack and Rakic, 1998), the size of the proliferative pool at the comparable embryonic stages is much larger in monkey than in mouse. However, it takes only a few days to account for the difference of an order of magnitude of cortical expansion between monkey and human (Figures 5c and 5d). If the length of the cell cycle in these two Old World primates is comparable, a delay in the onset of the second phase of a few days that allows three to four extra rounds of mitosis would result in a 23-fold to 24-fold increase in founder cells, which would generate an 8–16 times larger number of columns and therefore the proportionally larger cortical surface (Figure 5c and 5d). In contrast, a 20-day longer duration of the second phase in human compared to monkey (E100 and E120) would add only about 10 more cells per ontogenetic column. Assuming that each column consists of about 100 neurons (Rakic, 1988b), such an addition would increase cortical thickness by only 10%. These numbers, as well as the descriptions of developmental events, are oversimplifications of more complex cellular processes that occur during this developmental period. For example, this model does not take into account the possible changes in the proportion of symmetrical cell divisions during the second phase, the growth in size of individual neurons, the contribution of glial cells and myelin, or the rate of cell death, all of which may also influence surface expansion to different extents in each species. However, these developmental and structural differences are relatively minor between the two Old World primate species that we selected for comparison. The enlargement of the number of radial units must be the most prominent and decisive evolutionary factor.

The developmental events described here indicate that the evolutionary expansion of the neocortex in primates could be attributed, to a large extent, to a change in genetic mechanisms that control the onset, cessation, rate, and/or mode of cell division (Rakic, 1995b). According to the proposed model, the species-specific size of the cortex is determined at early stages by the pool of founder cells before corticogenesis starts, and before there is any input from the periphery. Although the evolutionary construction of the mammalian brain may require as many genes as were needed for all morphogenetic and metabolic functions in phyletic history (John and Gabor Miklos, 1988), a small modification of a regulatory gene or genes may have played a significant role in the evolutionary expansion of the neocortex, as has presumably occurred in other bodily systems (Medawar, 1953). Therefore, the explanation for cortical expansion between mammalian species rests predominantly upon the process of heterochrony whereby changes in the timing of developmental events increases the number of founder cells and consequently the surface of the cortical plate (Rakic, 1995b; Kornack and Rakic, 1998).

4.14.6 The Protomap Hypothesis of Cortical Parcellation

In addition to expanding more than 1000 times in surface area during evolution, the neocortex also became divided into more complex and more distinct cytoarchitectonic maps by both the differential growth of existing areas and the introduction of novel ones. For example, compared to small-brained mammals (e.g., insectivores, rodents), large-brained primates, including macaque monkeys and humans, display a larger number as well as more pronounced differences between cytoarchitectonic features, distribution of neurotransmitter receptor complements, and neuronal circuitry (Rakic and Singer, 1988). How might such differences emerge? Again, to get some insight into how this could have occurred during evolution, we have devised

scenarios based on advances in understanding ontogenetic development.

As predicted by the radial unit hypothesis and the data reviewed above, the initial number of proliferative units that generate prospective cortical columns in a given species is likely to be set up early in embryogenesis by a few regulatory genes that control cell production. However, the final pattern and relative size of cytoarchitectonic subdivisions of the neocortex are probably regulated by a different set of genes and, in addition, it must be coordinated through reciprocal cell–cell interactions with various afferent systems. Ten years ago, one of us proposed a protomap hypothesis of cortical parcellation (Rakic, 1988b). This hypothesis postulates that intersecting gradients of molecules might be expressed across the embryonic cerebral cortex that guide and attract specific afferent systems to appropriate cortical regions where they can interact with the responsive set of cells. The prefix 'proto' indicates the malleable character of this primordial map, as opposed to the generally accepted *tabula rasa* hypothesis, which considers the cortical plate as an undifferentiated primordium that is shaped and subdivided entirely by afferents, as advocated effectively by Otto Creutzfeldt in the mid-1970s (Creutzfeldt, 1977). However, in the past decade, there has been increasing evidence of differential gene expression across the embryonic cerebral wall that indicates prospective subdivisions of the neocortex. Some of these molecules may be expressed in a region-specific manner before and/or independently of the input (Kuljis and Rakic, 1990; Barbe and Levitt, 1991; Arimatsu *et al.*, 1992; Ferri and Levitt, 1993; Cohen-Tannoudji *et al.*, 1994; Levitt *et al.*, 1997; Acampoa *et al.*, 1999; Donoghue and Rakic, 1999a, 1999b; Gitton *et al.*, 1999a, 1999b; Rubenstein and Rakic, 1999). This independence is supported by finding that animals devoid of input from periphery (as in experimentally induced and congenital anophthalmia) nevertheless develop region-specific cytoarchitecture and appropriate local and topographic connections (Kaiserman-Abramof *et al.*, 1989; Kuljis and Rakic, 1990; Kennedy and Dehay, 1993; Rakic and Lidow, 1995; Bourgeois and Rakic, 1996; Miyashita-Lin *et al.*, 1999; Grove and Fukuchi-Shimogori, 2003; reviewed in special issues of *Cerebral Cortex*: Rubenstein and Rakic, 1999; O'Leary and Borngasser, 2006).

There is an emerging body of evidence for the existence of areal differences in the duration and rate of cell production in the macaque ventricular zone that can be detected prior to overt cell differentiation (Rakic, 1976; Dehay *et al.*, 1993). Finally, regional differences in the embryonic cerebral wall (either in the subplate or cortical plate) seem to be capable of attracting axons that originate from distinct diencephalic nuclei (Kostovic and Rakic, 1990; De Carlos and O'Leary, 1992; Agmon *et al.*, 1995; Letang *et al.*, 1998). This selective attraction may be due a gradient of recognition molecules distributed across the cerebral wall (Boncinelli *et al.*, 1993; O'Leary and Koester, 1993; Bulfone *et al.*, 1995; Simeone, 1998; Acampora *et al.*, 1999; Donoghue and Rakic, 1999a, 1999b).

It should be emphasized that these hypothetical primordial protomaps merely provide an oriented blueprint and a biological potential that, in turn, is translated into a species-specific archetype of neural connections through reciprocal interactions between interconnected levels. Numerous studies have shown that perturbation of afferent connections and local circuitry result in changes within these regions (e.g., O'Leary and Stanfield, 1989; Rakic, 1981, 1988b; Rakic *et al.*, 1991; Algan and Rakic, 1997), implicating cellular connectivity as a necessary but not sufficient determinant of regional specification. These interactions begin before birth (Rakic, 1981) and therefore may be influenced by spontaneous electrical activity (Shatz, 1996). However, numerous experimental studies in the developing brain indicate that basic species-specific topology develops autonomously and that neural activity may play more of a permissive rather than an instructive role (reviewed in Chair, 1999). Therefore, input from sensory receptors at the periphery may not be essential for the establishment of basic species-specific connections (Kuljis and Rakic, 1990; Rakic, 1995b).

In the primate visual system, such interactions continue until the time of puberty (Bourgeois and Rakic, 1993), when most structural and biochemical properties become stabilized through visual stimulation from the environment. Such interactions are bidirectional, i.e., from photoreceptors at the periphery toward the central structures in the cortex, and back from the cortex toward the retina. Disruption, or even a short delay in a single communication step in either direction may cause a cascade of reactions affecting heterogeneous cell classes, leading to an abnormal organization of the entire system and, consequently, to abnormal function (Hubel *et al.*, 1977; Rakic, 1983; Bourgeois and Rakic, 1996; Shatz, 1996). Thus, once connected with the appropriate input, neurons at each visual center may have a species-specific response, depending on the complement of genes that become differentially expressed.

The enlargement of cortical surface area or even the differential expansion of the individual areas in

themselves is not sufficient to account entirely for the elaboration of cortical connectivity that occurred during evolution (Rakic, 1995b). The increase in cortical surface by the introduction of new radial units, as well as the expansion and elaboration of areas, provides only an opportunity for creating novel input/target/output relationships with other structures that, if heritable, may be subject to natural selection. The new synaptic relationships resulting from these neuronal interactions may be adverse or neutral, or may enhance the capacity for behavioral adaptation. As pointed out by Jacob (1977), a new structural feature does not have to be optimal, but must be good enough to provide a survival advantage for the species. For example, reducing mortality by advantages of increased cognitive capacity and thereby prolonging the sexually active period of individuals with a larger cortex might improve their lifetime reproductive success and propagate this trait. Although the introduction of novelty in evolution by the process of heterochrony has been proposed to explain morphological changes of non-neural organs of the body (Alberch *et al.*, 1979), understanding the role of heterochrony in the phylogenetic development of the brain presents special problems because of the complex interplay among multiple epigenetic factors that regulate gene expression during development (Edelman, 1988; Purves, 1988; Changeux and Chavaillon, 1995; Rakic, 1995b). During the genesis of the cerebral cortex, such cellular interactions probably play a more significant role than in any other organ, and this, as well as the paucity of crucial comparative developmental studies, is perhaps why progress in this field has been slow.

In summary, modern developmental neurobiology provides insight into how the cerebral cortex develops and how it may have evolved. The genes and morphoregulatory molecules that control the production, migration, and appropriate allocation of neurons to their final positions within the developing cortical plate are being identified at a rapid rate and their functions tested both *in vitro* and *in vivo* in transgenic animals. The data suggest that the early stages of corticogenesis determine the species-specific size and basic pattern of organization that set the stage for the formation of the final pattern of synaptic connections whose functional values are then tested by natural selection.

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4.15 Brain Size in Primates as a Function of Behavioral Innovation

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Glossary

behavioral innovation	A novel learned behavior not previously found in the population.
social learning	Learning from another animal or its products.

4.15.1 Introduction: What is Behavioral Innovation?

A behavioral innovation can be operationally defined as "a new or modified learned behavior not previously found in the population" (Reader and Laland, 2003b). Thus, innovations are novel, learned acts. The focus on learned acts excludes accidental, one-off behaviors and emphasizes behavior patterns more likely to be consequential to the animal. The focus on innovations as novel to the group rather than the individual distinguishes the creation of a novel behavior pattern (innovation) from social acquisition of the new behavior. This definition contrasts with that used in the human innovation literature (e.g., Rogers, 1995), which considers socially acquired novel behavior patterns as innovations.

Innovation taps into what many consider an important aspect of cognitive sophistication: the flexibility to try and learn something new (Eysenck, 1995; Reader and Laland, 2003a). Innovators must depart from an established behavioral repertoire and locate a novel problem to be solved or a novel solution to an old problem (Kummer and Goodall, 1985; Reader and Laland, 2003b). Innovation therefore combines problem solving with the tendency to seek new solutions or new problems. The 'problems' solved may range in apparent cognitive complexity, from inclusion of a new item in the diet to the use of novel tools. Thus, innovation is likely to involve a number of underlying cognitive, perceptual and motivational processes, and perhaps also a variety of neural substrates (Greenberg, 2003; Reader and Laland, 2003b). This broad definition of innovation is deliberate, and is appropriate at this early stage of investigation in order to encourage research demarcating innovation into its possible component processes on the basis of empirical data, rather than on arbitrary distinctions such as perceived cognitive complexity (Reader and Laland, 2003b).

Innovation is of significance to several research fields. To the psychologist, understanding innovation is an important part of understanding learning and problem solving. To the social learning theorist, a complete understanding of the spread of novel behaviors through groups will also involve an understanding of how the novel behavior arises in the first place. To the ecologist, shifts to novel resources, novel habitats, or novel mate choice preferences are of great theoretical and applied interest (Sol, 2003; Sol *et al*., 2005). To the evolutionary biologist, of interest are the potentially dramatic evolutionary consequences of such innovations, particularly, perhaps, when innovations spread by social learning (Wyles *et al*., 1983; Nicolakakis *et al*., 2003; Sol, 2003; West-Eberhard, 2003). Innovation itself may be under natural, sexual, and cultural selection, and Baldwin (1896) raised the possibility that adaptive behavioral innovations may lead to selection favoring unlearned versions of the same behavior (for supporting theoretical models and human data, see Holden and Mace, 1997; Ancel, 1999; West-Eberhard, 2003).

Moreover, innovation can provide a useful measure of behavioral flexibility. For taxa like primates and birds, where there is a history of reporting deviations from the established population

behavioral repertoire, one can count the number of reports of innovation for large numbers of species (Lefebvre *et al.*, 1997; Reader and Laland, 2002). With appropriate correction for possible confounding variables (such as research effort) we have a general index with which we can examine the evolution and evolutionary consequences of one aspect of behavioral flexibility. In particular, we can examine the neurological, cognitive, and ecological correlates of innovation. Such analyses can provide useful clues for the role of behavioral flexibility in brain evolution, the co-evolutionary history of cognitive capacities, and the ecological pressures favoring an innovative strategy.

4.15.2 Innovation and Cognitive Evolution

The comparative studies of nonhuman primates described below are detailed fully by Reader and Lefebvre (2001), Reader and Laland (2002), Reader (2003), and Reader and MacDonald (2003), and examples of the innovations collated can be found in Reader and Laland (2001). For exposition, we present figures of across-species data, with each datum representing one species. However, such across-species analyses are potentially flawed if species show similar characteristics to each other because of common ancestry, not independent evolution, thereby violating the assumption of data independence (Harvey and Pagel, 1991). We employed the independent contrasts technique to account for this difficulty (Felsenstein, 1985; Purvis and Rambaut, 1995). Such techniques assume that while two species may be similar to each other because of common ancestry, the differences between them represent independent evolution (Harvey and Pagel, 1991).

4.15.2.1 Neurological Correlates of Innovation

The brain does not evolve as a unitary structure (Barton and Harvey 2000; see Mosaic Evolution of Brain Structure in Mammals). Thus, it is sensible to focus on the brain areas thought to be involved in the psychological processes of interest. In the case of primate innovation, these are generally thought to be the neocortex and striatum, together the so-called 'executive brain' (Oakley, 1979; Jolicoeur *et al.*, 1984; Keverne *et al.*, 1996). Figure 1 shows that there is a correlation between relative executive brain volume and innovation rate: species with large executive brains relative to their body size are more frequently reported as innovators. The relative brain measure used in Figure 1 is the ratio of executive brain to brainstem volume. Note that there is debate over the most appropriate methods for taking body size into account in comparative brain studies (Deaner *et al.*, 2000). We did not find relationships between innovation and every alternative measure of relative executive brain volume (Reader and Laland, 2002). However, the relationship remained significant when body mass was included in a multiple regression with innovation rate and executive brain ratio, suggesting that the correlation is not due to the confounding effect of body mass (Reader and Laland, 2002).

These results are consistent with the idea that cognitive capacity and brain volume are linked, if the reasonable assumption is made that innovation rate is a measure of cognitive capacity (see also Section 4.15.2.2). These correlational data do not, however,

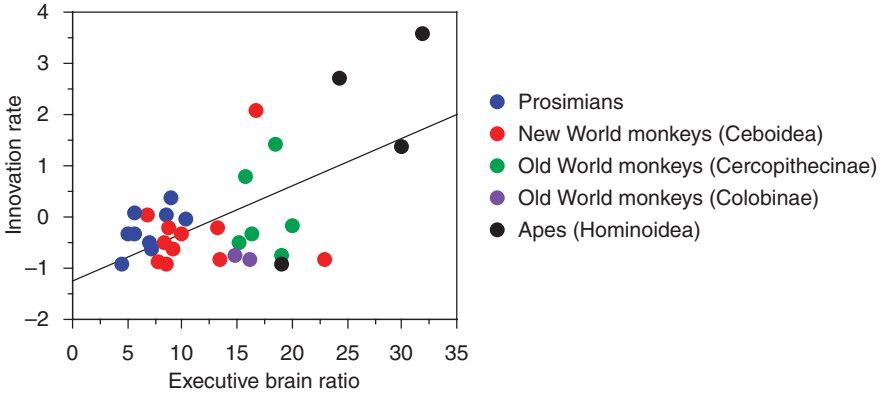

Figure 1 Innovation rate and relative executive brain ratio (executive brain volume/brainstem volume) are correlated. Innovation rate was corrected for differences in research effort by taking the residuals from a natural log-log plot of innovation rate against research effort, thus corrected innovation rate can take positive and negative values. For exposition the figure shows across-species data, with colored symbols indicating taxa. Independent contrast analysis gave similar results (Reader and Laland, 2002). Adapted from Reader, S. M. and Laland, K. N. 2002. Social intelligence, innovation and enhanced brain size in primates. *Proc. Natl. Acad. Sci. USA* 99, 4436–4441.

allow us to distinguish between innovation as an adaptation, an exaptation, or a nonadaptive byproduct of another selection pressure that promoted brain enlargement. Moreover, the fact that innovation correlates with brain volume casts scant light on the neuropsychological processes underlying innovation: the question of why neural volume and cognitive capacity should be linked remains open (Bolhuis and Macphail, 2001; Deary, 2001).

4.15.2.2 Cognitive Correlates of Innovation

Social learning and tool use frequencies can be collated from published literature in the same manner as innovation frequency. Innovation rate correlates with both social learning and tool use frequency (Figure 2; Reader and Laland 2002). These relationships remain significant when the confounding effect of executive brain volume is taken into account: executive brain ratio correlates with both social learning and tool use rates (Reader and Laland, 2002). While tool use and innovation can be identified by observational studies, observational reports of social learning must be treated with caution (Galef, 1992; Reader and Laland, 2002). Innovation rate also correlates with performance in laboratory individual learning tasks, though the number of species available for analysis was small and no attempt was made to correct for phylogenetic relationships (Riddell and Corl, 1977; Reader and MacDonald, 2003). Notwithstanding these caveats, we have evidence for different cognitive measures evolving together, which would suggest that they are all part of one general intelligence or that these 'intelligences' are modular but the modules have evolved together (Fodor, 1983; Tooby and Cosmides, 1989; Byrne and Whiten, 1997).

4.15.2.3 Social and Ecological Correlates of Innovation

Many socioecological factors may favor an innovative brain, and the factors favoring brain enlargement have received considerable attention (see The Evolution of Encephalization). Social intelligence hypotheses, for example, focus on dealing with the rapidly changing social world, with more advanced cognitive abilities evolving to cope with the demands of social living, to gain information from others, and create unpredictability in personal behavior – unpredictability that may be key in outwitting other individuals (Byrne and Whiten, 1988). Ecological intelligence hypotheses instead emphasize the role of variables such as foraging ecology in determining brain evolution (Milton, 1988).

The selection pressures favoring innovation specifically have not received the same attention as those favoring brain enlargement. Innovation may be beneficial in both social and ecological domains. For example, innovations may be particularly effective methods to deceive or manipulate others (Kummer and Goodall, 1985; Byrne, 2003), and shifts to novel foods or foraging techniques may be vital to animals in impoverished circumstances (Reader and Laland, 2001). Social learning, a component of social intelligence, may be favored in ecologically innovative species if the acquisition of innovations by social means provides a cheaper, more reliable, or less risky alternative to innovating for oneself. The correlation between social learning and innovation rates described above would support this idea. However, such information scrounging by group members will often reduce the benefits of innovation to the innovator. Moreover, although innovations are reported in both social and

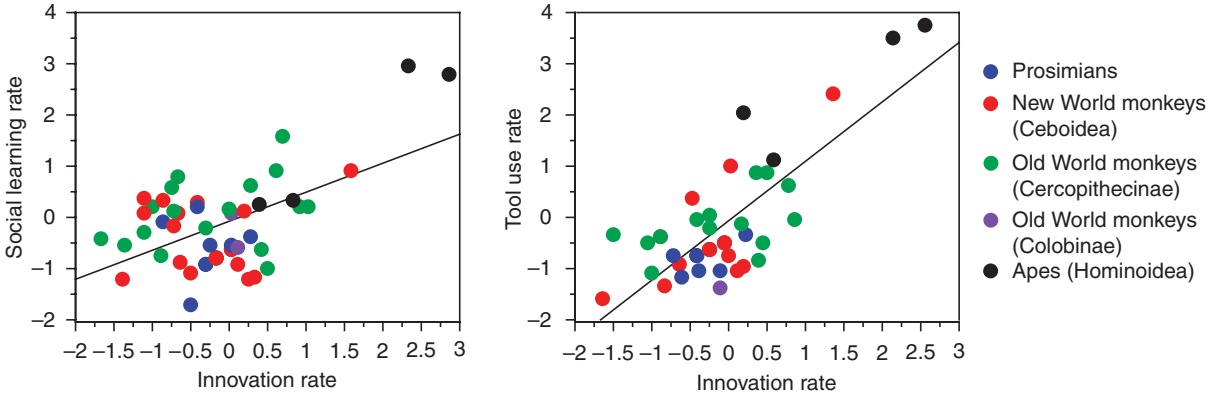

Figure 2 Innovation rate correlates with reported frequencies of social learning ($r^2_{adj} = 0.29$, $p < 0.0001$) and tool use ($r^2_{adj} = 0.60$, $p < 0.0001$). All frequencies are corrected for research effort (see legend, Figure 1). Reanalysis of the data from Reader and Laland (2002), excluding species where social learning, tool use, or innovation were not reported. Relationships remain significant when executive brain ratio is included as a covariate. For exposition the figures show across-species data, with colored symbols indicating taxa. Independent contrast analyses gave similar results (Reader and Laland, 2002).

ecological contexts, very few have been observed spreading through primate groups (Kummer and Goodall, 1985; Reader and Laland, 2001; Laland and Hoppitt, 2003). Thus, the hypothesis that social intelligence and innovation have had feedback effects on each other's evolution remains unproven.

Clues to the circumstances promoting the evolution of innovation might come from observational and experimental studies of when and where animals innovate. If innovation consistently occurs in harsh or marginal habitats (e.g., the edge of the species range; K. MacDonald, personal communication), in changed or poor conditions (e.g., cold periods), or in individuals of poor competitive ability (Reader and Laland, 2001) this might suggest that innovation has an adaptive advantage in conditions of range expansion, climatic variability, or social competition, respectively. In birds, innovative species are known to be more likely to become established when introduced to new habitats than less innovative species (Sol *et al*., 2005). At present, primate analyses do not support a link between climatic variability and innovation rate, nor a correlation between the geographical range of a species and innovation rate (Reader and MacDonald, 2003). Correlations between these variables might have been expected if innovation is prompted by variation in current conditions, or if variable conditions had selected for a propensity to innovate. Social competition clearly has an effect on innovation, and much innovation appears to be performed by peripheral, low-ranking individuals, perhaps limiting the social learning of innovations by the rest of the group (Reader and Laland, 2001).

Social intelligence hypotheses link living in larger or more complex groups with increased cognitive demands (Deaner *et al*., 2000). One line of evidence for social intelligence ideas comes from correlations between primate group size and relative brain volume (Deaner *et al*., 2000). As brain volume is assumed to be an indirect cognitive measure, this begs the question of which cognitive capacities are favored by group life – innovation, deception, and social learning are likely candidates. Such cognitive capacities would be expected to correlate with both group size (group living being the selection pressure favoring their evolution) and relative brain volume. However, in contrast to the above expectations, three behavioral correlates of brain volume do not correlate with social group size: primate social group size does not predict innovation, social learning, or deception rates (Reader and Lefebvre, 2001; Byrne and Corp, 2004; S. M. Reader, unpublished data). If these findings are robust, they raise the question of whether other, as yet unrecognized, cognitive or noncognitive correlates of brain size were favored by group living.

4.15.3 Discussion: Cognitive Convergence – Are Primates Special?

Confidence in the generality of the relationships between innovation and other variables described in primates will be strengthened if they are also found in other animal taxa. Lefebvre *et al*. (1997; see The Evolution of Encephalization) pioneered the study of the co-evolution of brain and innovation in birds, and we can compare findings in birds and primates to examine the evidence for convergent cognitive evolution in two groups that diverged more than 250 Mya (Lefebvre *et al*., 2004; Jarvis and The Avian Brain Nomenclature Consortium, 2005). In both birds and primates innovation rate correlates with the volumes of higher order and multimodal integration brain areas: the mesopallium and nidopallium in birds and the neocortex in primates. Far from being reptilian in size, birds typically have relative brain sizes closer to those of mammals, and show similar cognitive sophistication (Jarvis and The Avian Brain Nomenclature Consortium, 2005). Moreover, in both primates and birds innovation rate correlates with tool use frequency and individual learning measures (Lefebvre *et al*., 2004). The fact that these neural and cognitive associations are observed in two independent groups, and at different taxonomic levels (species were compared in primates, and parvorders in birds), suggests a striking pattern of convergent evolution.

A common assumption to virtually all cognitive explanations for brain enlargement is that large brains afford greater behavioral flexibility. Comparative analyses of innovation allow this assumption to be tested, and have raised the possibility that behavioral flexibility played a pivotal role in the evolution of enlarged brains. What of the future? Understanding the psychological, neural, and genetic processes underlying innovation will be key to improving understanding of the evolution of innovation and behavioral flexibility. It is still not clear what processes underlie innovation in humans and other animals, or whether the same processes are involved in both. For example, do animals actively seek novel solutions to problems, or do they profit from accidents? If the former, what are the determinants of creativity? How do experience, development, and the socioecological environment influence innovativeness? Is innovativeness heritable, and, if so, what are its heritable components? These are all questions amenable to experimental study,

and will be instrumental in understanding how and why brain enlargement and behavioral flexibility are linked.

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4.16 Cerebral Cortical Folding Patterns in Primates: Why They Vary and What They Signify

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Glossary

FEF	Frontal eye fields.
LIP	Lateral intraparietal area.
MRI	Magnetic resonance imaging.
MST	Medial superior temporal area.
MT	Middle temporal area.
PALS	Atlas population average, landmark and surface-based atlas.
V1	Visual area V1.

4.16.1 Introduction

As the dominant component of the primate brain, the cerebral cortex is remarkably variable in its pattern of convolutions. Lissencephalic primates, such as the owl monkey and galago, have small brains and only a few shallow cortical sulci. In larger-brained primates, such as the highly gyrencephalic human, most of the cortex is buried in a complex, irregular array of sulci whose pattern is species-specific but also varies markedly from one individual to the next within a given species.

These observations give rise to many questions. Why does the degree of folding vary so much across species, and why does it increase systematically with brain size? What accounts for the variability in the pattern within and across species? What is the relationship between the arrangement of cortical areas and the pattern of folds in any given individual or species?

This article provides a developmental and comparative framework for addressing these questions. Many aspects of cortical folding can be understood in terms of a basic set of developmental processes that include the differentiation into distinct cortical areas, the formation of long-distance connections, and the generation of folds by mechanical tension along these axons. Differences among species may largely reflect the characteristic arrangement of cortical areas and their connections.

4.16.2 Why Is the Cortex Folded?

Cerebral cortex is a thin sheet that surrounds a core of subcortical structures, including various nuclei (e.g., thalamus, basal ganglia), the ventricles, and a surrounding shell of subcortical white matter. The factors regulating the total volume and surface area of the cortex differ in many ways from those regulating the aggregate volume of the subcortical core. Nonetheless, cortical and subcortical development are coupled, insofar as the cortical sheet is effectively shrink-wrapped around the subcortical core. With these points in mind, the correlation between cortical folding and overall brain size can be attributed to several systematic relationships revealed by allometric studies.

1. *Disproportionate cortical expansion.* As overall brain size increases, cerebral cortical gray matter expands disproportionately compared to subcortical nuclei (Jerison, 1982; Hofman, 1989). This differential growth is attributable to a disproportionately extended period of cortical neurogenesis in larger-brained mammals (Finlay and Darlington, 1995; see The Evolution of Sensory and Motor Systems in Primates).
2. *Staying thin.* As cortical volume (V_{cort}) increases, total cortical surface area (A_{cort}) increases much more rapidly than average cortical thickness ($T_{cort} = V_{cort}/A_{cort}$). For example, in comparing human and macaque cerebral cortex, there is a 10-fold difference in surface area but only a

1.5-fold difference in average cortical thickness (Hofman, 1989; see Captured in the Net of Space and Time: Understanding Cortical Field Evolution).

3. *Limited wiring*. White matter underlying cerebral cortex includes axons of long-distance corticocortical connections plus axons associated with corticosubcortical pathways. This subcortical white matter is a very small fraction of total subcortical volume in lissencephalic animals. It increases with total brain size (V_{brain}) much more rapidly than does the volume of cortical gray matter ($V_{WM} \propto V_{brain}^{1.37}$ vs. $V_{cort} \propto V_{brain}^{1.05}$), yet remains less than cortical gray matter volume even in the largest-brained species (Frahm *et al.*, 1982; Hofman, 1989).

To appreciate the implications of these relationships, note that the surface area $A_{subcort}$ bounding the subcortical domains would be minimal if the aggregate volume (subcortical white matter, gray matter, and ventricles) were configured as a perfect sphere, in which case $A_{subcort}^{min} = 4.84\ V_{subcort}^{2/3}$. If cortical surface area is less than this minimum ($A_{cort} < A_{subcort}^{min}$), then a completely smooth cortex would only partially enclose the subcortical components, and the resultant brain would perforce be lissencephalic (Figure 1a). On the other hand, if cortical surface area substantially exceeds the subcortical minimum ($A_{cort} \gg A_{subcort}^{min}$), then the subcortical core must take on an irregular shape, including peaks and valleys that mesh with a folded cortical sheet and thereby achieve an appropriate shrink-wrapping of the cortex (Figure 1b). By this argument, the approximate degree of cortical folding is predictable largely on the basis of the number of neurons, average neuronal size, and average connectivity patterns of major cortical and subcortical domains. This conclusion does not rely on any particular mechanistic hypothesis about the mechanical forces that cause a specific pattern of folds to emerge.

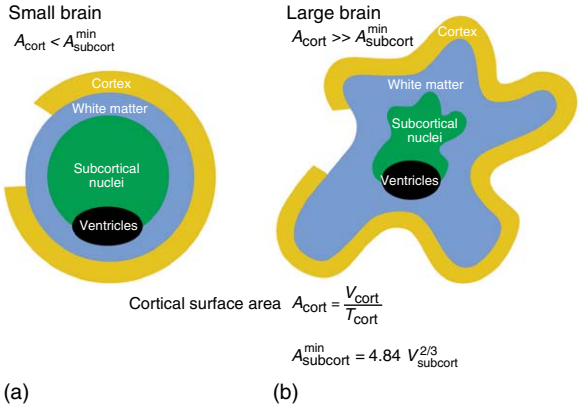

Figure 1 Schematic drawing of cortical and subcortical components in small vs. large brains. a, In lissencephalic brains, subcortical nuclei constitute a large fraction of total brain volume, and the cortical sheet lacks sufficient surface area to completely envelop the subcortical domains. b, In gyrencephalic brains, the increase in cortical surface area greatly outstrips the increase in subcortical nuclei and ventricular domains. Even though subcortical white matter also increases greatly, the surface area of subcortical regions matches cortical surface area only if the subcortical domain is highly nonspherical.

4.16.3 A Tension-Based Hypothesis of Cortical Folding

Five observations set the stage for a general hypothesis for how and where cortical folding occurs. The first four relate to morphogenetic events that occur during embryonic cortical development.

1. Cerebral cortex initially forms as a completely smooth sheet, as a result of neuronal proliferation along the ventricular surface followed by outward radial migration (Rakic, 2003). Each hemisphere is like an inflated balloon, in which elevated intraventricular pressure generated by the production of cerebrospinal fluid keeps the hemisphere inflated (Desmond and Jacobson, 1977; Miller *et al.*, 1987).
2. The cortical sheet becomes regionally specialized into a species-specific mosaic of many distinct cortical areas. This results from a complex set of molecular signaling processes that remain poorly understood (Grove and Fukuchi-Shimogori, 2003).
3. A high percentage of cortical neurons send axons into what becomes the white matter and begin to establish precise long-distance connections that are appropriate for their areal identity (Schwartz *et al.*, 1991; Coogan and Van Essen, 1996).
4. Cortical folding begins around the time that initial long-distance pathways are being established and continues over an extended period of late prenatal and early postnatal maturation, during which time cortical surface area increases rapidly.
5. Neurons grown in tissue culture extend neurites that generate substantial mechanical tension (Dennerll *et al.*, 1988; Lamoureux *et al.*, 1992), suggesting a biologically plausible mechanical force for inducing cortical folds.

   The fifth observation discussed above is of a different type and relates to mechanical forces exerted by axons and dendrites.

If, as seems likely, the axons of cortical neurons generate tension *in vivo* akin to that generated by neurites *in vitro*, then the aggregate forces associated

with long-distance corticocortical pathways would tend to bring strongly interconnected regions closer together, thereby forming a gyral fold in between. In contrast, weakly interconnected regions would lack sufficient strength to force an intervening gyral fold and in many cases would tolerate an intervening sulcal fold that actually increased their separation. This tension-based hypothesis of cortical folding is part of a more general theory of morphogenesis throughout all parts of the nervous system (Van Essen, 1997). A related aspect of this theory is that radially oriented tension along the apical dendrites of pyramidal cells keeps the cortex thin as it grows in volume. This radial anisotropy would make the developing cortex mechanically stiffer along the radial axis and would therefore lead to preferential expansion along the path of least resistance (in the tangential plane). Finally, tension associated with the pathways linking cortex with subcortical structures may keep cortex tightly shrink-wrapped around subcortical structures while having less influence on the specific pattern of folds.

Tension-based cortical folding provides a plausible basis for understanding species differences in folding patterns, as well as individual differences within a species. Alternative models of cortical folding have been proposed (Richman *et al.*, 1975; Smart and McSherry, 1986; Welker, 1990), such as the hypothesis that differential growth of superficial versus deep layers causes buckling of the cortex, but they do not easily account for the specificity of folding patterns. The remainder of this article will focus on evolutionary comparisons that are predicated on tension-based cortical folding.

4.16.4 Folding Patterns in Primates

4.16.4.1 Surface Representations

The diversity of cortical folding patterns in primates is illustrated in Figure 2, using surface reconstructions of the right hemisphere in a monkey (rhesus macaque), great ape (chimpanzee), and human (see also Sereno and Tootell, 2005). The chimpanzee MRI data came from the study of Rilling and Insel (1999), accessed via the fMRI Data Center (see 'Relevant Websites'). Each surface was generated from a structural MRI (top row, coronal slices) using the SureFit segmentation algorithm (Van Essen *et al.*, 2001); surface contours (blue) resulting from the segmentation are overlaid on the MRI slices. Lateral and medial views of the fiducial (3D) surfaces (middle and lower rows of Figure 2) reveal, as expected, progressively greater complexity in macaque, chimpanzee, and human cortex. The pastel colors indicate cortex assigned to different lobes; dark shading indicates sulcal (buried) cortex, determined using an automated method that is part of the SureFit segmentation process.

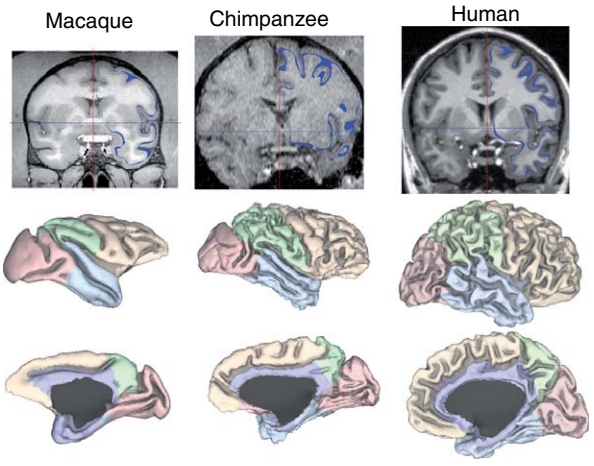

Figure 2 Structural MRI and surface reconstructions of three primate species. The quality of the chimpanzee structural MRI is considerably lower than that for the other species, but a high-quality fiducial surface reconstruction was nonetheless obtained with the aid of extensive manual editing. Data for this and following figures are available at the SumsDB database (see 'Relevant Websites'). The exact views seen in individual panels can be recreated using the scene selection option in WebCaret (no download required) or in Caret (data download required).

The fiducial surface of the macaque has a surface area of 108 cm^2. Chimpanzee cortex is about threefold greater in extent (317 cm^2), and human cortex is another threefold greater (960 cm^2; Van Essen, 2005a; see also Jouandet *et al.*, 1989; Tramo *et al.*, 1995; Van Essen and Drury, 1997). The percentage of buried cortex increases much more modestly (46% in the macaque, 54% in the chimpanzee, and 60% in humans). Regarding different lobes, the frontal lobe can be delineated most precisely and is also of intrinsically high interest. The frontal lobe occupies 26% of macaque cortex but is substantially more dominant in both the chimpanzee (34%) and human (36%), consistent with previous volumetric analyses (Semendeferi and Damasio, 2000).

To compare the arrangement of identified sulci in different species, it is advantageous to view inflated surfaces (with shallow sulci smoothed out) along with flat maps (Figure 3). The inflated surfaces are displayed in oblique dorsolateral and ventromedial views to better visualize the sulcal patterns. Each surface shows a map of sulcal depth, in which darker shades represent more deeply buried regions (i.e., more distant from gyral cortex that forms the cerebral hull). The far right column of Figure 3 shows a population average of 12 individual inflated human right hemispheres that were registered to the PALS surface-based atlas (Van Essen, 2005b). The average sulcal

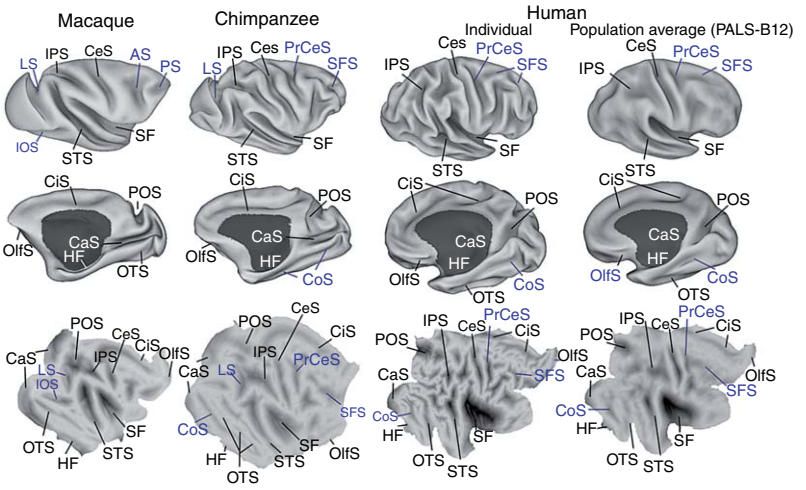

Figure 3 Sulcal depth maps for three primate species on inflated surfaces and flat maps. Inflated maps are viewed from a dorsolateral perspective in the top row and a ventromedial perspective in the middle row. Sulci common to all three species are labeled in black; sulci found in only one or two species are labeled in blue. Note that the posterior half of the occipitotemporal sulcus in the chimpanzee is located more medially than the human OTS and instead has a trajectory more like that of the collateral sulcus. Other examples of interspecies differences in geographically corresponding sulci are noted in the main text. PrCeS, precentral sulcus; SFS, superior frontal sulcus; CoS, collateral sulcus.

depth displayed on the PALS atlas is blurry in regions of high variability and has higher contrast in regions of low variability. Individual variability in the pattern of convolutions is well known to be much less in the macaque. In the chimpanzee, the degree of variability appears to be intermediate, based on visual inspection of published brain photographs and drawings (Holloway *et al.*, 2003; Gannon *et al.*, 2005).

4.16.4.2 Geographic Correspondences

Sulci that have a common location, orientation, and general shape in different species can be considered geographically corresponding. It is common practice in such cases to assign the same name, even though geographic correspondence does not imply that the sulci contain the same set of functional areas. Indeed, examples of functional noncorrespondence are discussed in Section 4.16.4.3.

Ten major sulci are present in all three species, based on geographic correspondence (Figure 3). Four of these are visible from the dorsomedial views in the top row of Figure 3: the central (CeS), superior temporal (STS), and intraparietal (IPS) sulci plus the Sylvian fissure (SF). Of these, the human IPS is the most variable across individuals (thus appearing blurred in the average sulcal depth map) and on average differs markedly from the chimpanzee and macaque. Six common sulci are visible from ventromedial views (middle row of Figure 3): the cingulate (CiS), parieto-occipital (POS), calcarine (CaS), occipitotemporal (OTS), and olfactory (OlfS) sulci, plus the hippocampal fissure (HF). Of the remaining four major sulci in the macaque, one (the lunate sulcus, LS) is prominent in both the macaque and chimpanzee but is typically absent in humans (and even when present is not prominent). The remaining three are the inferior occipital (IOS), arcuate (AS), and principal (PS) sulci, which cannot be unambiguously identified in either chimpanzee or human cortex (see below). The collateral sulcus is prominent in the chimpanzee and human, but is absent in the macaque.

Table 1 Size of individual sulci as percent of total cortex

Sulcus	*Macaque*	*Chimpanzee*	*Human*
Superior temporal	7.9%	5.9%	4.0%
Sylvian fissure	6.8	6.5	9.2
Calcarine	6.7	3.2	1.8
Intraparietal	4.0	3.3	3.8
Cingulate	2.5	3.0	3.4
Central	2.4	4.1	3.1
Parieto-occipital	2.1	2.7	2.1
Hippocampal fissure	1.9	0.5	0.4
Occipitotemporal	1.6	0.5	1.7
Olfactory sulcus	0.07	0.1	0.5
Total surface area	108 cm^2	317 cm^2	960 cm^2

Table 1 shows the size of each sulcus as a percentage of total cortical surface area and reveals marked interspecies variability in the relative sizes of sulci. Most notably, the STS is the largest sulcus in the macaque (8% of fiducial surface area), slightly larger than the SF (7%), and several times larger than the central sulcus (2.4%). In contrast, the STS in humans (4%) is less than half the size of the SF (9%) and is only about one-third larger than

the central sulcus (3%). Results for the chimpanzee are intermediate, as the STS (6%) is slightly smaller than the SF (6.5%) and half again larger than the central sulcus (4%). The STS extends more dorsally relative to the SF in the macaque than it does in humans, consistent with its greater relative size. The calcarine sulcus is also notably variable. It is the third largest sulcus in the macaque (7%, slightly smaller than the SF), whereas it is the sixth largest (3%) in the chimpanzee and the tenth largest in humans (2%). The calcarine sulcus also varies in shape, having a characteristic T-shaped posterior bifurcation in the macaque and chimpanzee that is lacking in humans.

4.16.4.3 Relation between Folds and Cortical Areas

A central issue in understanding the significance of cortical folding is the degree to which geographically corresponding sulci (sharing a common name) also correspond functionally (i.e., contain a similar collection of cortical areas). Reasonably good functional correspondences are demonstrable for a few sulci, such as the central sulcus (which contains somatosensory area 3 and motor area 4) and the calcarine sulcus (which contains area V1). The situation is more complex for the Sylvian fissure, which contains primary auditory and gustatory areas in both humans and macaque. In view of its larger relative size, the human Sylvian fissure may contain many areas that are absent in the macaque. For some sulci, such as the STS discussed below, the mismatch between geographic and functional correspondence may be even more severe.

Before discussing additional specific examples, it is useful to note that in general our understanding of the identity and arrangement of distinct cortical areas remains fragmentary in all primate species, even the intensively studied macaque monkey. The total number of distinct cortical areas in the macaque may be around 100 (Lewis and Van Essen, 2000; Van Essen, 2004). However, a reasonable consensus regarding areal identity and location exists for only a minority of these candidate areas; for most of parietal, temporal, and prefrontal cortex, several competing partitioning schemes remain in widespread use. In the chimpanzee, there are very few studies of cortical partitioning schemes for using modern architectonic approaches to reassess the classic partitioning scheme of Bonin and Bailey (1950). In humans, the total number of cortical areas may be substantially larger than in the macaque (perhaps as many as 200), but for most regions of cortex, there are major uncertainties regarding the basic layout of areas (Van Essen, 2004, 2005a). Human visual cortex shows striking similarities to the macaque in the topographic organization of early areas (especially V1–V3 and middle temporal (MT)) but pronounced species differences are evident in several nearby visual regions (Grill-Spector and Malach, 2004; Van Essen, 2004, 2005a). In general, speculations about candidate homologies between macaque and human are fraught with uncertainty and controversy for most regions of cortex.

Despite these caveats, interesting comparative insights can nonetheless be gleaned by focusing on a few select regions and areas of occipitotemporal, parietal, and frontal cortex. In each of these regions, current evidence points to major differences between macaque and human cortex in terms of functional organization and relationship of function areas to folding patterns.

If cortical connectivity patterns are the primary determinants of cortical folding patterns, is it possible to work backward and infer the arrangement of cortical areas and their connections by precisely analyzing the exact pattern of cortical folds? Unfortunately, finding the inverse solution is not feasible, as many possible connection patterns could give rise to virtually identical folding patterns. Nevertheless, analysis of folding patterns does provide some constraints on what are the most likely general connectivity patterns. In this regard, it is useful to include the chimpanzee in a discussion of relationships between areas and folding patterns despite the paucity of direct evidence about its functional organization.

4.16.4.3.1 Gyral folds along the V1/V2 border V1 is the largest cortical area in the macaque, occupying 10 cm^2 (10% of neocortex). In the chimpanzee, V1 is more than twice as large as in the macaque (25 cm^2) and thus nearly as large in percentage size (8%; see Figure 4 legend for details). Human V1 is about the same absolute size as in the chimpanzee (24.0 ± 4.9 cm^2; range 14.4–33.6 cm^2, Andrews *et al.*, 1997; 21 cm^2, Stensaas *et al.*, 1974) and thus is much smaller in percentage size (average 2.5%). In macaque and human cortex, neighboring area V2 is nearly as large as V1 and has a mirror-symmetric topographic organization (Van Essen *et al.*, 1986; Dougherty *et al.*, 2003), so it is likely that these characteristics also apply to chimpanzee V2. In the macaque and other primates where connectivity has been studied, V1 and V2 are powerfully interconnected (Felleman and Van Essen, 1991); it seems likely that this generalizes to humans and great apes.

The perimeter of area V1 runs along a largely continuous gyral ridge in all three species (Figure 4). In the macaque (Figures 4a and 4d), this gyral ridge adjoins five major sulci (the calcarine, parieto-occipital, lunate, inferior occipital, and occipitotemporal sulci). The pattern is similar in the chimpanzee, except for the absence of an inferior occipital sulcus (Figures 4b and 4e; see also Holloway *et al.*, 2003). In humans, V1 is largely contained within the calcarine sulcus (Figure 4c) and the surface area of V1 is correlated with the size of the calcarine sulcus in different individuals (Andrews *et al.*, 1997). The absence of a prominent lunate or inferior occipital sulcus (Figure 4f) is thus correlated with the more restricted relative extent of human V1.

In all three species, folding of occipital cortex in general brings topographically corresponding loci in V1 and V2 into close proximity. Hence, it is indicative of compact wiring in this region, and it is consistent with tension-based cortical folding. The interspecies differences in folding in this region may reflect differences in connectivity and/or organization of nearby extrastriate areas.

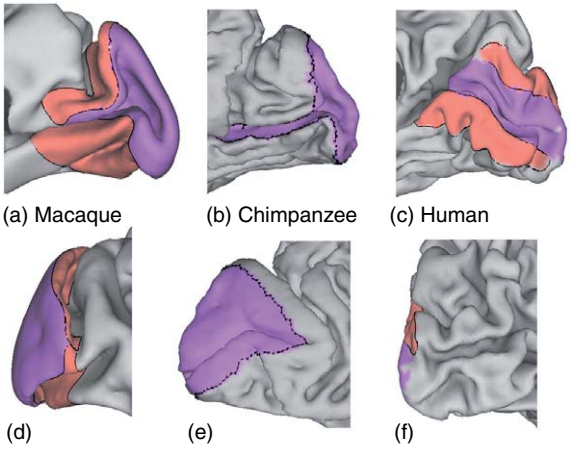

Figure 4 Relationships of areas V1, V2, and MT to folding patterns. For the chimpanzee, V1 was drawn on the surface map based on selected histological sections illustrated by Holloway *et al.* (2003).

4.16.4.3.2 Area MT and a functional mismatch in the STS MT is a small visual area that has been identified in many primates by its distinctive myeloarchitecture and/or functional specialization for motion processing (Born and Bradley, 2005). In the macaque, MT is located on the posterior bank of the STS (red in Figures 5a and 5d), whereas in humans MT+ (MT plus part of the medial superior temporal (MST) complex) is situated several centimeters posterior to the STS (Figures 5c and 5f). The more posterior location of human MT may in part be attributed to the more medial disposition of V1 and the smaller relative size of the STS. However, it is also striking that the region between MT and primary auditory cortex (on the posterior bank of the Sylvian fissure, light blue in Figure 5) is greatly expanded in humans compared to the macaque (Van Essen, 2005a). This region, including the human STS, includes cortex involved in multisensory processing (Beauchamp, 2005) and in specialized processing for tool-related functions (Lewis, 2006). In the chimpanzee, cortical folding in lateral occipitotemporal cortex (Figures 5b and 5e) is similar to that in the macaque, with the STS lying immediately anterior to the lunate sulcus. Hence, it is plausible to suggest that chimpanzee MT may lie on the posterior bank of the STS (i.e., more like the macaque than the human location).

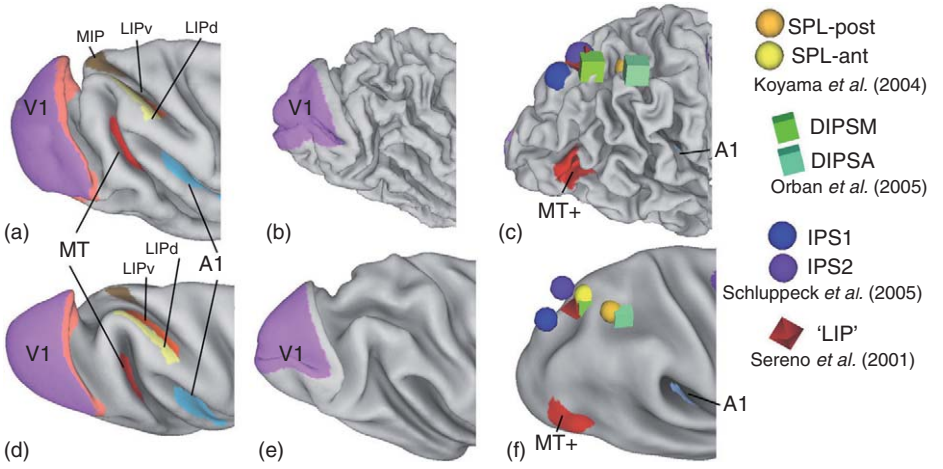

Figure 5 Relationship of selected occipitotemporal and parietal areas to folding patterns. SPL, superior parietal lobe; DIPSM, dorsal intraparietal sulcus area medial; DIPSA, dorsal intraparietal sulcus area anterior; LIP, lateral intraparietal area.

4.16.4.3.3 Parietal cortex and a functional mismatch in the IPS In the macaque, parietal areas associated with vision are located in the inferior parietal lobule, lateral and posterior to the fundus of the IPS. The most intensively studied parietal areas, LIPd and LIPv (Figures 5a and 5d, often considered together as LIP) can be activated during saccade and attention-shift tasks as well as by passive visual stimulation in both single-unit and fMRI studies. In human fMRI studies, a variety of vision-related tasks activate parietal cortex, but mainly the superior parietal lobule, medial to the fundus of the IPS. Figure 5c shows the stereotaxic coordinates for the centers of some of these regions, including attention- and delayed-saccade-related areas IPS1 and IPS2 (Schluppeck *et al.*, 2005; Silver *et al.*, 2005); saccade-related SPL-post and SPL-ant regions (Koyama *et al.*, 2004); attention-related area LIP (Sereno *et al.*, 2001); and motion-related regions DIPSM and DIPSA (Orban *et al.*, 2005). Most of these studies have suggested a potential homology between macaque areas LIPd/v and one or more of the vision-related regions identified in Figure 5. Given the complexity and diversity of these data, it is premature to argue strongly for a homology at the level of individual areas. Nonetheless, it seems plausible that the clusters of human and macaque parietal visual areas have a common evolutionary origin and are thus homologous at a regional level (Orban *et al.*, 2004). If so, an important implication is that the fundus of the IPS in the macaque (the boundary between visual and somatosensory domains) corresponds not to the human IPS fundus but rather to a more medial strip within the superior parietal lobule. This in turn would signify that cortex of the inferior parietal lobule has expanded disproportionately in the human lineage compared to the macaque. Morphologically, the chimpanzee parietal lobe is intermediate but arguably closer to the macaque pattern than to the human pattern (e.g., the postcentral sulcus is only modest in extent). Hence a plausible speculation is that the relative expansion of the human inferior parietal lobule occurred mainly after the split from the chimpanzee lineage.

4.16.4.3.4 Differential expansion of prefrontal cortex In the macaque, dorsal frontal cortex contains only two sulci (the arcuate and principal sulci) that run approximately orthogonally to one another (Figures 6a and 6d). The chimpanzee and human both have a much more complex folding pattern (commensurate with their larger relative size) that includes precentral and superior frontal sulci as well as major folds in dorsolateral prefrontal cortex.

The frontal eye fields (FEF) represent a key functional landmark for the frontal lobes, analogous in this respect to area MT in occipitotemporal cortex. In the macaque, FEF is centered in area 8 on the anterior bank of the arcuate sulcus (Bruce *et al.*, 1985); neuroimaging studies suggest that there may be two saccade-related foci plus a partially overlapping pursuit-related region (Baker *et al.*, 2006), but also that FEF may extend posteriorly into area 6 (Koyama *et al.*, 2004). In humans, several studies have identified FEF activations centered mainly in area 6 in and near the anterior bank of the precentral sulcus (Corbetta *et al.*, 1998; Koyama *et al.*, 2004; Hagler and Sereno, 2006; Figures 6c and 6f). Because the human precentral sulcus is much farther posterior in the frontal lobe than is the arcuate sulcus in the macaque, it suggests a disproportionate expansion of prefrontal cortex in

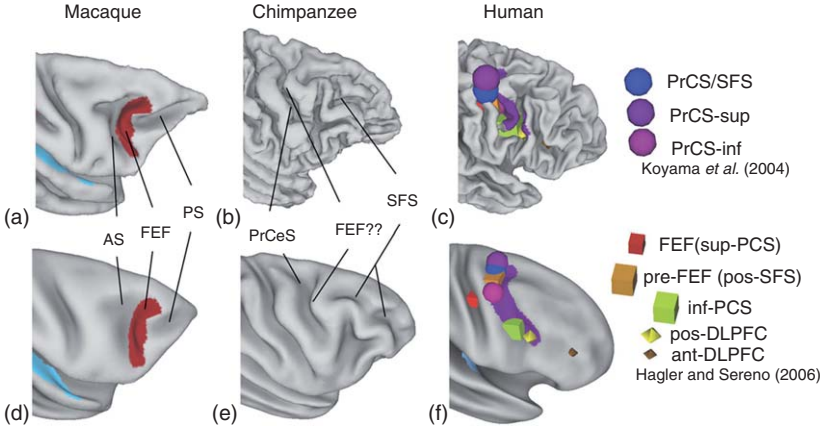

Figure 6 Relationship of selected frontal areas to folding patterns. PCS, PrCeS, and PrCS, precentral sulcus; DLPFC, dorsolateral prefrontal cortex.

humans (Van Essen *et al.*, 2005). Recently, Hagler and Sereno (2006) reported additional maps of visual working memory in more anterior regions (DLPFC in Figures 6c and 6f).

For the chimpanzee, one interesting hypothesis is that the expansion of the frontal lobe relative to the macaque occurred primarily in dorsolateral prefrontal cortex. By this scenario, the chimpanzee precentral and superior frontal sulci may be equivalent to the macaque arcuate and principal sulci, respectively, and the chimpanzee FEF may be located on the anterior bank of the precentral sulcus (identified as FEF?? in Figures 6b and 6e). However, this is only one of many plausible hypotheses regarding the evolution of frontal cortex in great apes.

4.16.5 Concluding Remarks

The central thesis of this article is that cortical folding may be a natural consequence of a tension-based mechanism for achieving compact wiring of cerebral circuits and that species differences in folding patterns can be related to the arrangement of cortical areas and their connections. While the discussion has focused on three representative primates, the hypothesis is generally applicable to all gyrencephalic primates. For example, cortical folding in the New World cebus monkey is remarkably similar to patterns in the Old World macaque even though their common ancestor was presumably lissencephalic (Rosa *et al.*, 2000). This type of evolutionary convergence could occur if brain size increased in both monkey lineages without dramatic changes in the mosaic of cortical areas. In other mammalian orders, there is great diversity in the general layout of cortical folds, suggestive of large differences in areal organization and connectivity.

Within a given species, it is well documented that the absolute size of a given cortical area can vary by twofold or more in the normal population (Andrews *et al.*, 1997). From the perspective of tension-based cortical folding, variability of this magnitude in the size of areas as well as connectivity patterns can account for variability in the pattern of folds, especially in balkanized regions where folding reflects competitive interactions among many small areas. In addition, specific neurological or psychiatric disease conditions such as Williams syndrome are associated with specific folding abnormalities that may reflect developmental effects on the size and/or connectivity of specific cortical areas (Van Essen *et al.*, 2006). In general, the mosaic of cortical areas and connectional patterns specified genetically and by early developmental interactions may be the primary determinants leading to a unique pattern of folds in any given individual.

The analyses illustrated in this article include qualitative evaluations based on simple visual inspection plus several quantitative measurements carried out on cortical surface models. Another useful strategy for interspecies comparisons involves surface-based registration between species, in which candidate areal homologies provide landmarks to constrain the registration. This allows the implications of any set of proposed homologies to be examined objectively, by assessing the alignment of geographic and/or functional data in intervening regions. Surface-based registration has been successfully carried out between macaque and human cortex using several combinations of candidate landmarks (Van Essen *et al.*, 1998, 2004; Astafiev *et al.*, 2003; Denys *et al.*, 2004; Orban *et al.*, 2005). The availability of chimpanzee surface models will allow the chimpanzee to be registered to both the macaque and the human atlas surfaces. Such comparisons will become of even greater interest if it eventually becomes feasible to carry out *in vivo* functional neuroimaging and/or postmortem architectonics on chimpanzees or other great apes.

As a final note, readers interested in viewing the data used in this study or in carrying out additional analyses can take advantage of the fact that all of the data sets are freely available in the SumsDB database (see 'Relevant Websites'). The data can be viewed online using the WebCaret visualization tools, without the need to download data or software. Alternatively, the data sets can be downloaded for visualization and analysis using Caret software.

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4.17 Cortical Commissural Connections in Primates

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4.17.1 Introduction

Following its appearance some half a billion years ago, bilaterality has been established as the organizational plan for the vast majority of animal life. This inevitably includes the brain, and the requirement for intercommunication of the right and left halves to achieve a unity of action (Doty, 2003). There is thus a deep evolutionary history of interhemispheric communication, and the forebrain commissures in primates reflect this stability. In their first-order anatomy, development, and characteristic sites of termination, they are typically mammalian. Aside from the variation with overall size of the brain, the primary organization of the primate commissures offers little to suggest evolutionary modulation. However, at the detailed level of the origin and termination of their intracortical components, there is substantial variance across primate species. These differences are also compounded, at least in macaques and humans, by a wide interindividual variance, to the degree that the pattern of callosal versus acallosal zones in the cortex of macaques has been characterized as a 'callosal fingerprint' (Van Essen *et al.*, 1982; see Figure 1). Here, evolution may be at work, but it will be an arduous task to delineate the species from the individual variation. Such analysis has only recently begun, so that designating species characteristics at the microlevel awaits much more extensive analysis than is presently available.

A review by Cumming (1970) traces the history of work and thought concerning the corpus callosum (CC) through the nineteenth and early twentieth centuries, and Tomasch (1954), in his landmark paper, provides a valuable appraisal of the state of knowledge of the corpus callosum (CC) just prior to the revolution wrought by the 'invention' of the split-brain procedure (Myers and Sperry, 1953). A number of other reviews will be helpful to provide a background for the present material (Doty and Negrão, 1973; Elberger, 1982; Cusick and Kaas, 1986; Innocenti, 1986; Aboitiz and Montiel, 2003).

4.17.2 Components of the Forebrain Commissural System

The forebrain commissures of primates consist of the CC, anterior commissure (AC), hippocampal commissure, and the basal telencephalic commissure (LaMantia and Rakic, 1990b). Evolutionary distinctions of primates versus other mammals are immediately at hand in this list since: (1) unlike the case in most eutherian mammals, where the AC is primarily an interhemispheric pathway for the olfactory system, in macaques, at least, the few olfactory fibers are concentrated in the basal telencephalic commissure, that abuts the anterior dorsal portion of the AC (LaMantia and Rakic, 1990b); (2) again, contrary to the case so far known in most mammals (Van Groen and Wyss, 1988), the dorsal hippocampal commissure has very limited connections to cornu ammonis, and instead includes substantial

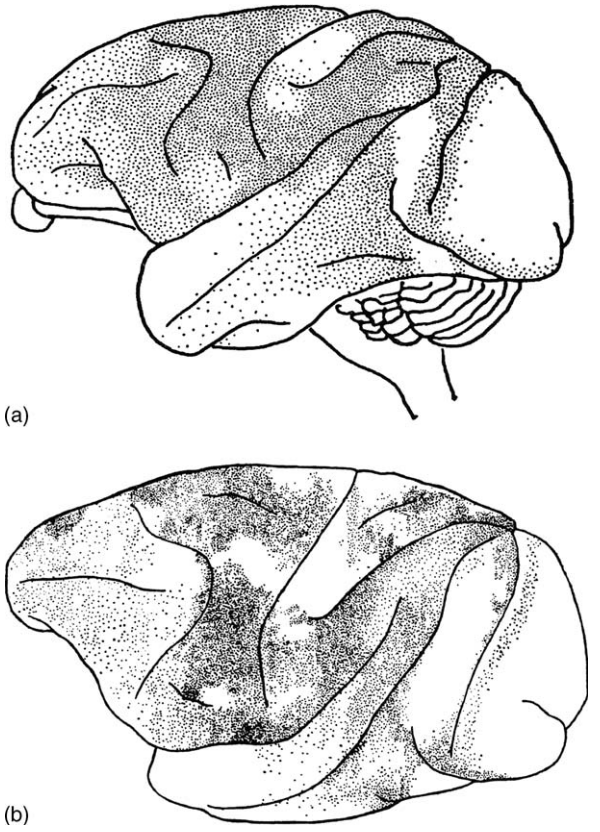

Figure 1 Two examples of the degeneration of terminals in macaque neocortex following total transection of the corpus callosum. The differences in (a) versus (b) can be ascribed not only to slight variation in procedures between the two laboratories, but to the uniqueness of the pattern between individual animals, as noted by Van Essen *et al.* (1982). The paucity of distribution to the inferior temporal lobe, however, is mostly attributable to its being the terminus for fibers of the AC. The degree and nature of the interdigitation between callosal and anterior commissural fibers remains to be determined, but there does seem to be some overlap. a, Reproduced from Myers, R. E. 1965. Organization of forebrain commissures. In: Ciba Foundation Study Group No. 20 – Functions of the Corpus Callosum (*ed.* E. G. Ettlinger), p. 142. Little Brown. b, Reproduced from Karol, E. A. and Pandya, D. N. 1971. The distribution of the corpus callosum in the rhesus monkey. *Brain* 94, 471–486, Oxford University Press.

connections from entorhinal cortex, presubiculum, and parahippocampal gyrus (Amaral *et al.*, 1984; Pandya and Seltzer, 1986; Demeter *et al.*, 1985, 1990).

Axons of the dorsal hippocampal commissure constitute only about 0.4% of the forebrain commissural total in macaques (*c.* 270000 fibers), the basal telencephalic commissure 0.3% (193000 fibers). This compares with 5.3%, 3.15 million fibers, in the AC, and 56 million in the CC (LaMantia and Rakic, 1990a, 1994).

In macaques, the hippocampal commissure lacks the ability to support interhemispheric transfer of learned visual discriminations. Two animals were prepared with CC, AC, and optic chiasm transected, sparing the hippocampal commissure (Rosene and Doty, unpublished observations). Intactness of the latter was confirmed by MRI and by retrograde axoplasmic transfer. One hemisphere was trained to distinguish a rewarded from a nonrewarded projected image, and the other hemisphere was then tested on its ability to learn the discrimination. In a series of 25 such discrimination tests, there was no evidence that the second hemisphere benefited from the learning by the first. In view of this failure to contribute significantly to interhemispheric visual transfer, and the small number of fibers composing the hippocampal and basal telencephalic commissures, they will be ignored in the remainder of this review, and the term 'forebrain commissures' will refer only to the CC and AC.

4.17.3 Evolutionary Stability of the Forebrain Commissures

Despite the differences just noted between primates and most eutheria, the CC and AC are highly similar across the mammalian order in their development and ultimate connections. Primates share with marsupials and other mammals four characteristic features:

1. In its fetal development, the human CC utilizes a series of proteins, for example, Robo/Slit, that hark back to the evolutionary origin of bilaterality and paired, symmetrical brains (Mineta *et al.*, 2003; Doty, 2003), a system that guides the commissural fibers in their 'six points of decision' as they migrate across the midline (Richards *et al.*, 2004; Ren *et al.*, 2004).
2. The forebrain commissures are unmyelinated at birth, progress gradually with myelinization as juveniles, but still leave a substantial proportion of interhemispheric fibers unmyelinated in adults.
3. Developmentally, the initial number of fibers is far greater than of those which ultimately survive ('exuberance').
4. Interconnection is avoided between one striate cortical area and the other.

4.17.3.1 Exuberance and Myelinization

In the marsupial wallaby, Ashwell *et al.* (1996) found that the total number of fibers in the AC and fasciculus aberrans peaked at about 63 million on postnatal day 148, and was down to 22 million in adults. Similarly, Berbel and Innocenti (1988)

report 67 million fibers in the cat CC at postnatal day 4, 32 million in adults. For macaques, the figures are 108 million fibers in the CC at birth, 56 million in the adult (LaMantia and Rakic, 1990a), and for the AC 11 million fibers becoming 3.3 ± 0.5 million in adults (LaMantia and Rakic, 1994). The phenomenon is dramatic in the tree shrew. Retrogradely labeled peristriate, CC neurons first become detectable at 7–9 days of age, peak in number by day 13, and 2 days later are reduced by 94% (Kretz and Rager, 1992).

This exuberant growth sends commissural axons into cortical areas and laminae from which they will ultimately be lost. Thus, the proliferation provides an inventory of fibers from which subsequent selective elimination sculpts the connectivity in the milieu of postnatal conditions (e.g., Innocenti, 1986; Shang *et al*., 1997). Indeed, there is a substantial body of studies examining how callosal connections are modulated by rearing conditions, lesions, etc. (e.g., Dehay *et al*., 1991; Meissirel *et al*., 1991; Sanchez *et al*., 1998; Reed *et al*., 2002). For the cat it has been shown that some of the neurons along the 17/18 border, that shed their CC axons during the postnatal period, survive to maintain intracortical association fibers (Innocenti *et al*., 1986).

Myelinization occurs postnatally, and has been followed electrophysiologically in cats as an increase in the complexity of the callosally evoked potential and its gradually diminishing latency (Grafstein, 1963). Myelinization, however, remains incomplete even in adults. Using electron microscopy, for the wallaby only 56–62% of AC fibers are myelinated (Ashwell *et al*., 1996). For the CC of the rat the figure is 54% (Seggie and Berry, 1972), rabbit 55% (Swadlow, 1985), cat 43–60% (Fleischhauer and Wartenberg, 1967; Koppel and Innocenti, 1983), macaque 70–94%, depending upon region of the callosum (LaMantia and Rakic, 1990b; Swadlow *et al*., 1980). For humans, with light microscopy and three brains of 50-year-old males, Tomasch (1954) estimated that the lowest proportion of myelinated fibers was in the genu, but the number, 60%, was little different from the remainder of the CC. He recognized that the histological procedure might have removed the myelin from fibers that were only lightly coated. Using electron microscopy on the brain of a 41-year-old human male, Aboitiz *et al*. (1992) found 84% myelinated in the genu, 95% elsewhere, that is, similar to the situation in macaques. On the other hand, the electron microscopic study of Olivares *et al*. (2001) on horse, cow, dog, cat, rabbit, and rat found a high degree of similarity for these species at about 70% myelinated in the splenium of young adults.

4.17.3.2 Avoidance of Striate Cortex

Striate cortex lacks interhemispheric connections in marsupials (Granger *et al*., 1985; Heath and Jones, 1971; Putnam *et al*., 1968; Sheng *et al*., 1990), as is true in most mammals. There is an exception in the case of species with laterally placed eyes, such as the tree shrew (Bosking *et al*., 2000), hedgehog (Gould and Ebner, 1978), squirrel (Gould, 1984), and rabbit (Swadlow and Weyand, 1981), where the CC makes homotopic connections with the striate representation of the ipsilateral visual field. The question has never been addressed as to what happens in these species when ocular vergence occurs, bringing different regions of visual space into correspondence. Interestingly, these CC connections ignore the orientation preference that characterizes later stages of intrastriatal processing (Bosking *et al*., 2000). Perhaps because primates have the largest binocular visual fields among mammals (Heesy, 2004), the decussation of the optic pathway is more complete, rendering this callosal input into striate cortex redundant, perhaps a true case of evolution in the primate callosal system. This singular absence of CC input to striate cortex in primates has been consistently noted, beginning with Beevor (1891) on the marmoset, van Valkenburg (1913), Clarke and Miklossy (1990) for human, and Bailey *et al*. (1941) for the chimpanzee. As shown in Figure 1, the finding is equally consistent in macaques (e.g., Curtis, 1940; McCulloch and Garol, 1941; Myers, 1962; Jacobson and Marcus, 1970; Karol and Pandya, 1971; Van Essen *et al*., 1982; Dehay *et al*., 1988; Chalupa *et al*., 1989), and squirrel monkeys (Gould *et al*., 1987). In the owl monkey, and particularly in galago (Newsome and Allman, 1980; Weyand and Swadlow, 1980; Cusick *et al*., 1984; Beck and Kaas, 1994), callosal cells and terminals are found farther into striate cortex, away from the representation of the vertical meridian, than in higher primates.

4.17.3.3 Other Acalossal Areas

A somewhat similar CC avoidance (Figure 1) is present for motor and somatosensory areas representing the hand and foot in primates, as well as other mammals (e.g., Karol and Pandya, 1971; Vogt and Pandya, 1978; Jones *et al*., 1979; Jones and Hendry, 1980; Killackey *et al*., 1983; Cusick and Kaas, 1986; Killackey and Chalupa, 1986; Rouiller *et al*., 1994; but see Iwamura, 2000). While striate cortex is the prime example of an acallosal cortical area, it and the distal extremities are by no means the only such areas. Indeed, the density of callosal cells and terminals varies widely

across the cortex, to give a patchy appearance in most mammals, including human (Clarke and Miklossy, 1990) and macaque (Figure1; Myers, 1962; Karol and Pandya, 1971; Cusick and Kaas, 1986). The variance in this pattern is so great that Van Essen *et al*. (1982), on the basis of the situation in macaques, propose that the distribution constitutes a 'callosal fingerprint' unique to each individual. The significance of these numerous acallosal regions in relation to those that receive forebrain commissural input remains essentially unexamined.

4.17.4 Protracted Course of Myelinization

As noted above, a rather large proportion of axons in the forebrain commissures remains unmyelinated. Yakovlev and Lecours (1967; Lecours, 1975), using the Weigert stain for myelin in human brains, found a continuing increase in CC myelinated fibers into the third decade. Data from MRI are consonant with this, a continuing increase in cross-sectional area of the human CC being seen from age 4 into the mid-20s (Giedd *et al*., 1996, 1999; Keshavan *et al*., 2002; Pujol *et al*., 1993; Rauch and Jenkins, 1994; Schaefer *et al*., 1990). Children were 2 years old before interhemispheric comparisons were behaviorally evident (Liégeois *et al*., 2000; see Primate Brain Evolution in Phylogenetic Context, Brain Size in Primates as a Function of Behavioral Innovation).

This exceedingly slow pace of myelinization presents something of a puzzle. What functional increment may be provided by the newly myelinated fibers, and how is this increment consolidated with existing circuitry? This problem is analogous to that of the continuing appearance of new neurons in the dentate gyrus throughout life (e.g., Kornack and Rakic, 2001), and possibly related through the generation of new oligodendrocytes. This protracted myelinization is not limited to the CC, but is seen in various cortical areas into the fifth decade (Kaes, 1907, cited by Lecours, 1975). The data of Aboitiz *et al*. (1996) are also compatible with an increase in fiber size and myelinization of the CC into middle age.

4.17.5 Laminar Organization and Regional Variance

Here is a point where evolution may enter, in the details of distribution of CC and AC cells and terminals. The variance exists not only in the commissural density from one to another functional area, but in the cortical laminae involved, and whether the projection is homo- or heterotopic. While a few examples are available, overall the data are far from sufficient to support any clear evolutionary theme.

Throughout the mammalian order, the primary site of CC and AC neurons is layer 3, with a sizable population in layer 5; and termination is typically directed to layer 4. The cells of origin are mostly pyramids, often slightly larger than adjacent cells forming ipsilateral connections (Innocenti, 1986). In macaque prefrontal cortex, the CC neurons have 34% and 25% longer apical and basilar dendrites compared to ipsilaterally projecting pyramidal cells, dendritic length being independent of whether the neuron lies supra- (75%) or infragranular (25%) (Soloway *et al*., 2002).

There is a clear tendency for grouping of the interhemispheric cells and terminations to form pseudocolumns (Bugbee and Goldman-Rakic, 1983; Cusick *et al*., 1984; Goldman-Rakic and Schwartz, 1982; Innocenti, 1986; Johnson *et al*., 1989; Jones *et al*., 1979; Schwartz and Goldman-Rakic, 1984). The CC connections that are homotopic are generally reciprocal, and tend to differ in their laminar locations compared to heterotopic projections. All this is well summarized and reviewed by Innocenti (1986). That heterotopic CC connections can be extremely diverse is shown by the example of fibers from a limited region of the right human inferotemporal lobe projecting into both Wernicke's and Broca's areas on the left (Di Virgilio and Clarke, 1997).

The ispi- and contralateral exchange between the ascending stages of visual processing in macaques provides a cogent illustration of the rich detail in such relations (Kennedy *et al*., 1986). 'Feedforward' connections are defined as those progressing from earlier to later stages of processing, and 'feedback' as occurring in the opposite direction; these exist for both ipsi- and contralateral projections. For instance, feedforward connections passing ipsilaterally from the border of striate cortex into area MT arise primarily from layer 3, with some input from layer 5, all projecting to layer 4. The ipsilateral feedback from MT to striate, on the other hand, arises primarily from layer 5, with some input from layer 3, and terminates in layers 3 and 6 of the striate cortex. Callosal input in the feedforward, heterotopic mode, from striate border to MT, is similar to that ipsilaterally: it too terminates sharply in layer 4 of MT, but arises exclusively from layer 3. The homotopic terminals of the callosal cells in the contralateral border area of the striate cortex avoid layer 4 contralaterally, and instead are limited to layers 3 and 5, rather similar to the ipsilateral feedback format. The heterotopic projections of the

callosal cells, also all in layer 3, forming the striate border and projecting into visual area 2, terminate much more diffusely, in all layers except 4, and strongly favor the cytochrome oxidase loci (see also Preuss and Kaas, 1996; Olavarria and Abel, 1996).

Distinct patterns are thus formed as to layer of origin and termination that depend upon the relations of the cortical areas involved. The pattern described by Kennedy *et al.* (1986) for macaques, as they discuss, differ from those found in New World monkeys and, particularly, from galago.

4.17.6 Subcortical Involvement

As is the case for marsupials (Heath and Jones, 1971), primates have extensive crossed connections between cortex and striatum, via the AC and fasciculus aberrans in the first and the CC in the latter. As is so far known, these crossed corticostriatal connections in primates emanate only from frontal and motor cortical areas, and seem to be absent from sensory areas (Jones *et al.*, 1977). For area 6, the contralateral projection to the putamen and head of the caudate nucleus is as numerous as is that ipsilaterally, with smaller contralateral numbers from area 8, and rare from area 4. All arise from lamina 5 (Jones *et al.*, 1977). McGuire *et al.* (1991) show that the ipsi- and contralateral projections are largely in register. They found that the CC projection from the area of the principal sulcus was weaker than ipsilaterally, but for the supplementary motor area (6?) and area 4 the density and extent of projection from the two sides were roughly equal, and directed mainly at the putamen.

There has been some dispute concerning the reality of crossed corticothalamic projections, but Dermon and Barbas (1994) have resolved this by showing that such connections proceed only from 'transitional' cortex, that is, cortex not being eulaminate in having six clearly developed laminae and a distinct layer 4. Thus, areas 13, 32, 24, and 25 have contralateral projections into ventralis anterior, medialis dorsalis, and the intralaminar nuclei. These crossed connections are reciprocated from these thalamic nuclei to the transitional cortex, but send no crossed connections to eulaminate cortical areas. It bears note that in the rat, Negyessy *et al.* (1998) demonstrated that these crossed corticothalamic fibers were primarily collaterals of the ipsilaterally projecting fibers.

Another instance of crossed projection can be found in the dorsal raphé. When striate cortex of macaques was unilaterally, but liberally, loaded with HRP, at any given level of the dorsal raphé 10–45% of the labeled neurons were contralateral to the injection (Doty, 1983). Thus, both sides of the raphé project to both striate cortices, but whether this is a case of collateralization remains to be examined.

4.17.7 Phrenology of the CC

The shape and area of the human CC in midline cross section is variable. The shape has been extensively studied, and assigned correlation with a rather wide number of variables, such as sex, various talents, handedness, and schizophrenia (see Smith, 2005). What is often overlooked is just how extreme the variation is, not just for the forebrain commissures, but for the human central nervous system generally. For instance, Andrews *et al.* (1997) demonstrated a twofold variation in the central visual system, commensurate at the different stages, for example, a small lateral geniculate nucleus was correlated with a small striate area. When examining the cross-sectional area of the human AC, Demeter *et al.* (1988) noted a sevenfold range, that was not related either to brain weight or cross-sectional area of the CC. Such variability in the AC may have a functional equivalent in that some patients undergoing transection of the CC, with sparing of the AC, can effect a degree of interhemispheric transfer of visual information, whereas others cannot (McKeever *et al.*, 1981), unlike macaques that have been uniformly capable of interhemispheric mnemonic transfer via the AC in the absence of the CC (Doty *et al.*, 1994).

A correspondingly large variation is found in the size of the human CC (e.g., Aboitiz *et al.*, 1996; Rauch and Jenkins, 1994; Jancke *et al.*, 1997; Giedd *et al.*, 1999). However, evidence for a clear sexual difference in form is often contradictory (Smith, 2005), although the midline cross-sectional area in human females is commonly smaller, corresponding to smaller average brain size. In a series of consecutive births of human males and females, 100 each, Hwang *et al.* (2004) found no gender differences in total area of the CC or its subregions. In line with many studies, Scamvougeras *et al.* (2003) observed a 1.5-fold variation in cross-sectional area for 14 monozygotic twins, twofold for 12 dizygotic twins, and concluded that heredity accounted for 94% of this size variance.

Even among eight macaques, the range is almost twofold, 55–95 million μm^2; but the smaller CC, from an animal only 2 years old, had 54.7 million axons, compared with the 17-year-old macaque with the largest CC that had only 36.9 million

(LaMantia and Rakic, 1990b). This, and other comparisons in their table, shows not only the great variation in area, but also that the area is not necessarily well correlated with the number of fibers.

4.17.8 Effect of Brain Size on the CC, and Its Consequences

As the number of neurons in a brain increases, and the neurons necessarily become farther apart, the volume devoted to the axons required to maintain a given level of interconnectivity between the neurons increases faster than does the volume devoted to the neurons *per se* (Ringo, 1991). The result is that the proportion of white versus gray matter increases as a function of brain volume, evident in a diverse series of primates including humans (Luders *et al.*, 2002; Rilling and Insel, 1999b; Striedter, 2005). This effect extends to the CC, where cross-sectional area is proportionately less the larger the brain, thus providing a clear example of evolution modulating interhemispheric connectivity (de Lacoste and Woodward, 1988; Olivares *et al.*, 2000; Rilling and Insel, 1999a; Striedter, 2005). Not only does the proportionate number of cross-connections diminish as the brain enlarges, the distance that they must traverse necessarily also increases. Thus, unless the diameter of the interhemispheric fibers were to keep pace with the greater distance in the larger brain, the time required for interhemispheric communication inevitably increases (Ringo *et al.*, 1994). The consensus is that the mean diameter of fibers in the CC is essentially constant among species (see Olivares *et al.*, 2001), and the average time for transmission across the CC must thus increase in step with size of the brain. In the human brain, this temporal delay for interhemispheric transmission offers a sufficient penalty that intrahemispheric computations are likely to be significantly more efficient than bihemispheric calculations, so that each hemisphere develops an intrahemispheric modus operandi, that is, hemispheric specialization reflects the solution to this problem (Ringo *et al.*, 1994).

It may be asked why the diameter of the CC fibers does not increase *pari passu* with size of the brain, but augmenting the diameter does itself require space that increases the distance for communication, etc. (Ringo, 1991). However, in so far as it has been examined, species with larger brains do have a few exceptionally large CC fibers that are absent in species with smaller brains. The best example is that of Olivares *et al.* (2001). In their series of rat, rabbit, cat, dog, cow, and horse, only the latter had a population of heavily myelinated fibers with a diameter of 1 μm or more in the splenium. A similar population of conspicuously large splenial fibers has also been observed in humans (Shoumura *et al.*, 1975) and macaques (Glickstein and Whitteridge, 1976), presumably subserving rapid integration of the two halves of the visual field.

It should be emphasized that whereas the temporal delay in interhemispheric communication with increasing brain size may be the root cause of hemispheric specialization, it has nothing to do with which hemisphere forms what manner of specialization. Handedness is a frequent index of hemispheric specialization, or dominance, in humans, but whether right or left need have no necessary relation to the forebrain commissures.

4.17.9 What Do the Forebrain Commissural Pathways Do?

4.17.9.1 Electrophysiology

The basic data derive almost entirely from experiments on cats and rodents. The classical observations of Asanuma and Okamoto (1959), on antidromically identified cells of the CC and pyramidal tract in the anesthetized cat, established that stimulation of the CC could inhibit the discharge of PT cells driven by peripheral nerve stimulation, after a latency of some 20–30 ms. Although subsequent work has shown that the vast majority of CC terminals are excitatory, disynaptic inhibition is a very common feature. Less than 1% of the CC fibers in the frontal areas of the cat and rat are GABAergic, the remainder being ostensibly glutamatergic (e.g., Conti and Manzoni, 1994; Fabri and Manzoni, 2004; Kumar and Huguenard, 2001; Cissé *et al.*, 2003).The functionally inhibitory influence of the CC is exemplified by the suppression that it exerts upon the discharge of units in V4 of macaques when the field of view impinges upon the visual field opposite to that activating the units (Desimone *et al.*, 1993). An inhibitory component remains a factor in the interpretation of many callosal effects. However, the details of the neuronal interplay in relation to the behavioral effect of the commissural influence remain largely unexplored, and beyond the scope of the present review.

4.17.9.2 Behavior

As is well known, it took a surgical trick, the split-brain preparation of Myers and Sperry (1953), to begin defining the behavioral contribution of the forebrain commissures. The ingenious feature was

to sever the optic chiasm, thereby allowing easy limitation of visual input to one or the other hemisphere, with subsequent complete or partial transection of the forebrain commissures to test the nature of interhemispheric communication. The loss of half or more of the visual input to each hemisphere in this procedure is commonly ignored and, so far as is known, is not relevant to the analyses performed. In split-brain patients (Gazzaniga *et al*., 1962), of course, the chiasm remains intact, and the addition of observations on humans greatly augmented the depth of understanding concerning interhemispheric communication (Doty, 1999).

A summary of this now vast literature cannot be attempted here but, instead, several examples are offered to suggest the range of the effects observed.

4.17.9.2.1 Transcortical monitoring by the amygdala This series of experiments in macaques (Figures 2 and 3) tested whether the amygdala in one hemisphere can communicate with the visual system of the other hemisphere across the splenium of the CC (Doty *et al*., 1973). Surprisingly, the result demonstrated that there is a continual intercomunication between these entities despite limitation of exchange to this restricted route, thus revealing a remarkable depth in the capability of interhemispheric processes.

Downer (1962) and Horel and Keating (1969) had already shown in split-brain macaques that the amygdala in one hemisphere could communicate commissurally with the visual system in the other. The procedure was thus modified to produce a blind hemisphere by severing the optic tract on one side, and surgically removing the amygdala in the other hemisphere (Figure 2; Doty *et al*., 1973). The forebrain commissures were then transected save for 5 mm of the splenium, around which a snare was placed. Macaques so treated retained an entirely normal reaction to the human presence, fleeing consistently and expertly when approached within a 3×5 m enclosure. When, however, under local anesthesia, the snare was pulled, transecting the remaining CC fibers and eliciting a blink, the animal was immediately transformed in its visual appraisal (Doty *et al*., 1973). Rather than fleeing the human presence, it endeavored to smell the approaching human hand (Figure 3), typical of the Klüver–Bucy syndrome (Bucy and Klüver, 1955). It struggled fiercely if seized, and leapt about the perches in the room with its previous agility. It is thus apparent that when only the splenium was intact, the 'seeing hemisphere', that lacked an amygdala, was continually in communication with the amygdala in the 'blind' hemisphere. The interesting question, still unapproached, is the direction of that interchange: whether the amygdala scans the activity callosally transmitted into its 'blind' visual system, or whether the signal crossing the splenium already contains the

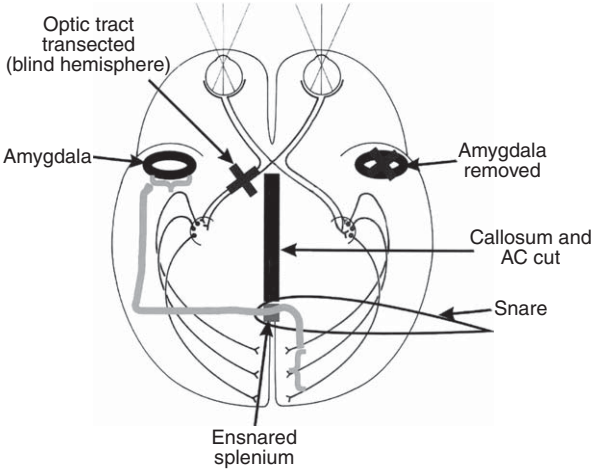

Figure 2 Schema for demonstrating the interhemispheric communication between amygdala and the cortical visual system in macaques. The amygdala has been removed on one side (also interrupting the AC), and the optic tract has been transected on the other, blinding that hemisphere. All of the CC has been cut save for 5 mm of the splenium, around which a snare has been passed. The thick gray line represents the putative path connecting the 'seeing' visual hemisphere with the contralateral amygdala. When the snare is pulled, transecting the remaining connections via the CC, interruption of that pathway then precludes the animal from visually judging the threat of human presence, that was fully manifest prior to pulling the snare (see Figure 3). Based on experiments of Doty, R. W., Negrão, N., Yamaga, K. 1973. The unilateral engram. *Acta Neurobiol. Exp. (Wars.)* 33, 711–728.

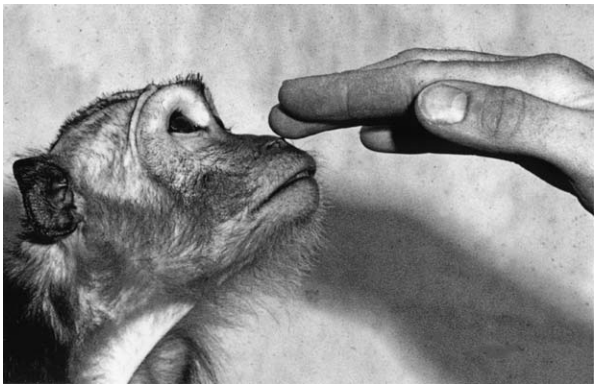

Figure 3 Macaque manifesting Klüver–Bucy syndrome a few minutes after interruption of the cortical visual–amygdalar interhemispheric path, as per Figure 2. Prior to this disconnection, the human hand would have been severely bitten, were it so available, in contrast to the olfactory exploration illustrated here. Visuomotor coordination retained its usual dexterity, in that the animal leapt skillfully from one perch to another, and were it touched or seized, it still reacted with full aggression and resistance. After Doty, R. W., Negrão, N., and Yamaga, K. 1973. The unilateral engram. *Acta Neurobiol. Exp. (Wars.)* 33, 711–728.

code for alerting the amygdala. The most remarkable feature remains the flexibility of the visual/emotional interhemispheric paths, that maintain a link that would seem to be redundant in normal circumstances.

4.17.9.2.2 Memory In everyday life, items are continually falling first within one visual field, and a moment later the next. The basic question is thus whether the memory traces formed by the input via one hemisphere/visual field are recognized in the other hemisphere/visual field, a process requiring interhemispheric transmission. Tested with a continuous series of words or intermingled nonverbalizable images, human subjects displayed essentially no differences in subsequent recognition regardless of which visual field or type of stimulus was used for the second viewing (Kavcic and Doty, 2002). At delays of 1–2 min, however, there was an interesting paradox. When words were first viewed via the left visual field/right hemisphere, they were subsequently better recognized via the right visual field/left hemisphere in these right-handed subjects, suggesting that the more secure linguistic memory trace had been established contralateral to the input, in the linguistically more facile hemisphere.

This question of which hemisphere actually holds the memory trace is difficult to assess, given the near-perfect efficiency of commissural communication and, in humans, the pronounced specialization of the individual hemispheres. It can, however, be addressed in macaques, where the commissural transmission can be blocked or eliminated. When intact macaques learned to respond to electrical excitation of striate cortex in one hemisphere, they unhesitantly responded to such stimulation of the contralateral striate cortex, so long as the AC or CC was intact (Doty *et al*., 1973). However, there was a dramatic dichotomy between the CC and AC in this regard. Using the 'snare' procedure alluded to above, it was arranged that only the splenium or the AC was intact during the training to excitation of striate cortex. In all the cases, the learned response was readily elicited from excitation of the contralateral striate cortex without further training. When the splenial snare was pulled, to complete the transection of the commissures, the response to contralateral stimulation was abolished, while excitation of previously untrained loci on the 'trained' side was unaffected.

The latter result encouraged the idea that the engram in this case was strictly unilateral, and suggested that the mnemonic role of the CC might be to restrict memory traces to a single hemisphere, thereby doubling the mnemonic storage capacity of the brain compared to that were engrams to be allowed bilaterality. So long as the commissures were intact, one hemisphere could activate the memory trace held unilaterally by the other. Unexpectedly, and still inexplicably, the situation was exactly the opposite on the nine animals in whom the AC remained intact during training, all of the CC having been severed (Doty *et al*., 1973). In this case, pulling the AC snare to complete the severance of the forebrain commissures had no effect upon the learned responses elicited by excitation of the contralateral, 'untrained' striate cortex, and they proceeded immediately upon first testing, without further training. Presumably, the critical difference lies in the fact that the AC serves the inferior temporal lobe, wherein an engram would have been induced from the input provided by the AC, but it would, of course, also have to be made accessible to the input via the 'untrained' striate cortex.

The unilateral engram via the CC may be unique to cases where the stimulus originates in the cortex, bypassing normal sensory pathways. Using normal visual input, Ringo (1993) demonstrated an unequivocal bilaterality of the memory trace. He used a delayed matching-to-sample task with split-chiasm macaques, retaining only AC or splenium of the forebrain commissures. Formation of or access to memory traces was restricted to one or the other hemisphere by mild tetanization of the corresponding medial temporal lobe during either the encoding or retrieval stages. If tetanization proceeded in opposite hemispheres during encoding and retrieval, performance was reduced to essentially chance levels. By manipulating the sequence of encoding and retrieving by the two hemispheres in relation to the tetanization, it could be shown that a unilateral trace was accessible from the other hemisphere. If only one eye/hemisphere viewed the target image, and during retrieval via the other eye/hemisphere the original hemisphere was tetanized, accuracy of retrieval was still above chance. Controls indicated that in the 5–10 s interval between encoding and retrieval the engram had in that case been passed to the initially nonviewing hemisphere, that is, created a bilateral engram. This was true with either the CC or AC.

A further example of mnemonic processing by macaques with split chiasm and possessing only the AC or the splenium for interhemispheric communication used memory for six visual targets, that were subsequently to be distinguished from nontarget items on each set of trials (Lewine *et al*., 1994). The critical point was that the target items were randomly distributed between (presented to) the hemispheres, as were the tests. So long as either the splenium or AC was intact, the hemispheric

distribution of targets and tests for accuracy was close to that of the intact animal, and, as expected, the reaction time was linearly related to the total number of targets held by the two hemispheres together. In other words, the commissures were extremely efficient in unifying the mnemonic load and the processing required. Once the commissures were totally severed, to produce a split-brain animal, the reaction time reflected the mnemonic load only of the hemisphere being queried; surprisingly, however, the accuracy of either hemisphere reflected the total mnemonic load held by the two hemispheres together.

This latter indication of subcortical participation in mnemonic processing was further examined in two split-brain macaques (Kavcic *et al*., 2000): first, to determine whether the two hemispheres could each acquire a memory trace when visual stimuli were presented to each simultaneously, and second, to assess the degree of hemispheric dominance when they were given a conflicting choice. Unexpectedly, it was found that when the two hemispheres viewed different images simultaneously, testing either hemisphere alone for recognition of 'its' image was consistently less accurate than when the repetition of the images was simultaneous and binocular. The interpretation follows that, while parallel processing by the two hemispheres is achieved at a high level, there is an additional factor. The binocularly acquired trace somehow predominates and, when one component is lacking upon monocular viewing, the distinction is noted as a less accurate match. Thus, even in the absence of the forebrain commissures, subcortical processes are in play to yield, in so far as possible, a bilaterally unified memory trace. Support for such supposition comes from observations by Marcel (1998) on human patients with unilateral loss of the geniculo calcarine pathway. When viewing Kanizsa illusory contour figures, part of which fell into the blind visual field, they nevertheless could make the appropriate decision as to the nature of the figure, amalgamating the subcortical component with that available through the intact side of their visual system. In other words, here too there was a unification of features available via bilaterally disparate channels, one of which required access at subcortical levels.

A further feature of subcortical exchange was displayed by the two split-brain macaques when consistently contradictory stimuli were employed (Kavcic *et al*., 2000). In the course of a running recognition task with colored images, the first viewing of an item, NEW, would be given to, say, the right hemisphere and, several trials later, that item would again appear for the right hemisphere, now as OLD, while simultaneously the left hemisphere viewed an item that for it was NEW. Prima facie, the situation is wholly ambiguous, one hemisphere cued to judge OLD, the other NEW. One animal 'solved' the situation by responding correctly to what the dominant hemisphere was viewing, gradually consolidating this behavior over the course of several days. The other animal remained confused. Throughout this testing period, it was the OLD stimulus that was always rewarded. The situation was then reversed, so that the stimulus always rewarded in this conflict situation was NEW. Over the course of some 200 trials, interspersed with other tests for several weeks, both animals were gradually able to effect a subcortical 'negotiation', whereby the hemisphere viewing an OLD image deferred 80% of the time to its mate if it was concurrently viewing a NEW image. This was achieved at some cost to accuracy, normally *c*. 90%, when the situation was such that both hemispheres were viewing an OLD image. There is thus a form of learning that can be effected between the hemispheres independently of the forebrain commissures. It is hypothesized that much of this interchange passes via the superior colliculus.

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4.18 The Hominin Fossil Record and the Emergence of the Modern Human Central Nervous System

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Glossary

anatomically modern human — Hominin fossil evidence that cannot be distinguished from the skeletal remains of contemporary modern humans.

brain specific mass — The average density of the brain.

brain tissue volume — The volume of the brain itself (i.e., brain volume minus the volume of the CSF within the ventricles of the brain and the volume of any meninges and cranial nerves that may adhere to the brain).

brain volume — Usually defined as the sum of the volume of the whole brain (including the ventricles) plus the leptomeninges (i.e., the arachnoid and pia mater).

clade (Gk. clados = *Branch)* — A grouping of taxa that contains no more and no less than all the descendants of the group's most recent common ancestor.

encephalization (syn. cephalization, céphalisation) — The process by which, for a given taxon, relative brain size becomes larger than predicted from the general scaling relationship between brain size and body size.

encephalization quotient (EQ) — The ratio of the observed brain size over the expected brain size, based on a general scaling relationship.

endocranial cast — A natural or artificial cast that uses the walls, roof, and floor of the cranial cavity as a mold.

endocranial volume (syn. cranial capacity, endocranial capacity)	The volume of the endocranial cavity. This is the sum of brain volume plus the volumes of the meninges, the extracerebral CSF (i.e., the CSF outside the ventricles), the intracranial (but extracerebral) vessels, and the cranial nerves within the cranial cavity.
hominin (L. homin = *Man)*	Vernacular for a species (or an individual specimen belonging to a taxon) within the tribe Hominini (e.g., *Paranthropus boisei* is a hominin taxon and KNM-ER 1470 is a hominin fossil cranium).
meningeal vessels	The meningeal arteries and veins that supply blood to, and drain most of the blood from, the neurocranium and the dura mater.
petalia	The greater anterior, posterior, or lateral protrusion of one cerebral lobe relative to the other.
suture (L. sutura = *A Seam)*	Fibrous joint between two bones of the cranial vault. The fibrous tissue in these joints gradually disappears as the bones fuse together.
virtual endocast	3-D digital cast of the neurocranial cavity, created using 2-D computed tomography slices.

4.18.1 Introduction

This article focuses on what the fossil evidence for human evolution can tell us about the evolution of the modern human central nervous system (CNS). Its scope is the hominin clade, which extends in time from the hypothetical common ancestor of chimpanzees and modern humans, via the appearance of the first hominins (*c.* 8–5 Mya), to the emergence a little over 190 kya ago of anatomically modern humans. We use the term 'hominin' for modern humans and all extinct species that are more closely related to modern humans than to any other living taxon, and the term 'panin' for living chimpanzees and all extinct species that are more closely related to chimpanzees than to any other living taxon.

The first section reviews the ways in which the fossil and archeological records can be used to help reconstruct the CNS of extinct hominin taxa. The second section reviews the relationships of modern humans, and outlines the organization of the hominin fossil record. The third section reviews each of the fossil hominin species. It uses a relatively speciose hominin taxonomy, but it also indicates how the same fossil evidence would be arranged if it were grouped into a smaller number of more inclusive taxa. Brief descriptions of each taxon are provided. There are two reasons why this section concentrates on the craniodental evidence. The first relates to the relative durability of the hard tissues of the head and neck of higher primates. Because they are generally hard and dense, they tend to preserve in larger numbers than do the bones of the limbs and trunk. This means that most species are defined on the basis of a set of distinctive craniodental traits. The second reason is that because, in this article, we focus on how the quality and scope of the fossil evidence for each taxon can contribute to our knowledge about the recent evolutionary history of the modern human CNS, this inevitably means emphasizing the cranial evidence. The fourth section of the article summarizes trends in the evolution of the hominin CNS. It summarizes the differences between the CNSs of modern humans and chimpanzees, and makes predictions about the CNS of the common ancestor of chimpanzees and modern humans. It addresses, among others, the following questions:

1. When did hominin brain size begin to increase beyond the levels we see in contemporary nonhuman higher primates?
2. At what stages in hominin evolution do we see grade shifts in brain morphology?
3. Were changes in brain size and shape restricted to our own genus, or did they occur in other hominin genera?

4.18.2 How can Fossil Evidence Be Used to Reconstruct the Recent Evolution of the Modern Human CNS?

4.18.2.1 Fossil Evidence Relevant for Reconstructing the Size and Shape of the Brain

The biggest obstacle to understanding the evolution of the human CNS is that the CNS is not preserved in the hominin fossil record. However, inferences can be made about the size and shape of the CNS from natural endocasts, from the fossilized morphology of the neurocranium, the cranial base, and the axial skeleton. Endocasts and neurocranial fossils convey information about the size, shape, external convolutional morphology, and blood supply of the brain. Cranial base morphology contains information about the brainstem, and the cranial

nerves and blood vessels that perforate it. Finally, the neural canal conveys information about the spinal cord which extends in adult modern humans from the atlas (C1) to the first (usually) or second lumbar vertebras. Across primate species, the cross-sectional area of the vertebral canal provides an indication of spinal cord dimensions – particularly in its most rostral aspect, but less so more caudally where a greater proportion of the canal is devoted to spinal nerves (MacLarnon, 1995).

Although endocasts look remarkably brain-like, an endocast is not a fossil brain, but rather a cast of the neurocranial cavity. A natural endocast is formed during fossilization as the cranial cavity fills with fine sediments that enter through the various foramina and fissures that perforate the floor of the cranial cavity. Similarly, a synthetic endocast is made by stopping these perforations and then lining the inner surface of the endocranial cavity with quick-drying latex. Once dry, the thin layer of flexible latex can be peeled off the endocranial surface and removed through the foramen magnum. It is also possible to create a three-dimensional (3-D) digital cast of the neurocranial cavity, called a virtual endocast, and this has become the method of choice for investigating delicate and/or fragmentary fossils (e.g., Falk *et al.*, 2005; Zollikofer *et al.*, 2005). Endocasts potentially preserve details of the convolutional morphology of the surface of the cerebral and cerebellar hemispheres that are imprinted through the three layers of meninges (from outside in: the dura, arachnoid, and pia mater). In addition, endocasts preserve the imprints of blood vessels and skeletal sutures.

Convolutional details are not always well preserved. The differential imprinting of convolutional detail may be due to taphonomy, the relative size of the brain, and the effects of ontogeny. In general, natural endocasts produce more details than synthetic endocasts. Falk (1980a) proposed two explanations for this: (1) natural endocasts may begin to form before the dura mater has fully disintegrated, so that any details present on it, but absent on the endocranial surface of the inner table of the neurocranium, are preserved; and (2) synthetic endocasts are often produced from crania that have been reconstructed from fragments, and the process of reconstruction can introduce morphological noise. The relatively small-brained *Australopithecus* endocasts are usually more detailed than endocasts made from crania assigned to larger-brained later *Homo* taxa. It is noteworthy that, for various groups of mammals, those with the largest brains within that group tend to produce the least-detailed endocasts, although this pattern does not hold true for absolute brain size across major groups (Radinsky, 1972, p. 176). This is related to the fact that endocranial volume increases more rapidly than brain size increases within primates (Martin, 1990, pp. 365, 392). The intensity of gyral impressions is also related to ontogeny. In modern humans there are few or no impressions on the endocranial aspect of the cranial vault before 1 year (Du Boulay, 1956). Gyral impressions are probably most marked during adolescence, and with increasing age basal markings become more prominent whereas vault impressions become fainter (Connolly, 1950, p. 291).

The convolutional details of endocasts are notoriously difficult to interpret (Symington, 1916; Holloway, 1966). A feature might be the impression of a sulcus, or a blood vessel, or a skeletal suture, or it might be an artifact, and observers might offer genuinely different interpretations of what the same feature represents (Connolly, 1950; Falk, 1980a). For example, over the last three decades, Holloway and Falk have been providing often conflicting interpretations of the same endocasts. Only a handful of researchers study the details of endocast morphology, and both Falk and Holloway have called on other paleoanthropologists to join the debate (Falk, 1987; Holloway *et al.*, 2004a).

Not all researchers are convinced that the detailed morphology of endocasts has functional relevance. Many paleoneurologists take it for granted that sulci delimit functional or somatotopic cortical areas (see Radinsky, 1972, and references therein). However, it has become clear that the primate brain exhibits a substantial amount of intraspecific variability in sulcal anatomy and cytoarchitectural boundaries (Geyer *et al.*, 2001; Rademacher *et al.*, 2001). In some cases, the relationship between sulcal landmarks and functional areas is maintained within a species (Holloway *et al.*, 2003), but in others it varies within species (Sherwood *et al.*, 2003). It might not be possible to make detailed interpretations of brain function from endocasts alone.

Information about fossil hominin brain evolution is not limited to the hard-tissue fossil record. Natural endocasts are a form of trace fossil that record, often in unusual detail, the endocranial morphology of an individual. Archeologists also claim that artifacts reveal information about the evolution of the hominin CNS. Tools, art, and other artifacts found in association with hominin remains provide direct evidence of the capacity of a species for specific behaviors, something that fossils cannot reveal.

The combination of paleontological and archeological evidence provides more insight into the brain function of fossil hominins than either of these two lines of evidence could generate on their own.

4.18.2.2 Data Interpretation

In a specimen of an extant taxon, the size and shape of the brain can be estimated from either the brain itself or, indirectly, from cranial measurements. In a fossil specimen, measurements are taken from either an endocast (natural or synthetic) or from a fossil neurocranium. In general, the same measurement methods are used for endocasts and whole brains, and similar methods are applied to extant and fossil neurocrania. However, there are several reasons why data taken from contemporary taxa and data taken from fossil specimens might not be comparable and suggestions have been made to correct for the discrepancies.

First, it is important to appreciate that the volume of an endocast is the volume of the neurocranial cavity (i.e., endocranial capacity or endocranial volume). Endocranial volume includes not only the volume of the brain, but also the space occupied by meninges, extracerebral cerebrospinal fluid (CSF), and cranial nerves.

Second, all fossils, including endocasts and cranial fossils, are imperfect representations of the hard tissues they represent. Problems include incompleteness and plastic deformation. Incomplete fossils require that assumptions are made about the size and shape of the missing parts, and deformation may be difficult to detect when the form of the undeformed brain is unknown. Matrix can fill and widen cracks, expanding the size of the fossil beyond the size of the original bone. Virtual methods have recently been developed to correct for such deformation.

Third, even well-preserved fossils present sampling problems that affect interspecific comparisons. For example, we do not know the chronological age of individual fossils, yet if the individual is immature it may be necessary to estimate the size of the equivalent adult. However, ontogenetic age is difficult to estimate even in extant animals, let alone in a fossil species for which we will never have a satisfactory reference sample. In addition, sexual dimorphism may introduce substantial variation in brain volume within a species. In hominin species with a sparse fossil record, overrepresentation of a particular sex might give an inaccurate impression of the species' true mean and range of brain size.

4.18.2.3 Brain Size Measures and Estimates

The brain sizes of fossils can be obtained from a variety of methods that vary in their accuracy and precision. For example, de Miguel and Henneberg (2001) reviewed the brain size estimates for OH 5 cited in the literature and found that for this relatively complete *Paranthropus boisei* fossil cranium 15 different endocranial volume estimates are given ranging from 500 to 750 cm^3. Holloway (1983b) has devised a useful system for indicating the reliability of endocranial volume measurements which uses a letter code for the method used and a number code for the reliability of the measurement.

4.18.2.3.1 Measurements of the volume of a brain or of a solid endocast by water displacement The preferred way to determine the volume of a brain or an endocast, natural or artificial, is by water displacement according to Archimedes' principle. For extant species this is done using postmortem brains, but how well does this method measure brain size? For postmortem samples, one or more of the following confounding factors needs to be taken into account: time from death to measurement, time from death to fixation, fixation method and preparation prior to measurement, whether the leptomeninges and CSF are included, adequacy of dissection, and shrinkage. For example, what Stephan and others (Stephan *et al.*, 1970, 1988; Stephan, 1981) call brain weight includes meninges, hypophysis, and any nerves still attached to the brain (Heiko Frahm, personal communication). Similarly, the largest brain mass data sets for modern humans (Dekaban and Sadowsky, 1978) and chimpanzees (Herndon *et al.*, 1999) include leptomeninges and CSF in the brain mass estimates. Jerison (1973) estimates that the effect of including or excluding variables such as these can cause measurements to differ by as much as 20%.

Holloway *et al.* (2004a, 2004b) found that weighing the water displaced by an endocast was a more consistent method than measuring its volume. Volumes measured using artificial endocasts might be underestimates of the true volume because endocasts are likely to have undergone shrinkage (Gingerich and Martin, 1981; Broadfield *et al.*, 2001).

4.18.2.3.2 Measurements of the volume of the endocranial cavity

4.18.2.3.2.(i) Packing methods Packing methods involve filling the cranial cavity with small particles such as mustard seed, sintered glass beads, or shotgun pellets, and then determining the volume of the packing material. Different types of fillers can produce slightly different endocranial volume estimates (Miller, 1991). For fossil crania, packing methods are sometimes preferred over water displacement of a latex endocast because the problem of endocast shrinkage is avoided. However, because of

variation in techniques for settling the packing material, these methods almost certainly underestimate the true endocranial volume (Gould, 1978, 1996).

4.18.2.3.2.(ii) Filling methods Filling methods are like packing methods, but involve a fluid rather than a solid. Uspenskii (1954) describes a method in which a rubber balloon is put into the cranial cavity and then filled with water. Similar values (mean difference 1.67 cm^3) were obtained with this method and with water displacement, but packing with millet seed resulted in smaller (mean difference 65.4 cm^3) values than those obtained using the balloon method (Uspenskii, 1954).

4.18.2.3.3 Estimating volumes from slices

4.18.2.3.3.(i) Cavalieri's principle Using Cavalieri's principle, it is possible to produce an unbiased estimate of total brain volume from measurements of the cross-sectional area of a sample of brain sections (Stephan, 1981, p. 3; Gundersen and Jensen, 1987). Cavalieri's principle can be used to determine brain volume from actual and virtual brains. Serial sections of brains mounted onto slides undergo shrinkage as a result of fixation and embedding, so volume measurements determined from slide-mounted sections need to be corrected accordingly. In early papers, the effect of shrinkage was overlooked. Stephan (1981) advised researchers to generate an individual conversion factor (C_{ind}) for a brain with known mass and known volume:

$$C_{ind} = \frac{\text{Volume of fresh brain}}{\text{Serial section volume}}.$$

Stephan (1981, p. 4) list conversion factors for specific types of fixation, ranging from 1.54 to 2.4. Aside from mismeasurement due to shrinkage, the only disadvantage with using sections as opposed to water displacement is that the former estimates volume, rather than measuring it. However, what the estimate loses in precision it may gain in accuracy, since imaging makes it possible to be sure that only brain tissue is included.

4.18.2.3.3.(ii) In vivo *MRI* Magnetic resonance imaging (MRI) has recently begun to be applied to comparative samples of living hominoid species in order to obtain volumes of both entire brains and particular brain regions (e.g., Rilling and Insel, 1999; Semendeferi and Damasio, 2000; Sherwood *et al.*, 2004). Comparison of MRI volumes with volumes obtained by water displacement have established that as few as 5–6 MRI slices per brain are enough to yield reliable estimates of mean brain volume, with a coefficient of error (CE) of approximately 5% (Mayhew and Olsen, 1991). The CE decreases as the number of slices increases (e.g., for 28 slices, CE < 1%).

There are several advantages to using *in vivo* MRI volumes over autopsy brain volumes. *In vivo* MRI brain volumes avoid biases inherent to using autopsy brains; for example, autopsy brain samples overrepresent aged individuals. *In vivo* MRI volumes are not affected by changes in brain volume due to the elapsed time between death and measurement or fixation. Peters *et al.* (1998) compared the results of cross-sectional studies in which human brain volumes were obtained either *in vivo* by MRI (or nuclear magnetic resonance, NMR) or from autopsy brains. They found large discrepancies between the means of the different samples (even in cases in which the same method was used), but they did not identify the way in which the autopsy and MRI volumes differed.

4.18.2.3.3.(iii) Postmortem MRI Peters *et al.* (2000) compared estimates of brain volume obtained from MRI and from water displacement in autopsy specimens. They found that provided thin MRI slices (1–1.25 mm) were used, MRI volumes did not differ significantly from water displacement volumes. However, MRI volumes were found to be overestimates when thicker slices (5 mm) were used.

4.18.2.3.3.(iv) CT slices and virtual endocasts A widely applicable and noninvasive way in which to accurately estimate fossil endocranial volumes is by using two-dimensional (2-D) computed tomography (CT) slices. It is possible to use these slices to obtain an endocranial volume in two ways which yield similar results: (1) either directly using Cavalieri's principle, or (2) through the construction of virtual endocasts (e.g., Conroy *et al.*, 1998). Increasingly popular, a 3-D virtual endocast is a 3-D model of the fossil constructed from the 2-D CT slices (Zollikofer *et al.*, 1998; Tobias, 2001; Zollikofer, 2002). For matrix-filled skulls, thresholding to distinguish between local object densities is the method used to separate the walls of the fossil neurocranium from the matrix at their interface (Conroy and Vannier, 1985; Conroy *et al.*, 1990; Zollikofer *et al.*, 1998). Fragmentary specimens are completed using mirror-imaged parts from the opposite side (e.g., Conroy *et al.*, 2000a), or scaled parts from another specimen (e.g., Zollikofer *et al.*, 1998). Once the virtual cranium has been created (e.g., Zollikofer *et al.*, 2005), it is possible to create a

virtual endocast. If there is uncertainty about the dimensions, several potential endocrania are created to establish a range of endocranial volumes, from which a most likely endocranial volume can be determined (Conroy *et al.*, 2000b). The virtual endocast technique was tested on 10 *Homo sapiens* crania whose endocranial volumes were measured using a mustard seed filler; it was found that the difference between the measured and virtual endocast volumes was around 2% (Conroy *et al.*, 1998).

4.18.2.3.4 Measuring incomplete endocasts

4.18.2.3.4.(i) Partial endocast method Tobias (1964, 1971, p. 64) introduced a method for estimating endocranial volume. The method, which has become known as the partial endocast method, involves taking a complete endocast with known endocranial volume, reducing it to the anatomy preserved in the fossil of interest, and then determining what proportion of the complete endocast is represented by the reduced endocast. This provides a conversion factor to estimate complete endocranial volume for the specimen for which there is only a partial endocast. This method was originally used to determine the volume of OH 7, the type specimen of *H. habilis*, and its large endocranial capacity (estimated by the partial endocast method to be 675–680 cm^3; Tobias, 1964), was one of the reasons given for including the new taxon in the genus *Homo* (Leakey *et al.*, 1964). This spawned a debate revolving around the reliability and taxonomic implications of the original estimate, in which alternative methods to determine endocranial volumes from partial endocasts were suggested (Pilbeam, 1969; Wolpoff, 1969, 1981; Holloway, 1983d; Vaisnys *et al.*, 1984).

4.18.2.3.4.(ii) Reconstructed endocast method Synthetic and natural endocasts are typically reconstructed using plasticene to fill in missing areas. If only small parts of bilateral structures are missing on one side the necessary reconstruction does not require much guesswork. Holloway (1973, 1975) distinguishes between minimal plasticene reconstruction (method A) and extensive plasticene reconstruction involving close to half the total endocast (method B). Endocast reconstruction should be re-evaluated as additional fossils are discovered, and new, improved, methods should be applied to existing endocasts, not just to newly discovered evidence. Holloway's method involves making one endocast reconstruction based on comparisons with specimens belonging to the same hypodigm, or to members of different fossil hominin hypodigms (e.g., *P. robustus* and *P. boisei*) with brains that are similar in size and shape. Reconstructions made independently by different researchers provide a test of reliability. For example, the differences between the endocranial volumes of Holloway's (914 cm^3) and Broadfield's (921 cm^3) reconstructions of the Sambungmacan 3 calvaria are minimal (Broadfield *et al.*, 2001).

4.18.2.3.5 Extrapolations from ecto- and endocranial linear metrics

Several formulas have been suggested to estimate brain volume from linear dimensions of the endocranial cavity of crania, or endocasts. MacKinnon *et al.* (1956) compared linear measurements of the cranial cavity taken from radiographic images to mustard seed endocranial capacities for 52 modern human crania. They devised the following formula, which predicts endocranial volume with an error of 0.62% (0.87 cc in a 1400 cc cranium):

$$V = 0.51[1/2(LHW - LBW)],$$

where L is endocast length from frontal pole to occipital pole, W is maximum width (usually taken at the level of the superior aspect of the temporal), B is the distance from bregma to basion, and H is the distance from vertex to the deepest portion of the cerebellar lobes.

Holloway (1973, p. 450) applied this formula to endocasts, but he replaced the value of 0.51 with f, a variable determined for each taxon:

$$V = f[1/2(LHW + LBW)].$$

It is not advisable to calculate endocranial volume from external head or cranial measurements. Simmons (1942) found that crania with similar external perimeter measurements had different internal capacities, and Wickett *et al.* (1994) found that head perimeter measurements were not significantly correlated with total brain size. Bookstein *et al.* (1999) found that some of the factors responsible for the differences between the external and the internal cranial form were independent, so the inability of external head dimensions to accurately predict endocranial volume is not surprising. Further, there are particular problems with applying formulas designed for modern humans on fossil hominins. Formulas that have been developed to estimate modern human endocranial volume, such as those of Welcker (1885), Pearson (1926), and Manouvrier (1898), do not provide accurate estimations of the cranial capacity of fossils (Olivier and Tissier, 1975). Olivier and Tissier (1975) developed formulas specifically designed for archanthropians and paleoanthropians, but the fact that members of the taxon *H. heidelbergensis* fall into both

categories suggests that the reliability of this approach is dependent on having a satisfactory taxonomy.

4.18.2.4 Comparing Different Types of Measurements and Estimates

Brain size can be measured as a volume or as a weight (or mass). European authors tend to use weight whereas American authors tend use mass, but in most cases these terms are used interchangeably. For consistency we will refer to masses in grams (g) and volumes in cubic centimeters (cm^3).

4.18.2.4.1 Brain tissue mass from brain mass Measurements reported as brain mass from autopsy brains typically include the leptomeninges (i.e., the arachnoid and pia mater) as well as whatever CSF remains in the ventricles (Peters *et al.*, 1998). Volumes taken from MRI or stained sections measure brain tissue volume from which the volume of the meninges and the CSF are excluded (Peters *et al.*, 1998). This is comparable to the net brain volume calculated by adding up brain volumes for various brain components (e.g., Stephan, 1981). In modern humans, the meninges and CSF are estimated to contribute an additional 183 g for males and 132 g for females (Peters *et al.*, 1998). Thus, for male modern humans:

$$\text{Brain tissue mass (g)} = [\text{Brain mass (g)}] - 183,$$

and for female modern humans:

$$\text{Brain tissue mass (g)} = [\text{Brain mass (g)}] - 132.$$

4.18.2.4.2 Brain mass from brain volume For a sample of 78 adult human brains, the brain tissue was found to have an average specific mass of 1.032 g cm^{-3} (Zilles, 1972). Thus,

$$\text{Brain mass (g)} = [\text{Brain volume } (cm^3)] \times 1.032.$$

The specific mass of the brain has also been determined by comparing rodent brain weights with volumes to give an average specific mass of 1.036 g cm^{-3} (Stephan, 1960). Thus,

$$\text{Brain mass (g)} = [\text{Brain volume } (cm^3)] \times 1.036.$$

This suggests that brain mass is *c.* 3% larger than brain volume (but see Jerison's argument below).

4.18.2.4.3 Brain volume from endocranial volume Brain volume and endocranial volume (= cranial capacity) are not identical. Endocranial volume is larger as it also includes meninges, CSF, and cranial nerves. Few data are available for actual brain volume and endocranial volume from the same specimen because it is difficult to remove the brain from the brain case without causing damage to either. Novel imaging techniques could improve our understanding of this relationship, although in practice different techniques are used to visualize soft (MRI) and hard (CT) tissues.

Pickering (1930) found a correlation between nonfixed brain volume as determined by water displacement and endocranial volume measured with mustard seed in a sample of 29 modern humans, using the following conversion formula ($r^2 = 0.805$):

$$\text{Brain volume} = (\text{Endocranial volume}) \times 0.8598.$$

In other words, approximately 14% of the endocranial volume does not represent brain volume.

4.18.2.4.4 Brain mass from endocranial volume Count (1947) suggested a value of 0.876 g cm^{-3} for brain mass/endocranial volume, so that:

$$\text{Endocranial volume } (cm^3) = [\text{Brain mass (g)}] \times 1.14,$$
$$\text{Brain mass (g)} = [\text{Endocranial volume } (cm^3)] \div 1.14.$$

Ruff *et al.* (1997) used an equation that acknowledged the allometric nature of the relationship between endocranial volume and brain mass (see Martin, 1990). Ruff *et al.* derived brain mass from endocranial volume using a regression based on brain masses from Stephan *et al.* (1970) and cranial capacities from Martin (1990) for 27 primate species ($r^2 = 0.995$):

$$\text{Brain mass (g)} = 1.476 \times [\text{Endocranial volume } (cm^3)]^{0.976}.$$

Jerison (1973, p. 30) does not recommend converting endocranial volumes into brain volumes or brain masses. Apparently, the specific gravity of the mammalian brain ranges from 0.9 to 1.1; for example, brain mass (in g) is approximately 5% larger than endocranial volume in insectivores (Bauchot and Stephan, 1967), whereas brain mass (in g) is approximately 3% smaller than endocranial volume in a cat (Jerison, 1973).

4.18.2.5 Indices for Estimating and Comparing the Relative Sizes of Brains

The relationship of brain size to body size, analyzed by Snell (1891), was the basis of Dubois' index of

cephalization (Dubois, 1897), which related brain size to (1) body size and somatic functions, and (2) the encephalization of psychic functions. Jerison and Martin have further investigated the relationship between brain size and body size, and have made contributions to the most widely used measure of relative brain size, the encephalization quotient.

4.18.2.5.1 Encephalization quotient Some researchers (e.g., Jerison, 1973; Martin, 1990) do not consider absolute brain size to be an appropriate way to compare the mental capacities of different species. Nor is it useful to compare the brain/body ratio, because this ratio decreases with increasing body size. The solution has been to plot log brain mass on log body mass, from which is derived the allometric formula:

$$E = kP^{\beta},$$

where E is brain size, P is body size, k is the allometric coefficient, and β is the allometric exponent. It has been suggested that different taxonomic groups tend to have a similar value for β (reflecting a consistent functional relationship), but different values for k (reflecting different grades; Martin, 1981). This is usually expressed as the log-transformed linear equation:

$$\log E = \log k + \beta(\log P).$$

The key variable β is typically referred to as the scaling coefficient.

For all mammals, Jerison (1961, 1973) observed that the relationship between brain size and body size was described by the equation

$$\text{Brain mass} = 0.12 \times (\text{Body mass})^{2/3}.$$

Jerison developed Dubois' proposal for an equation to quantify encephalization (Dubois, 1897), and derived what he referred to as the encephalization quotient (EQ):

$$\text{EQ} = (\text{Brain mass})/[0.12 \times (\text{Body mass})^{2/3}].$$

Encephalization occurs when there is a departure from the general relationship between brain size and body size. Encephalization occurs in mammals and birds, but is rare in other vertebrates. Encephalization is explained by Jerison's (1973) additive theory of brain size: $E = E_v + E_c$, where E is brain size, E_v is brain size determined by body size, and E_c is associated with improved adaptive capabilities. According to his theory, if $E_c = 0$, then the brain is of a size sufficient for somatic maintenance. If one assumes there is a relationship between brain size and neuron number, and if $E_c > 1$, then the brain has extra neurons designated to deal with extracorporeal pressures. The presence of extra neurons is referred to as encephalization.

A scaling coefficient value of 2/3 (or 0.66) was suggested for several sets of mammals (Snell, 1891; Jerison, 1955, 1961, 1973; Gould, 1975), but a later study suggested that a scaling coefficient of 3/4 (or 0.75) is more appropriate (Martin, 1981). Based on this, Ruff *et al.* (1997) used the following equation to generate EQ in hominins:

$$\text{EQ} = \text{Brain mass}/[11.22 \times (\text{Body mass})^{3/4}].$$

A scaling coefficient of approximately 3/4 (i.e., 0.78) was also described for a comprehensive sample of primates (Bauchot and Stephan, 1969), although subsets of primates have a wider range of values. The scaling coefficient of nonhuman hominoids (i.e., the apes) is much lower (e.g., EQ = 0.58; Bauchot and Stephan, 1969).

4.18.2.5.2 Other standards for brain size comparison Although brain size is most often considered in relation to body mass, other standards for comparing brain size have been used. Some authors suggest that the scaling relationship between brain mass and body mass is a surrogate measure for some underlying variable (e.g., Harvey and Krebs, 1990). CNS or CNS-related standards of comparison are sometimes preferred because they vary less intraspecifically, and measure brain versus nervous system information flow. All standards have advantages and disadvantages.

Krompecher and Lipak (1966) were the first to suggest scaling brain size against the mass of another CNS structure (the spinal cord); subsequently, Passingham (1975) scaled brain mass to a CNS-related structure (the foramen magnum). The latter method has the advantage that it uses a hard-tissue structure that is occasionally preserved in the hominin fossil record. The absolute size of the spinal cord gives an indication of total neuronal input and output to the brain. In fact, an index of brain size to nonbrain CNS size provides a direct measure of Jerison's extra neurons. On the other hand, it has been suggested that body mass is better for use in scaling relationships precisely because, unlike the CNS or CNS-related structures, it is independent of brain mass (Stephan *et al.*, 1988). Radinsky (1967) suggested that foramen magnum area was a good estimate of body size, although it is less variable within a species than is body mass. However, others have suggested that foramen magnum area is linked more closely with brain size than with overall body size (Jerison, 1973; Gould, 1975; Martin, 1981). In fact, the relationship of the size of a

given CNS or CNS-related structure to body mass is variable. For example, the relationship between body size and foramen magnum/medulla size may be strongly influenced by specializations such as adaptation to water (Stephan and Dieterlen, 1982; Stephan and Kuhn, 1982).

Finally, it has been suggested that CNS structures make better standards because they vary less within a species than body mass does (Radinsky, 1967). One reason why body mass varies so much is that it comprises several components, including muscle mass and adipose tissue, which are themselves variable (Pitts and Bullard, 1968). Muscle tissue, which is well innervated, and other components of fat-free mass scale more closely to CNS mass than does the mass of the less well-innervated adipose tissue (Schoenemann, 2004). This finding has implications for certain questions about the scaling of brain size to body size. For example, the difference between male and female brain mass might be partially explained by the fact that male body mass contains proportionally more muscle (Manouvrier, 1903; Ankney, 1992; Gould, 1996). Along these lines, when scaled to fat-free mass, it has been suggested that the very muscular Neanderthals would have much smaller relative brain size than would modern humans (Schoenemann, 2004).

4.18.2.5.3 Measure of brain organization: A/S ratio Scaling brain size to other appropriate parts of the CNS gives a direct indication of the size of the brain in relation to the amount of input and output. Hebb (1949) advocated replacing brain/body size comparisons with an A/S (association cortex/primary sensory cortex) ratio, since the primary sensory areas are related to input from an animal's surroundings, whereas association areas are involved in higher-level cognitive processing. This procedure is also advocated by Holloway, who discussed in detail the problems associated with basing intelligence on brain/body size relationships (Holloway, 1968, 1979; Holloway and Post, 1982).

4.18.2.6 Major Lines of Fossil Evidence for CNS Evolution, Plus Other Endocranial Morphology

Particular attention is paid to fossil anatomy that can be used to make inferences about the functional anatomy of the CNS. In addition, non-CNS related aspects of endocranial morphology are included.

4.18.2.6.1 CNS-related fossil evidence The categories described below are the major lines of evidence that can be used to infer CNS evolution from the hominin fossil record. Their comparative contexts are data from extant primates from the endocranium and brain, and from the vertebral column and spinal cord. There is a dearth of data about the CNS of extant hominoids, so most inferences should be treated as preliminary. The extant hominoid data tend to be based on very small samples (e.g., one or two individuals per species, and often the same individuals are used in several studies), and, with respect to the cerebral cortex, rely on gross morphological landmarks as proxies for functional regions (see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates).

4.18.2.6.1.(i) Absolute and relative brain size Sample mean EQs are calculated using the formula of Ruff *et al.* (1997), which is based on Martin (1981), and using the calculation for estimating brain mass from endocranial volume of Ruff *et al.* (1997), based on Martin (1990). A list of endocranial volumes used here is available from the authors upon request; mean body mass estimates are from Skinner and Wood (in press). For more information, see Section 4.18.2.5.1.

4.18.2.6.1.(ii) Left occipital right frontal (LORF) petalia This asymmetrical pattern, with a wider and more posteriorly protruding left occipital pole, and a wider right frontal lobe, is typical of modern humans, and is statistically significantly related to right-handedness; that is, left-handed and ambidextrous people are more likely to be symmetrical or have the opposite pattern (Le May, 1976). Although petalias are also common in great apes (Le May, 1976; Le May *et al.*, 1982), they are less frequent than in humans and rarely involve both the frontal and the occipital lobes (Holloway and de Lacoste-Lareymondie, 1982).

4.18.2.6.1.(iii) Orbital surface of frontal lobe The orbital surface of the frontal lobe is blunt and expanded in modern humans. In contrast, it is beaked and pointed in African apes. This region corresponds to cytoarchitectural area 10, which is involved in planning future actions, abstract thinking, and undertaking initiatives (Semendeferi *et al.*, 2001). A regression of nonhuman primate area-10 volumes against brain volumes shows that humans have a larger than expected area-10 volume, but the residual (6%) is less striking than for other regions (Holloway, 2002).

4.18.2.6.1.(iv) Fronto-orbital sulcus The fronto-orbital (or orbitofrontal) sulcus typically incises the orbitolateral border of the frontal lobe of African apes, but is rarely present on modern human brains (Falk, 1980b). Due to the opercular

expansion of the frontal lobe in humans, this sulcus has probably been shifted so far posteriorly that it now comprises the anterior limiting sulcus of the insula, giving modern human brains a distinctly shaped lateral edge of the frontal lobe (Connolly, 1950, p. 330). The human frontal lobe (Semendeferi *et al.*, 1997; Semendeferi and Damasio, 2000) and its cortex (Semendeferi *et al.*, 2002) have volumes expected for an ape of similar brain size. It has been suggested that the human prefrontal cortex is larger than expected for a primate with a similar sized brain (Deacon, 1997), and that it has a higher than expected white/gray matter ratio (Schoenemann *et al.*, 2005), but these inferences are not reliable (Semendeferi *et al.*, 2002; Sherwood *et al.*, 2005). Note, however, that even if the frontal lobe did not become relatively larger, it is still possible for the prefrontal cortex to become relatively larger within it.

4.18.2.6.1.(v) Broca's cap region Broca's cap as seen on endocasts represents portions of Brodmann areas 47 and 45 (Broadfield *et al.*, 2001). Broca's cap overlaps with (but does not exactly correspond to) Broca's language area. Broca's area corresponds to the Brodmann cytoarchitectural areas 45 and 44 (respectively, 'pars triangularis' and 'pars opercularis' of the inferior frontal gyrus) (Aboitiz and Garcia, 1997). In the majority of modern humans, the left hemisphere is dominant for language (see The Evolution of Language Systems in the Human Brain), and Broca's region on the left hemisphere is asymmetrically enlarged in comparison to the contralateral areas 45 and 44 (Amunts *et al.*, 1999). Although an enlarged Broca's cap is a characteristic of modern humans, it does occur, albeit more rarely, in apes (Holloway, 1996). Questions persist about whether the African ape Broca's area homologue exhibits modern humanlike asymmetry (Holloway, 1996; Cantalupo and Hopkins, 2001; Sherwood *et al.*, 2003). Investigators have drawn attention to modern humanlike Broca's cap asymmetry in fossil hominin endocasts, in particular to those specimens in which the left side is larger than its homologue on the right. They also describe overall size and convolutional detail, particularly in fossils where only one hemisphere is present. Because an asymmetry in which the left Broca's area is larger than the right (L > R) is related to right-handedness is a characteristic of most modern humans, attention is drawn to cases in which Broca's area L > R asymmetries are found along with LORF L > R asymmetries.

4.18.2.6.1.(vi) Temporal poles Modern human endocasts have anteriorly expanded, laterally pointed temporal poles (Falk *et al.*, 2005), in contrast to African apes, which have rounded temporal poles. In modern humans, the anterior lateral temporal pole, particularly in the left hemisphere, is involved in human face recognition and naming (Damasio *et al.*, 1996; Grabowski *et al.*, 2001). The corresponding monkey area, TG, also functions in visual learning and recognition (Horel *et al.*, 1984; Nakamura and Kubota, 1995).

4.18.2.6.1.(vii) Lunate sulcus

4.18.2.6.1.(vii).(a) Primary visual cortex reduction The lunate sulcus (LS) is within the secondary visual area, close to the anterior border of the primary visual cortex. Modern humans have a more posteriorly located primary visual cortex than do the great apes. Most modern human brains lack an LS, but when present it is situated more posteriorly than it is in the great apes. A regression of occipital lobe volumes against mean brain volumes from small samples of diverse primate species suggests that modern humans have substantially less (−121%) primary visual cortex than expected for a nonhuman primate of similar brain size (Holloway, 1992). Although chimpanzees typically have a relatively larger primary visual cortex than do modern humans, a minority of chimpanzees show repositioning of the LS to a more modern humanlike posterior position (Holloway *et al.*, 2003). Holloway *et al.* (2003) used this point to argue that the hypothetical panin–hominin common ancestor must also have had within its population individuals with reduced primary visual cortices, so you would expect this condition in early hominins such as *Australopithecus afarensis*. The LS may be unique among the cortical sulci visible on endocasts in that it might provide information about the proportion of cortex allocated to distinct functional categories, and provides an estimate of the aforementioned ratio of association to sensory cortex (Holloway, 1966, 1968).

4.18.2.6.1.(vii).(b) Parietal expansion The posterior location of the LS indicates relative reduction of the primary visual cortex and relative expansion of the posterior parietal association cortex. The posterior parietal lobe is concerned with several aspects of sensory processing and sensorimotor integration (Lynch, 1980; Hyvarinen, 1981). The superior parietal lobule subcomponent is involved in visuomotor tasks, including finger movements (Shibata and Ioannides, 2001). The inferior parietal lobule subcomponent is involved in language and calculation abilities, and it is greatly expanded in humans compared to monkeys (Simon *et al.*, 2002). Derived modern human behaviors involving the posterior

parietal lobe include enhanced social behavior, including communication, tool-making, and tool use (Holloway *et al.*, 2004a). Bruner *et al.* (2003) suggested that the unique globular shape of the neurocranium of *H. sapiens* is related to an additional expansion of the parietal lobe in modern humans. Bruner (2004) associated the manufacture of more sophisticated tools and refined language ability with this difference.

4.18.2.6.1.(viii) Posterior cranial fossa A cerebellar quotient (CQ = actual/predicted value) was obtained when recent modern human cerebellar volume (determined from posterior cranial fossa volume) was regressed against brain volume (determined from endocranial capacity) minus cerebellar volume (Weaver, 2001, 2005). Extant hominoid brain data suggest that the modern human cerebellum is smaller than would be expected for an ape of similar brain size (Rilling and Insel, 1998; Semendeferi and Damasio, 2000). The difference between modern human and great ape relative cerebellar volumes is statistically significant, although less dramatic when considered along with the range of inferred relative cerebellar volumes found within the hominin fossil record (Weaver, 2005). The cerebellum of modern humans is relatively larger than that of some earlier hominins, perhaps because its size is linked to the complexity of cognitive functions (Weaver, 2005).

4.18.2.6.1.(ix) Thoracic vertebral canal Differences in thoracic vertebral canal size between humans and nonhuman primates have been related to unique aspects of breathing in human speech (MacLarnon, 1993). The thoracic part of the vertebral canal and the spinal cord segments which it encases (in modern humans, T2–S2) are enlarged in modern humans compared with nonhuman primates. It is inferred that this difference is due to an increase in the size of the anterior horns of the spinal cord and of nerves stemming from the segments which innervate the mid or lower trunk region. Some of these nerves innervate intercostal muscles and a set of abdominal muscles that are responsible for the fine control of breathing in modern human speech (Campbell, 1968, 1974; Gould and Okamura, 1974). Modern human speech involves long, punctuated, and modulated utterances (Draper *et al.*, 1959; Campbell, 1968; Hixon and Weismer, 1995), which require respiratory control mechanisms far beyond those necessary for nonhuman primate vocalizations. For example, it appears that in modern human speech the exhalatory portion of the breathing cycle is extended (Borden and Harris, 1984); this is in contrast to primate vocalizations, which drop in pitch during their duration (MacLarnon and Hewitt, 1999, 2004). MacLarnon and Hewitt (1999) explored several alternate hypotheses for increased thoracic vertebral canal size in humans, including postural control for bipedalism, endurance running, and parturition, but found that none were congruent with the fossil and neurological evidence.

4.18.2.6.2 Other endocranial morphology Endocasts preserve information about cranial venous sinuses and meningeal arteries. These may have taxonomic significance, although there is no evidence that their morphology is related to brain function (Holloway *et al.*, 2004a).

4.18.2.6.2.(i) Cranial venous sinuses Modern humans, apes and most hominins have a dominant transverse-sigmoid cranial venous sinus system. In contrast, a subgroup of hominin taxa have a dominant occipital/marginal (O/M) sinus system; that is, the occipital and marginal sinus complex is enlarged, and the transverse and sigmoid sinus complex is reduced (Falk and Conroy, 1983). The functional relevance of variations in the cranial venous sinus system is not clear. The radiator hypothesis (see Constraints on Brain Size: The Radiator Hypothesis) interprets the cranial blood drainage specializations of hominin taxa as an epigenetic adaptation to bipedalism (Falk, 1986, 1990). These specializations include an enlarged O/M sinus system, multiple hypoglossal canals, and augmented emissary vein foramina. African apes have none of these anatomical features, except multiple hypoglossal canals. Within the hominin clade, different combinations of these traits occur. Both *Au. afarensis* and *Paranthropus* (*P. boisei* and *P. robustus*) have high frequencies of an enlarged O/M sinus system, and the latter has high frequencies of posterior condyloid foramina and multiple hypoglossal canals, but low frequencies of mastoid and parietal foramina. There is a trend in the hominin clade toward the modern human condition (e.g., an increase in the number of mastoid and parietal foramina in *H. erectus* and later *Homo*), and a decrease in the frequencies of an O/M venous sinus system (in *Au. africanus* and *Homo*). Multiple hypoglossal canals are found in *Pan* and generally within the hominin clade. Braga and Boesch (1997a) investigated the incidence of these venous channels in African apes and hominins. They did not find statistically significant differences between *Paranthropus* and African apes with respect to the frequency of condylar canals, nor did they find statistically significant differences between

Paranthropus, *Australopithecus*, and African apes with respect to the frequency of divided hypoglossal canals, mastoid canals, and parietal and occipital foramina. Further, they suggest that, if differences did exist, these would probably be due to differences in brain size between African apes and hominins. The statistical arguments are set out in subsequent papers (Braga and Boesch, 1997b; Falk and Gage, 1997).

4.18.2.6.2.(ii) Meningeal arteries Descriptions of meningeal vessels are confused by the fact that several descriptive systems have been used to categorize the patterns of these vessels, and homologies between the vascular systems of different species are uncertain (see Falk, 1993; Grimaud-Herve, 1997; Holloway *et al.*, 2004a). Meningeal vessels are meningeal arteries and veins that supply blood to the neurocranium and also to the dura mater (Holloway *et al.*, 2004a). Of the three components of the meningeal arterial system (anterior, middle, and cerebellar), the middle has undergone the greatest change in hominin evolution (Falk, 1993; Grimaud-Herve, 1994; Holloway *et al.*, 2004a). In modern humans, the middle meningeal artery is divided into an anterior (bregmatic) branch which primarily supplies the frontal region, and a posterior (lambdatic) branch which primarily supplies the parietal region (Netter, 1997). In addition, there is an obelic (middle) branch, which varies in its relationship to the other two branches, and which serves as the basis for Adachi's (1928) simplistic classification of human meningeal artery configurations. This system has influenced paleoanthropologists, but it is not applicable to nonhuman taxa because it assumes that meningeal arteries enter the middle cranial fossa through its floor, which is the case for nearly all modern humans. In the great apes some or all of the meningeal arteries may enter through the back of the orbit (Falk, 1993).

4.18.3 The Hominin Fossil Record

4.18.3.1 Defining Hominins

Molecular biology has revolutionized our knowledge of the relationships within the great ape clade of the Tree of Life. Relationships between organisms can now be pursued at the level of the genome instead of having to rely on morphology (traditional hard- and/or soft-tissue anatomy, or the morphology of proteins) for information about relatedness. Comparisons of the DNA of organisms have been made using two methods. In DNA hybridization, all the DNA is compared, but at a relatively crude level. In DNA sequencing, the base sequences of comparable sections of DNA are determined and then compared. The results of hybridization (e.g., Caccone and Powell, 1989) and sequencing studies of both nuclear and mtDNA (e.g., Bailey *et al.*, 1992; Horai *et al.*, 1992; Gagneux and Varki, 2001; Wildman *et al.*, 2002, 2003) are virtually unanimous in suggesting that modern humans and the African apes are more closely related to each other than any of them is to the orangutan. They also suggest that modern humans and modern chimpanzees, i.e., commom chimpanzees and bonobos, both (belonging to the genus *Pan*) are more closely related to each other than either is to the gorilla (Miyamoto *et al.*, 1987; Sibley and Ahlquist, 1987; Goodman *et al.*, 1990, 1998; Goodman, 1999; Salem *et al.*, 2003; Wildman *et al.*, 2003; Ruvolo, 2004; but see Ruano *et al.*, 1992; Deinard and Kidd, 1999; Barbulescu *et al.*, 2001). Phylogenetic analysis of genome-wide gene expression profiles of the anterior cingulate cortex (ACC) also supports these relationships (Uddin *et al.*, 2004).

Thus, if we accept that the hominin twig of the Tree of Life may extend back in time to *c.* 8 Mya, and that the earliest unambiguous hominin is probably *Au. anamensis* (see below), then between 8 and 4 Mya we would expect to find primitive hominin and primitive panin taxa, and close to 8 Mya we should expect to see evidence of the common ancestor of panins and hominins. Not all of these primitive taxa, be they hominins, panins, or members of another clade, are direct ancestors of modern humans and chimpanzees. Some will belong to extinct panin and hominin subclades and it is also possible there were major clades for which we have no living representative.

4.18.3.2 Terminology

Paleoanthropologists have differed, and still do differ, in the way they classify the higher primates. We have tried to avoid using technical terms, but some are necessary in order to understand the implications of the different classifications. Linnean taxonomic categories immediately above the level of the genus (i.e., the family, the subfamily, and the tribe) have vernacular equivalents that end in 'id', 'ine', and 'in', respectively. In the past, *H. sapiens* has been considered to be distinct enough to be placed in its own family, the Hominidae, with the other great apes grouped together in a separate family, the Pongidae. Thus, modern humans and their close fossil relatives were referred to as

'hominids', and the other great apes and their close fossil relatives were referred to as 'pongids' (Table 1).

However, this scheme is inconsistent with the overwhelming evidence that modern humans and chimpanzees are more closely related to each other than either is to the gorilla or to the orangutan. Some researchers advocate combining modern humans and chimps in the same genus (e.g., Wildman *et al.*, 2003), which, according to the rules of zoological nomenclature, must be *Homo*. We adopt a less radical solution. The taxonomy we prefer lumps all the great apes including humans into a single family, the Hominidae (Table 1), and recognizes three subfamilies within the Hominidae: Ponginae for the orangutans, Gorillinae for the gorillas, and Homininae for modern humans and chimpanzees. The latter subfamily is broken down into two tribes: Panini (or panins) for chimpanzees, and Hominini (or hominins) for modern humans. The latter is further broken down into two subtribes: one, Australopithecina, which includes only extinct hominin genera; and the other, Hominina, for the genus *Homo*, which includes the only living hominin taxon, *H. sapiens*. Thus, modern humans are hominids (family), hominines (subfamily), and then hominins (tribe). Modern humans and all the fossil taxa judged to be more closely related to modern humans than to chimpanzees are called hominins; the chimpanzee equivalent is panin. We use 'australopith' when we refer to taxa belonging to the subtribe Australopithecina.

Table 1 A traditional taxonomy and a modern taxonomy that take account of the molecular and genetic evidence that chimpanzees are more closely related to modern humans than they are to gorillas. Extinct taxa are given in bold type

Traditional taxonomy
Superfamily Hominoidea (hominoids)
- Family Hylobatidae (hylobatids)
  - Genus *Hylobates*
- Family Pongidae (pongids)
  - Genus *Pongo*
  - Genus *Gorilla*
  - Genus *Pan*
- Family Hominidae (hominids)
  - **Subfamily Australopithecinae (australopithecines)**
    - **Genus *Ardipithecus***
    - **Genus *Australopithecus***
    - **Genus *Kenyanthropus***
    - **Genus *Orrorin***
    - **Genus *Paranthropus***
    - **Genus *Sahelanthropus***
  - Subfamily Homininae (hominines)
    - Genus *Homo*

Modern taxonomy
Superfamily Hominoidea (hominoids)
- Family Hylobatidae (hylobatids)
  - Genus *Hylobates*
- Family Hominidae (hominids)
  - Subfamily Ponginae
    - Genus *Pongo* (pongines)
  - Subfamily Gorillinae
    - Genus *Gorilla* (gorillines)
  - Subfamily Homininae (hominines)
    - Tribe Panini
      - Genus *Pan* (panins)
    - Tribe Hominini (hominins)
      - **Subtribe Australopithecina (australopiths)**
        - **Genus *Ardipithecus***
        - **Genus *Australopithecus***
        - **Genus *Kenyanthropus***
        - **Genus *Orrorin***
        - **Genus *Paranthropus***
        - **Genus *Sahelanthropus***
      - Subtribe Hominina (hominans)
        - Genus *Homo*

4.18.3.3 Organizing the Hominin Fossil Record

The classification of the hominin fossil evidence is a controversial topic. Some researchers favor recognizing relatively few taxa, whereas others think that more species and genera are needed to accommodate the observed morphological diversity. We use a relatively speciose (or splitting) taxonomy, but we also provide an example of a less-speciose (or lumping) taxonomy so that readers can appreciate how the evidence for human evolution would look if interpreted in a different way. The taxa included in the two taxonomies are listed in Table 2. Some of the taxon names are used in different senses in the speciose and less-speciose taxonomies. When we refer in the text to the hypodigm (the fossil evidence referred to that taxon) of one of these taxa in the speciose taxonomy we generally use the less-inclusive interpretation of the taxon, *sensu stricto* (*s.s.*). To save space we omit the *sensu stricto* if we are not making a particular distinction between members of the taxon *sensu lato* and members of the taxon *sensu stricto* (e.g., *Au. afarensis* means *Au. afarensis, s.s.*). When we refer to the same taxon in the more inclusive taxonomy (i.e., the hypodigm is larger), the Linnean binomial is followed by *sensu lato* (e.g., *Au. afarensis sensu lato* or *Au. afarensis s.l.*). This indicates that we are using the taxon name in a 'looser' sense. Table 2 demonstrates how the less-speciose taxonomy maps onto the more-speciose one.

The temporal spans of the taxa in the speciose taxonomy are illustrated in Figure 1. The age of the first and last appearances of any taxon in the fossil record (called the first-appearance datum, or FAD, and last-appearance datum or LAD) almost certainly underestimate the temporal range of the

Table 2 Speciose (splitter) and less-speciose (lumper) hominin taxonomies and skeletal representation of the taxa in the speciose taxonomy

Speciose taxonomy	*Age (Mya)*	*Type specimen*	*Crania*	*Dentition*	*Axial*	*Upper limb*	*Lower limb*
S. tchadensis	7.0–6.0	TM 266-01-060-1	X	X			
O. tugenensis	6.0	BAR 1000'00		X		X	X
Ar. kadabba	5.8–5.2	ALA-VP-2/10	X	X		X	X
Ar. ramidus s.s.	4.5–4.4	ARA-VP-6/1	X	X		X	ff
Au. anamensis	4.2–3.9	KNM-KP 29281	ff	X		X	X
Au. afarensis s.s.	3.9–3.0	LH 4	X	X	X	X	X
K. platyops	3.5–3.3	KNM-WT 40000	X	X			
Au. bahrelghazali	3.5–3.0	KT 12/H1		X			
Au. africanus	3.0–2.4	Taung 1	X	X	X	X	X
Au. garhi	2.5	BOU-VP-12/130	X	X		?	?
P. aethiopicus	2.5–2.3	Omo 18.18	X	X			
P. boisei s.s.	2.3–1.3	OH 5	X	X		?	?
P. robustus	2.0–1.5	TM 1517	X	X		X	X
H. habilis s.s.	2.4–1.6	OH 7	X	X	X	X	X
H. rudolfensis	1.8–1.6	KNM-ER 1470	X	X			?
H. ergaster	1.9–1.5	KNM-ER 992	X	X	X	X	X
H. erectus s.s.	1.8–0.2	Trinil 2	X	X	?	?	X
H. antecessor	0.7–0.5	ATD6-5	X	X			
H. heidelbergensis	0.6–0.1	Mauer 1	X	X			X
H. neanderthalensis	0.2–0.03	Neanderthal 1	X	X	X	X	X
H. sapiens s.s.	0.19–present	None designated	X	X	X	X	X
H. floresiensis	0.090–0.012	Liang Bua 1	X	X	X	X	X

Less-speciose taxonomy	*Age (Mya)*	*Taxa included from long taxonomy*
Ar. ramidus s.l.	7.0–4.4	*Ar. ramidus s.s., Ar. kadabba, S. tchadensis, O. tugenensis*
Au. afarensis s.l.	4.5–3.0	*Au. afarensis s.s., Au. anamensis, Au. bahrelghazali, K. platyops*
Au. africanus	3.0–2.4	*Au. africanus*
P. boisei s.l.	2.5–1.3	*P. boisei s.s., P. aethiopicus, Au. garhi*
P. robustus	2.0–1.5	*P. robustus*
H. habilis s.l.	2.4–1.6	*H. habilis s.s., H. rudolfensis*
H. erectus s.l.	1.9–0.2	*H. erectus s.s., H. ergaster*
H. sapiens s.l.	0.7–present	*H. sapiens s.s., H. antecessor, H. heidelbergensis, H. neanderthalensis*
H. floresiensis	0.090–0.012	*H. floresiensis*

Skeletal representation key: X, present; ff, fragmentary specimens; ?, taxonomic affiliation of fossil specimen(s) uncertain.

taxa. Nonetheless, FADs and LADs provide an approximate temporal sequence for the hominin taxa. The heights of the columns of those taxa with good, well-dated, fossil records (e.g., *Au. afarensis* and *P. boisei*) are a reasonable estimate of the temporal range of those taxa, but the heights of other columns marked with an asterisk (e.g., *S. tchadensis* and *Au. bahrelghazali*) reflect uncertainties about the age of the taxon because either the sample size is too small, or because the estimates of temporal range are not reliable.

For various reasons it is very unlikely that we have a complete record of hominin taxonomic diversity, particularly in the pre-4-Mya phase of hominin evolution. This is because intensive explorations of sediments of this age have been conducted for less than a decade, and because these investigations have been restricted in their geographical scope. Thus, the fossil evidence we are working with in the early phase of hominin evolution is almost certainly incomplete. More taxa are likely to be identified. We should bear this in mind when formulating and testing hypotheses about any aspect of hominin evolution, especially the evolution of bipedalism. We have not used lines to connect the taxa because the constraints of existing knowledge suggest that there are only two relatively well-supported subclades within the hominin clade, one for *Paranthropus* taxa, and the other for post-*H. ergaster* taxa belonging to the *Homo* clade. Without more well-supported subclades it is probably unwise to try to identify specific taxa as ancestors or descendants of other taxa.

4.18.3.4 Review of Individual Hominin Fossil Taxa

Each hominin taxon is placed in one of six informal grades (Huxley, 1958) based on a combination of brain and postcanine tooth size and on inferred

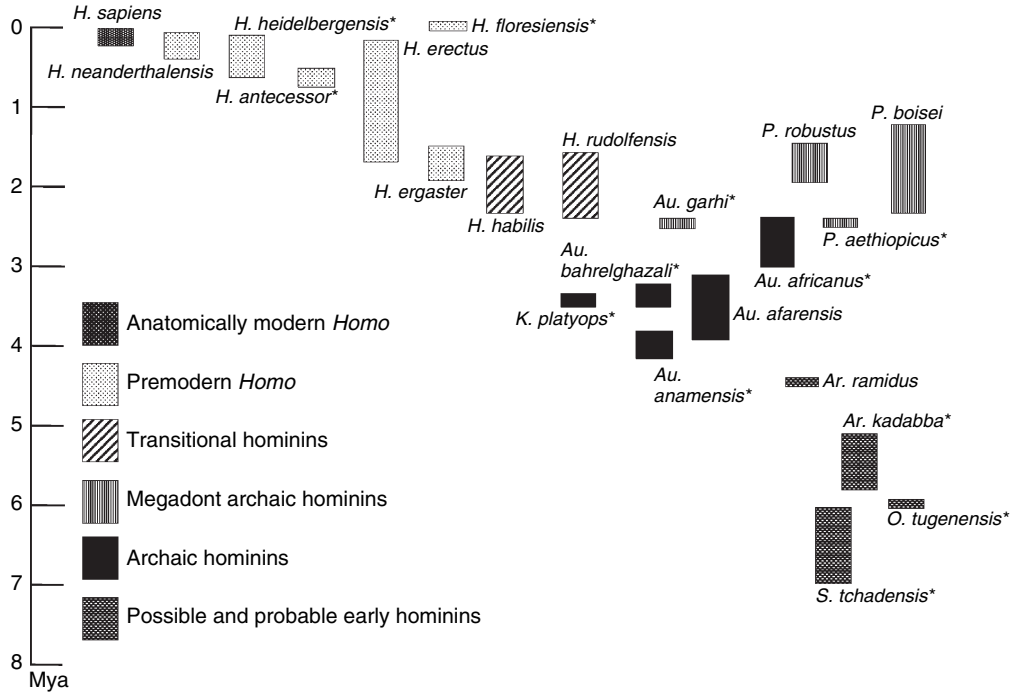

Figure 1 Speciose hominin taxonomy.

locomotor mode. The six grades are: possible and probable primitive hominins (PH); archaic hominins (AH); megadont archaic hominins (MAH); transitional hominins (TH); premodern *Homo* (PMH); and anatomically modern *Homo* (AMH) (Figure 1). Several taxon samples are too small to do other than make an informed guess about the grade of the taxon. Within each grouping, the taxa are listed in order of their first appearance in the fossil record.

Unless homoplasy (shared morphology not derived from the most recent common ancestor) is more common than we anticipate, there is little doubt that recent hominin taxa (i.e., post-*H. ergaster* taxa in group PMH) are more closely related to modern humans than to chimpanzees. These taxa all have absolutely and relatively large brains, they were obligate bipeds, and they have small canines, slender jaws, and small chewing teeth. However, the closer we get to the split between hominins and panins the more difficult it is to find features we can be sure fossil hominins possessed and fossil panins, or taxa in any other closely related clade, did not. In the early stages of hominin evolution it may be either the lack of panin features or relatively subtle differences in the size and shape of the canines, or in the detailed morphology of the limbs, that mark out hominins.

We are conscious that many readers might be unfamiliar with the details of the hominin fossil record, so we provide basic information about the morphology of each taxon. The formal way to cite a taxon name is to give the Linnean binomial, followed by the name(s) of the authors and the date of the paper that introduced the taxon. Conventionally, this citation is not placed in parentheses. However, when a taxon has been moved from its initial genus, the original reference is placed within parentheses followed by the revising reference: for example, *Homo erectus* (Dubois, 1892) Weidenreich, 1940. Further details about most of the taxa and a more extensive bibliography can be found in Wood and Richmond (2000). Recent relevant reviews of most of these taxa can be found in Hartwig (2002) and Wood and Constantino (2004).

4.18.4 Review of Hominin Taxa

4.18.4.1 Possible and Probable Primitive Hominins

This category includes one species, *Ardipithecus ramidus*, which is almost certainly a member of the hominin clade, one species, *Sahelanthropus tchadensis*, which is a possible hominin, and two species, *Ardipithecus kadabba* and *Orrorin tugenensis*, which may be hominins.

Taxon name. Sahelanthropus tchadensis Brunet *et al.*, 2002.

Temporal range. *c*. 7–6 Mya.

Initial discovery. TM 266-01-060-1 – an adult cranium; Toros-Menalla, Chad, 2001 (Brunet *et al*., 2002).

Type specimen. As above.

Source(s) of the evidence. Toros-Menalla, Chad, Central Africa.

Nature of the evidence. A distorted cranium, although an attempt to correct the distortion has been made in a virtual reconstruction (Zollikofer *et al*., 2005), plus several mandibles and some teeth.

Characteristics and inferred behavior. Cranial: A chimp-sized animal displaying a novel combination of primitive and derived features. Much about the base and vault of the cranium is chimp-like, but the relatively anterior placement of the foramen magnum, the presence of a supraorbital torus, the lack of a muzzle, the small, apically worn canines, the low, rounded molar cusps, the relatively thick tooth enamel, and the relatively thick mandibular corpus (Brunet *et al*., 2002) suggest that *S. tchadensis* does not belong in the *Pan* clade. Postcranial: No evidence. Conclusion: It is either a primitive hominin, the common ancestor of hominins and panins, or it belongs to a separate clade of hominin-like apes.

CNS-related fossil evidence. The endocranial volume is estimated to be between 350 and 370 cm^3, the smallest of any adult hominin, and within the range of extant chimpanzees.

Taxon name. *Orrorin tugenensis* Senut *et al*., 2001.

Temporal range. *c*. 6.0 Mya.

Initial discovery. KNM LU 335– left mandibular molar tooth crown; Tugen Hills, Baringo, Kenya, 1974 (Pickford, 1975).

Type specimen. BAR 1000'00 – fragmentary mandible; Tugen Hills, Baringo, Kenya, 2000 (Senut *et al*., 2001).

Source(s) of the evidence. The relevant remains come from four localities in the Lukeino Formation, Tugen Hills, Kenya.

Nature of the evidence. The 13 specimens include three femoral fragments.

Characteristics and inferred behavior. Cranial: The discoverers admit that much of the critical dental morphology is "ape-like" (Senut *et al*., 2001, p. 6). Postcranial: The femoral morphology has been interpreted (Pickford *et al*., 2002; Galik *et al*., 2004) as suggesting that O. *tugenensis* is an obligate biped, but some researchers interpret the radiographs and CT scans of the femoral neck as indicating a mix of bipedal and nonbipedal locomotion. Conclusion: O. *tugenensis* could prove to be a hominin, but it is more likely that it belongs to another part of the adaptive radiation that included the common ancestor of panins and hominins.

CNS-related fossil evidence. None.

Taxon name. *Ardipithecus kadabba* Haile-Selassie *et al*., 2004.

Temporal range. 5.8–5.2 Mya.

Initial discovery. ALA-VP-2/10 – partial mandible; Alayla, Western Margin, Middle Awash, Ethiopia, 1997.

Type specimen. As above.

Source(s) of the evidence. Middle Awash and Gona, Ethiopia.

Nature of the evidence. Mandible, teeth, and postcranial evidence.

Characteristics and inferred behavior. Cranial: The upper canine and lower first premolar morphology is less ape-like than that of O. *tugenensis*, but more ape-like than that of *Ar. ramidus*. The researchers who found it suggest that it closely approaches the extant and fossil ape condition (Haile-Selassie *et al*., 2004, p. 1505); they also suggest that the morphology of the canine–premolar complex of *S. tchadensis*, O. *tugenensis*, and *Ar. kadabba* is so similar that they may belong to one genus, or even one species (Haile-Selassie *et al*., 2004). Postcranial: *Ar. kadabba* has been suggested to be a biped on the basis that the shape of the proximal articular surface of the fourth proximal phalanx of the foot resembles that of *Au. afarensis*, but similar morphology may also be found in the quadrupedal African apes. Conclusion: *Ar. kadabba* is probably a member of an extinct clade closely related to hominins and panins.

CNS-related fossil evidence. None.

Taxon name. *Ardipithecus ramidus* (White *et al*., 1994) White *et al*., 1995.

Temporal range. *c*. 4.5–4.4 Mya.

Initial discovery. ARA-VP-1/1 – right M^3; Aramis, Middle Awash, Ethiopia, 1993 (White *et al*., 1994). (NB: if a mandible, KNM-LT 329, from Lothagam, Kenya, proves to belong to the hypodigm, then this would be the initial discovery.)

Type specimen. ARA-VP-6/1 – associated upper and lower dentition; Aramis, Middle Awash, Ethiopia, 1993 (White *et al*., 1994).

Source(s) of the evidence. A site called Aramis in the Middle Awash region of Ethiopia. A second suite of fossils, including a mandible, teeth, and postcranial bones, recovered in 1997 from five localities in the Middle Awash, that range in age from >5.7 to 5.2 Mya were initially allocated to this taxon (Haile-Selassie, 2001), but they were

subsequently transferred to *Ar. kadabba* (see above).

Nature of the evidence. The published evidence consists of isolated teeth, a piece of the base of the cranium, and fragments of mandibles and long bones. A fragmented associated skeleton has been found and is being prepared and reconstructed.

Characteristics and inferred behavior. Cranial: The remains attributed to *Ar. ramidus* share some features in common with living species of *Pan*, others that are shared with the African apes in general, and, crucially, several dental and cranial features are shared only with later hominins such as *Au. afarensis*. Its chewing teeth are relatively small, and the thin enamel covering on the teeth suggests that the diet of *Ar. ramidus* may have been closer to that of the chimpanzee than to that of modern humans. Postcranial: Judging from the size of the shoulder joint, *Ar. ramidus* weighed about 40 kg. The position of the foramen magnum suggests that the posture and gait of *Ar. ramidus* was respectively more upright and bipedal than is the case in the living apes. Conclusions: The discoverers initially allocated the new species to *Australopithecus* (White *et al.*, 1994), but they subsequently assigned it to a new genus, *Ardipithecus* (White *et al.*, 1995). Of the hominin taxa in this category, *Ar. ramidus*, is the most likely to be an early hominin.

CNS-related fossil evidence. A crushed cranium has been reported, but no details have been published.

4.18.4.2 Archaic Hominins

This group, which includes all the remaining hominin taxa not conventionally included in *Homo* and *Paranthropus*, subsumes two genera, *Australopithecus* and *Kenyanthropus*. As it is used in this and many other taxonomies, *Australopithecus* is almost certainly not a single clade, but until researchers can generate a reliable phylogeny there is little point in revising its generic terminology.

Taxon name. *Australopithecus anamensis* Leakey *et al.*, 1995.

Approximate time range. *c.* 4.2–3.9 Mya.

Initial discovery. KNM-KP 271 – left distal humerus; Kanapoi, Kenya, 1965 (Patterson and Howells, 1967).

Type specimen. KNM-KP 29281 – an adult mandible with complete dentition and a temporal fragment that probably belongs to the same individual; Kanapoi, Kenya, 1994.

Source(s) of the evidence. Allia Bay and Kanapoi, Kenya.

Nature of the evidence. The evidence consists of jaws, teeth, and postcranial elements from the upper and lower limbs.

Characteristics and inferred behavior. Cranial: The main differences between *Au. anamensis* and *Au. afarensis* relate to details of the dentition. In some respects the teeth of *Au. anamensis* are more primitive than those of *Au. afarensis* (e.g., the asymmetry of the premolar crowns, and the relatively simple crowns of the deciduous first mandibular molars), but in others (e.g., the low cross-sectional profiles, and bulging sides of the molar crowns) they show similarities to *Paranthropus* (see below). Postcranial: The upper limb remains are australopith-like, but a tibia attributed to *Au. anamensis* has features associated with obligate bipedality.

CNS-related fossil evidence. None.

Taxon name. *Australopithecus afarensis* Johanson *et al.*, 1978.

Approximate time range. *c.* 4–3 Mya.

Initial discovery. AL 128-1 – left proximal femur fragment; Hadar Formation, Afar, Ethiopia, 1973 (Johanson and Taieb, 1976).

Type specimen. LH 4 – adult mandible; Laetoli, Tanzania, 1974.

Source(s) of the evidence. Laetoli, Tanzania; White Sands, Hadar, Maka, Belohdelie and Fejej, Ethiopia; Allia Bay, West Turkana, and Tabarin, Kenya.

Nature of the evidence. *Au. afarensis* is the earliest hominin to have a comprehensive fossil record including a well-preserved skull, several crania, many lower jaws, and sufficient limb bones to be able to estimate stature and body mass. The collection includes a specimen, AL-288, that preserves just less than half of the skeleton of an adult female.

Characteristics and inferred behavior. Cranial: It has incisors that are much smaller than those of extant chimpanzees, but the premolars and molars of *Au. afarensis* are relatively larger than those of the chimpanzee. Postcranial: The hind limbs of AL-288 are substantially shorter than those of a modern human of similar stature. The range of body mass estimates is from 25 to >50 kg. The upper limb, especially the hand, retains morphology that most likely reflects a significant element of arboreal locomotion. The size of the footprints, the length of the stride, and stature estimates based on the length of the limb bones suggest that the standing height of adult individuals in this early hominin species was between 1.0 and 1.5 m. Direct evidence of the

locomotion of either *Au. afarensis* or another synchronic hominin comes from the 3.6 Mya hominin footprint trails at Site G in the Laetolil Formation, at Laetoli, Tanzania. If we assume that the skeletal evidence from Hadar and the trace fossil evidence from Laetoli represents one taxon, and if we weigh the primitive and derived characteristics observed in the Hadar pedal remains and in the Laetoli footprints, the form and function of the *Au. afarensis* locomotor system was not modern humanlike.

CNS-related fossil evidence. Endocranial: *Au. afarensis* is represented by at least 15 specimens that preserve endocranial anatomy and some for which endocranial volume can be estimated. The estimated mean adult endocranial volume is 446 cm^3 ($n = 5$; range 387–550). The range is interpreted as reflecting a substantial degree of sexual dimorphism. The average of the two largest crania (probable males) is 125 cm^3 greater than that for the three smallest crania (probable females). These values are larger than the average endocranial volume of a chimpanzee, and if the estimates of the body size of *Au. afarensis* are approximately correct then relative to estimated body mass the brain of *Au. afarensis* is substantially larger than that of *Pan*. The sample mean EQ for *Au. afarensis* is 2.5. Holloway has long argued that the primary visual cortex is relatively reduced in size in *Au. afarensis*, as is apparent in two specimens, AL 162-28 (Holloway, 1983a; Holloway and Kimbel, 1986) and AL 288-1 (Holloway *et al.*, 2004a). However, he allows that another specimen (AL 333-45) may have a proportionately larger, more ape-like, primary visual cortex. Other evidence presented by Holloway *et al.* for brain organization in *Au. afarensis* is ambiguous. There may be evidence of a slight left occipital petalia in one specimen (AL 333-45), but a juvenile specimen (AL 333-105) retains the ape pattern of an inferior frontal orbital sulcus (Holloway *et al.*, 2004a). On the other hand, Falk (1985b) argues that *Au. afarensis* (particularly AL 162-28) has an entirely ape-like sulcal pattern with no evidence of a relatively reduced primary visual cortex, and with an ape-like condition of a cerebellum projecting posteriorly beyond the occipital lobe, a suggestion Holloway and Kimbel (1986) and Holloway *et al.* (2004a) dismiss as a misinterpretation due to the way the fossil was oriented. Vertebral canal: This species is represented by four sets of vertebras. The most complete set of vertebras includes 15 vertebral elements belonging to AL 288-1, of which the thoracic vertebras have been used to make inferences about the spinal cord. *Au. afarensis* retains small (relative to modern human) thoracic vertebral canals in cross-sectional area, suggesting a small thoracic spinal cord, and by inference a lack of fine control of breathing for speech, and thus the lack of a complex vocal language ability (MacLarnon and Hewitt, 1999). Behavioral interpretations: Both Holloway and Falk recognize that the brain of *Au. afarensis* shows some degree of reorganization toward the modern human condition. However, a point of contention between these authors has been the relative location of the LS, and the coincident reduction of the primary visual cortex and expansion of the parietal association cortex cortex. Some *Au. afarensis* brains have a posteriorly placed LS, but some chimpanzee brains also have a posteriorly placed LS (Figure 2b) (Holloway *et al.*, 2003). Holloway *et al.* (2003) argue that the hypothetical panin–hominin common ancestor must also have had within its population individuals with reduced primary visual cortices, so you would expect this condition in early hominins such as *Au. afarensis*.

Other endocranial morphology. Cranial venous sinuses: Eight *Au. afarensis* from Hadar (AL 333-45, Al 333-105, AL 333-114, AL 333-116, AL 162-28, AL 444-2, AL 288-1, and AL 439-1) have an enlarged, and thus presumably dominant, occipital marginal sinus system (Kimbel *et al.*, 2004; Falk *et al.*, 1995). However, LH 21, and probably AL 224-9 and AL 427-1b, lack an enlarged occipital marginal sinus system. Meningeal vessels: Present, but not diagnostic.

Taxon name. *Kenyanthropus platyops* Leakey *et al.*, 2001.

Approximate time range. *c.* 3.5–3.3 Mya.

Initial discovery. KNM-WT 38350 – left maxilla fragment; Lomekwi, West Turkana, Kenya, 1998 (Leakey *et al.*, 2001).

Type specimen. KNM-WT 40000 – a relatively complete cranium but it is criss-crossed by matrix-filled cracks; Lomekwi, West Turkana, Kenya, 1999 (Leakey *et al.*, 2001).

Source(s) of the evidence. West Turkana, and perhaps Allia Bay, Kenya.

Nature of the evidence. The initial report lists the type cranium and the paratype maxilla plus 34 specimens – including three mandible fragments, a maxilla fragment, and isolated teeth – some of which may also belong to the hypodigm, but at this stage the researchers are reserving their judgment about the taxonomy of many of these remains (Leakey *et al.*, 2001). Some of them have only recently been referred to *Au. afarensis* (Brown *et al.*, 2001).

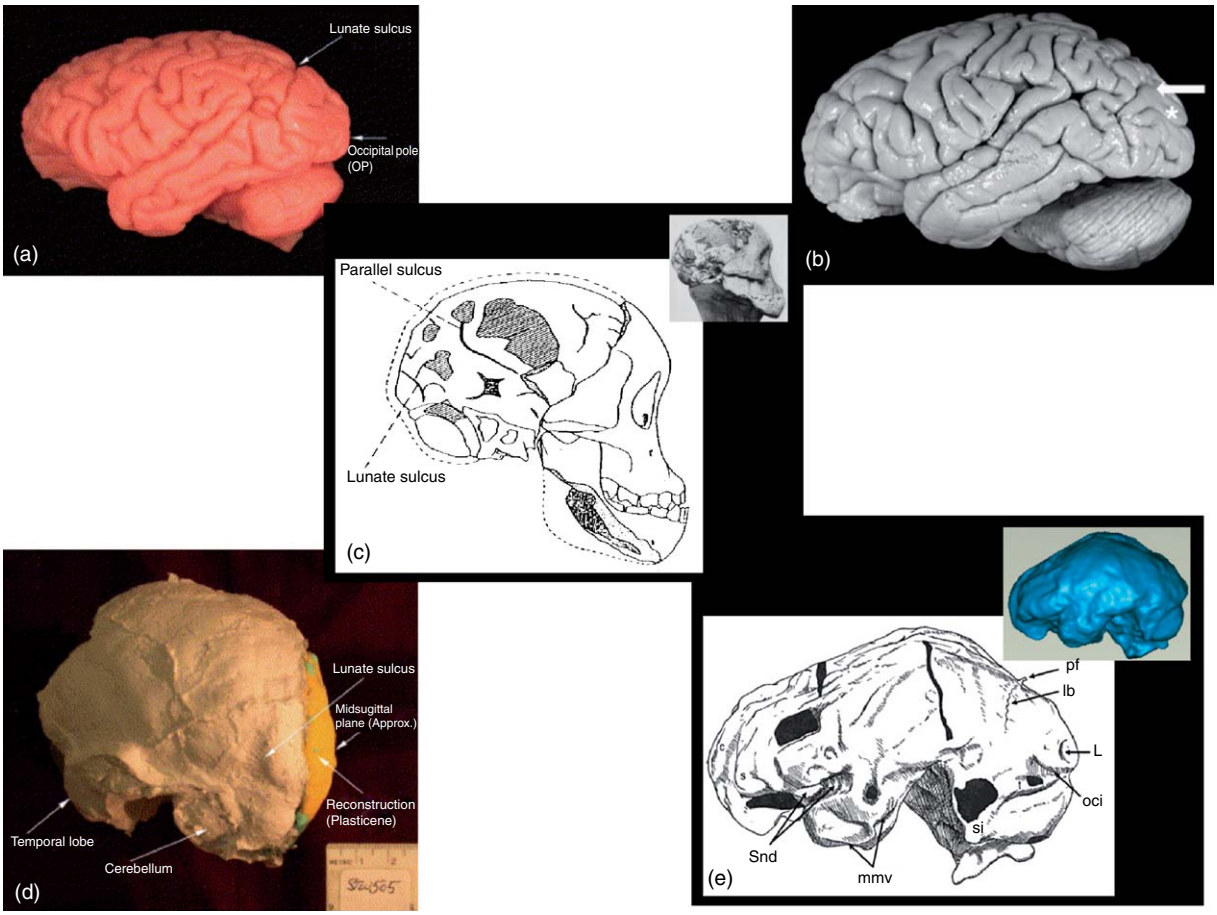

Figure 2 a, Left lateral view of a typical chimpanzee brain cast showing relatively anterior position of the LS (Holloway *et al.*, 2004b). b, Left lateral view of unusual chimpanzee brain with the LS in a more posterior position (* indicates lateral calcarine fissure) (Holloway *et al.*, 2003). c, Right lateral view of a natural endocast and sketch of juvenile *Au. africanus* (Taung) (Dart, 1925). d, Left oblique view of *Au. africanus* (Stw 505) partially reconstructed endocast showing the location of the LS (Holloway *et al.*, 2004b). e, Left lateral view of virtual endocast and sketch of *H. floresiensis* (LB1) showing the location of the LS (Falk *et al.*, 2005). Images are not shown to the same scale. a and d, Reproduced from Holloway, R. L., Clarke, R. J., and Tobias, P. V. 2004b. Posterior lunate sulcus in *Australopithecus africanus:* Was Dart right? *C. R. Palevol.* 3, 287–293, Elsevier. b, Reproduced from Morphology and histology of chimpanzee primary visual striate cortex indicate that brain reorganization predated brain expansion in early hominid evolution, *Anat. Rec.*; Holloway, R. L., Broadfield, D. C., and Yuan, M. S.; Copyright © 2003, Wiley-Liss. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. c, Reproduced from Dart, R. A. 1925. *Australopithecus africanus:* The man-ape of South Africa. *Nature* 115, 195–199, with permission from Nature Publishing Group. e, Reprinted with permission from Falk, D., Hildebolt, C., Smith, K., *et al.* 2005. The brain of LB1, *Homo floresiensis. Science* 308, 242–245. Copyright 2005 AAAS.

Characteristics and inferred behavior. Cranial: The main reasons why Leakey *et al.* (2001) did not assign this material to *Au. afarensis* are its reduced subnasal prognathism, anteriorly situated zygomatic root, flat and vertically orientated malar region, relatively small but thick-enameled molars, and the unusually small M^1 compared to the size of the P^4 and M^3. Some of the morphology of the new genus, including the shape of the face, is *Paranthropus*-like, yet it lacks the postcanine megadontia that characterizes *Paranthropus*. Postcranial: No evidence. Conclusion: The authors note that the face of the new material resembles that of *H. rudolfensis* (see below), but they rightly point out that the postcanine teeth of the latter are substantially larger than those of KNM-WT 40000. *K. platyops* apparently displays a hitherto unique combination of facial and dental morphology. Some researchers contend that KNM-WT 40000 is an *Au. afarensis* cranium that is distorted by the matrix-filled cracks.

CNS-related fossil evidence. No estimate of endocranial volume is given, although it is apparently australopith-like in size.

Taxon name. *Australopithecus bahrelghazali* Brunet *et al.*, 1996.

Approximate time range. *c.* 3.5–3.0 Mya.

Initial discovery. KT 12/H1 – anterior portion of an adult mandible; Koro Toro, Chad, 1995 (Brunet *et al.*, 1996).

Type specimen. As above.

Source(s) of the evidence. Koro Toro, Chad.

Nature of the evidence. Published evidence is restricted to a fragment of the mandible and an isolated tooth.

Characteristics and inferred behavior. Cranial: Its discoverers claim that its thicker enamel distinguishes the Chad remains from *Ar. ramidus*, and that its smaller mandibular symphysis and more complex mandibular premolar roots distinguish it from *Au. afarensis*. Otherwise, there is too little evidence to infer any behavior. Postcranial: No evidence. Conclusion: This taxon may be a regional variant of *Au. afarensis*.

CNS-related fossil evidence. None.

Taxon name. *Australopithecus africanus* (Dart, 1925).

Approximate time range. *c*. 3.0*–2.4 Mya. *NB: it remains to be seen whether the associated skeleton Stw 573 from Member 2 (Clarke and Tobias, 1995; Clarke, 1998, 1999, 2002) and the 12 hominin fossils recovered from the Jacovec Cavern since 1995 (Partridge *et al.*, 2003) belong to the *Au. africanus* hypodigm. Samples of quartz grains from Member 2 and the Jacovec Cavern have recently been dated to *c*. 4.2–4.0 Mya using ratios of the ^{26}Al and ^{10}Be radionuclides (Partridge *et al.*, 2003).

Initial discovery. Taung 1 – a juvenile skull with partial endocast; Taung (formerly Taungs), now in South Africa, 1924.

Type specimen. As above.

Source(s) of the evidence. Most of the evidence comes from two caves, Sterkfontein and Makapansgat, with other evidence coming from caves at Taung and Gladysvale.

Nature of the evidence. This is one of the best, if not the best, fossil record of an early hominin taxon. The cranium, mandible, and dentition are well sampled. The postcranium and particularly the axial skeleton is less well represented in the sample, but there is at least one specimen of each of the long bones. However, many of the fossils have been crushed and deformed by rocks falling onto the bones before they were fully fossilized.

Characteristics and inferred behavior. Cranial: *Au. africanus* had relatively large chewing teeth and apart from the reduced canines the skull is relatively ape-like. Postcranial: Overall, the postcranial remains of *Au. africanus* suggest that this hominin could engage in both arboreal and bipedal locomotion. The Sterkfontein evidence suggests that males and females of *Au. africanus* differed substantially in body size, but probably not to the degree they did in *Au. afarensis* (see above). Conclusion: This is a hominin with a mixed locomotor mode, part climbing and part bipedal walking.

CNS-related fossil evidence. Endocranial: *Au. africanus* is represented by at least 15 specimens representing the neurocranium, many of which are natural endocasts. *Au. africanus* endocasts are unique among hominin endocasts in that they preserve a great deal of convolutional detail (Falk, 1987). This species has the most studied (and debated) endocast morphology of any fossil hominin species. The estimated mean adult endocranial volume for *Au. africanus* is 460 ($n = 9$; range 428–515). The sample mean EQ for *Au. africanus* is 2.8. An early claim for reorganization of the cerebral cortex, in the form of a relatively reduced primary visual cortex and relatively expanded parietal–occipital association areas, was made by Dart (1925) on the basis of a humanlike posteriorly located LS on the Taung child endocast (Figure 2c). This observation is the focus of a long-standing debate over the identification and location of the LS, including support for Dart's view (Holloway, 1975), one author arguing for a very posterior position (Schepers, 1946), others arguing for an anterior position (Keith, 1931; Le Gros Clark *et al.*, 1936; Falk, 1980b, 1985a), and some noting that it is impossible to know the position with certainty (Le Gros Clark, 1947; Holloway, 1985; Tobias, 1991). The debate over the location of the Taung LS developed into a larger debate between Falk and Holloway about whether brain reorganization preceded brain size increase. On the one hand, Holloway (Holloway, 1975, 1983a, 1984, 1985, 1988b) and Holloway *et al.* (2004a, 2004b) maintain that Taung and other *Au. africanus* specimens demonstrate aspects of humanlike brain organization. Holloway does not maintain that a particular line clearly represents the LS, but states that for Taung there is no good evidence for a LS in a typical anterior pongid position (Holloway *et al.*, 2004a, p. 97). In addition, Holloway *et al.* claim that two other specimens (Stw 505 and Sterfontein Type 3) also show a relative reduction in primary visual cortex size, based on the position of the LS (see Figure 2d) (Holloway *et al.*, 2004a, p. 97). In addition, they find evidence of a humanlike brain morphology associated with right-handedness. Two specimens (Sts 5 and Sterfontein type 2) have Broca's cap regions which demonstrate a trend toward a modern humanlike pattern, and one of these (Sts 5) displays a slight LORF petalia (Holloway *et al.*, 2004a). On the

other hand, Falk (1980b, 1983b, 1985a) maintains and defends her interpretation of a more anterior, ape-like, position for the LS in Taung and other *Au. africanus* individuals. In addition, Falk points to other ape-like features of *Au. africanus* endocasts, most significantly the fronto-orbital sulcus. Falk (1980b) claims that Taung is ape-like in the position and size of the fronto-orbital sulcus at the lateral edge of frontal lobe (but see Holloway, 1981b). Five other *Au. africanus* endocasts (Sts 60, Type 2, Type 3, Sts 58, Sts 1017) are considered to have similar ape-like sulcal patterns, although based on much less evidence (Falk, 1980b). However, Falk *et al.* (2000) have pointed to derived, modern humanlike aspects of *Au. africanus* brain morphology that differentiate this species from *Paranthropus*. The orbital surface of the frontal lobe is blunt and expanded in *Au. africanus* (Sts 5, Stw 505, and see also the more fragmentary Type 2 and Sts 60) just as in *H. sapiens* (Falk *et al.*, 2000). Anteriorly expanded, laterally pointed temporal poles also characterize *Au. africanus* and *H. sapiens* to the exclusion of *Paranthropus* (Falk *et al.*, 2000). Vertebral canal: One set of 15 vertebras (Sts 14) and two individual vertebras (Sts 65, Sts 73) have been referred to this species. *Au. africanus* thoracic vertebral canals have a small cross-sectional area relative to those of modern humans. Behavioral interpretations: In the original description of the type specimen, Dart argued that *Au. africanus* demonstrates humanlike brain reorganization on the basis of the position of the LS. Although Falk does not concede the position of the LS, Falk *et al.* (2000) do agree that in other respects the *Au. africanus* brain has been reorganized relative to African ape brains. There are several examples showing an early start to a trend toward modern humanlike cortical reorganization (Falk *et al.*, 2000). The enlarged orbital surfaces of the frontal lobes in *Au. africanus* are thought to indicate an expansion of area 10 prior to brain size increase. The anterior lateral regions of the temporal poles, which show a modern humanlike expansion in *Au. africanus*, are involved in visual learning and recognition. In addition, *Au. africanus* (Sts 5) is more modern humanlike than ape-like in the size and shape of its olfactory bulbs (Falk *et al.*, 2000). In modern humans, olfactory bulbs are less than half the size of those of the apes. Furthermore, the fraction of functioning olfactory receptor genes is reduced in modern humans relative to apes (Gilad *et al.*, 2003a), a finding that corroborates behavioral data that suggest modern humans do not rely on their sense of smell as much as apes do. Reduction of olfactory bulb size, and corresponding changes in the dependence on smell (Gilad *et al.*, 2003b), may have arisen early in hominin evolution. Falk *et al.* (2000) point to shared morphology in *Au. africanus* and *H. sapiens* to the exclusion of *Paranthropus* as evidence of an *Au. africanus–Homo* lineage, but this is a highly contested proposal (e.g., Johanson and White, 1979). In spite of evidence for brain reorganization, there is no very strong evidence for brain morphology that can be related to language capacity. Moreover, the small vertebral canals in the thoracic region suggest a lack of fine breathing control, and thus the lack of complex vocal language ability. This conclusion is similar to that for *Au. afarensis*.

Other endocranial morphology. Cranial venous sinuses: Most researchers who have studied the cranial venous sinuses of *Au. africanus* suggest that it retains a symplesiomorphic dominant transverse sigmoid sinus and lacks an enlarged O/M sinus (e.g., Sts 5, Sts 19, Sts 26, MLD 1 and MLD 37/38) (Tobias and Falk, 1988; Conroy *et al.*, 1990; Falk *et al.*, 1995). Two exceptions are Taung and Stw 187a, which have evidence for a dominant O/M sinus system (Tobias and Falk, 1988; Kimbel *et al.*, 2004). Falk *et al.* (1995) argue that this unusual feature contributes to the uncertainty of Taung's taxonomic affinity. Holloway *et al.* (2004a) suggest that MLD 1 has an O/M sinus drainage system. Meningeal vessels: Saban (1983) drew distinctions between *Australopithecus* (i.e., *Au. africanus*) and *Paranthropus* (*P. boisei* and *P. robustus*) with respect to the configuration of meningeal vessels, but this interpretation has since been rejected (Falk, 1993; White and Falk, 1999). Saban (1983) showed that two *Au. africanus* specimens (Sts 60, Taung) have middle meningeal vessels that bifurcate into simple anterior and posterior branches, with no middle branch, in contrast to *Paranthropus* in which a middle branch is present.

4.18.4.3 Megadont Archaic Hominins

This group includes hominin taxa included in the genus *Paranthropus* and one other taxon, *Au. garhi*. The genus *Paranthropus* was reintroduced when cladistic analyses suggested that three of the four species in this grade formed a clade. Two genera, *Zinjanthropus* and *Paraustralopithecus*, are subsumed within the genus *Paranthropus*.

Taxon name. *Paranthropus aethiopicus* (Arambourg and Coppens, 1968) Chamberlain and Wood, 1985.

Approximate time range. *c.* 2.5–2.3 Mya.

Initial discovery. Omo 18.18 (or 18.1967.18) – an edentulous adult mandible; Shungura Formation, Omo Region, Ethiopia, 1967.

Type specimen. As above.

Source(s) of the evidence. Shungura Formation, Omo region, Ethiopia; West Turkana, Kenya.

Nature of the evidence. The hypodigm includes a well-preserved cranium from West Turkana (KNM-WT 17000), several mandibles (e.g., KNM-WT 16005), and isolated teeth from the Shungura Formation. No postcranial fossils have been assigned to this taxon.

Characteristics and inferred behavior. Cranial: Similar to *P. boisei* (see below) except that the face is more prognathic, the cranial base is less flexed, the incisors are larger, and the postcanine teeth are not so large or morphologically specialized. When this taxon was introduced in 1968 it was the only megadont hominin in this time range. With the discovery of *Au. garhi* (see below) it is apparent that robust mandibles with similar length premolar and molar tooth rows are being associated with what are claimed to be two distinct forms of cranial morphology.

CNS-related fossil evidence. Endocranial: One almost complete cranium is the only source of information about the CNS of *P. aethiopicus*. The estimated endocranial volume is 410 cm^3 (Walker *et al.*, 1986). Holloway *et al.* (2004a) describe a slight LORF petalial pattern, but no claims of humanlike brain organization have been made. Falk *et al.* (2000) suggest that this specimen retains aspects of ape-like brain morphology not found in *Au. africanus*. The olfactory bulb of *P. aethiopicus* is ape-like in size and shape, in contrast to *Au. africanus* (Falk *et al.*, 2000). *P. aethiopicus* has an ape-like beak-shaped orbital surface of the frontal lobe, in contrast to *Au. africanus* and *Homo*. The *P. aethiopicus* temporal lobe is ape-like in both size and shape and contrasts with the morphology seen in *Au. africanus* and *Homo*, but it shares this trait with other *Paranthropus* taxa (Falk *et al.*, 2000). Holloway (1988a) suggests that the cerebellum of this specimen is chimpanzee-like in that it is posteriorly protruding and laterally flaring, in contrast to the more modern humanlike tucked-under cerebellum of *P. robustus* (SK 1585) and *P. boisei* (OH 5). The ape-like morphology and small brain size are consistent with the conclusion that this species was more ape-like than humanlike in terms of its cognition.

Other endocranial morphology. Cranial venous sinuses: The lack of transverse sigmoid sinus grooves in KNM-WT 17000 is taken (by default) as evidence for an enlarged O/M sinus system in *P. aethiopicus* (Brown *et al.*, 1993).

Taxon name. *Paranthropus boisei* (Leakey, 1959) Robinson, 1960.

Approximate time range. *c.* 2.3–1.3 Mya.

Initial discovery. OH 3 – deciduous mandibular canine and molar; Olduvai Gorge, Tanzania, 1955 (Leakey, 1958).

Type specimen. OH 5 – adolescent cranium; Olduvai Gorge, Tanzania, 1959 (Leakey, 1959).

Source(s) of the evidence. Olduvai and Peninj, Tanzania; Omo Shungura Formation and Konso, Ethiopia; Koobi Fora, Chesowanja, and West Turkana, Kenya; Melema, Malawi.

Nature of the evidence. *P. boisei* has a comprehensive craniodental fossil record. There are several skulls (the one from Konso being remarkably complete and well preserved), several well-preserved crania, and many mandibles and isolated teeth. There is evidence of both large and small-bodied individuals, and the range of the size difference suggests a substantial degree of sexual dimorphism. There are no postcranial remains that can, with certainty, be assigned to *P. boisei*.

Characteristics and inferred behavior. Cranial: *P. boisei* is the only hominin to combine a massive, wide, flat, and face, massive premolars and molars, and small anterior teeth. The face of *P. boisei* is larger and wider than that of *P. robustus,* yet their brain volumes are similar. The mandible of *P. boisei* has a larger and wider body or corpus than any other hominin (see *P. aethiopicus* above). The tooth crowns apparently grow at a faster rate than has been recorded for any other early hominin. The fossil record of *P. boisei* extends across about 1 My, during which there is little evidence of any substantial change in the size or shape of the components of the cranium, mandible, and dentition (Wood *et al.*, 1994). Postcranial: There is, unfortunately, no postcranial evidence that can with certainty be attributed to *P. boisei*.

CNS-related fossil evidence. Endocranial: The *P. boisei* neurocranium is represented by 11 specimens: OH 5 (Olduvai Gorge); Omo L-338Y-6 and Omo-323-1976-896 (Omo Shungura Formation); KGA-10-525 (Konso); KNM-ER 23000, KNM-ER 406, KNM-ER 407, and KNM-ER 732 (Koobi Fora); KNM-CH 304 (Chesowanja); and KNM-WT 13750 and KNM-WT 17400 (West Turkana). The mean adult endocranial volume for *P. boisei* is 488 cm^3 ($n = 10$; range 400–545 cm^3). The sample mean EQ for *P. boisei* is 2.5. Four or five *P. boisei* (KGA-10-525, KNM-ER 23000, KNM-WT 13750, OH 5, and possibly Omo L338Y-6) endocasts have slight LORF petalial patterns (Holloway *et al.*, 2004a). For all other *P. boisei* endocasts, either it is not possible to determine whether a petalia exists,

or this information has not been reported. For one specimen (KNM-WT 17400) the Broca's cap region is larger on the left side than on the right side, but it is not clear whether or not this is due to distortion (Holloway *et al.*, 2004a). Three *P. boisei* have convolutional details in the occipital region, suggesting a reduction in the relative size of the primary visual cortex size (KGA-10-525, KNM-ER 23000, Omo L338Y-6). For all other *P. boisei*, it is either not possible to determine the key occipital landmarks, or this information has not been reported. The temporal lobe of KNM-WT 17400 is ape-like in its size and shape. This contrasts with morphology seen in *Au. africanus* and *H. sapiens*, but it shares this morphology with other *Paranthropus* taxa (Falk *et al.*, 2000). Three *P. boisei* endocasts (KNM-ER 2300, KNM-WT 17400, and OH 5) have a pointed rostral frontal lobe. They share this morphology with *P. aethiopicus* and the great apes, and it contrasts with the condition in *Au. africanus* and modern humans (Falk *et al.*, 2000). Vertebral canal: No evidence. Behavioral interpretations: There is more and better evidence for a more modern humanlike pattern of brain organization in *P. boisei* than in *Au. afarensis*. This may indicate that not only did *Paranthropus* brain size expand over time (Elton *et al.*, 2001), but some brain reorganization may have occurred in this lineage. Certainly, the impact of brain size on brain morphology (e.g., Jerison, 1975) could be a contributing factor to this trend.

Other endocranial morphology. Cranial venous sinuses: The cranial venous sinus system of *P. boisei* has been given much attention. Tobias (1967) first noted that OH 5 had an enlarged occipital marginal sinus system, a trait it shares with most other scorable *P. boisei* specimens: that is, KNM-CH 304 (Gowlett *et al.*, 1981), KNM-ER 23000 (Brown *et al.*, 1993), KNM-ER 407 (Day, 1976), and KNM-ER 732 (Leakey *et al.*, 1972). A probable exception to this is Omo L-338Y-6, because several authors (Rak and Howell, 1978; Holloway, 1981a; Kimbel, 1984; Holloway *et al.*, 2002) have failed to confirm the presence of an enlarged O/M sinus system in this specimen (cf. Falk *et al.*, 1995). The fossil KGA-10-525 lacks an O/M sinus system (Suwa *et al.*, 1997; Holloway *et al.*, 2004a), although it has been suggested that transverse sinus grooves are also missing (Suwa *et al.*, 1997; White and Falk, 1999; cf. Holloway *et al.*, 2004a). Meningeal vessels: Saban (1983) found that *P. boisei* (KNM-ER 407), like *P. robustus*, has three major branches of the middle meningeal vessels: anterior, middle, and posterior. However, Saban found only simple anterior and posterior branches for Omo 338y-6, now considered to belong to *P. boisei*, but originally thought to be an non-megadont australopith (Walker and Leakey, 1988; White and Falk, 1999).

Taxon name. *Paranthropus robustus* Broom, 1938.

Approximate time range. *c.* 2.0–1.5 Mya.

Initial discovery. TM 1517 – an adult, presumably male, cranium and associated skeleton; Phase II Breccia, now Member 3, Kromdraai B, South Africa, 1938.

Type specimen. As above.

Source(s) of the evidence. Kromdraai, Swartkrans, Gondolin, Drimolen, and Cooper's caves, all situated in, or near to, the Blauuwbank Valley, near Johannesburg, South Africa.

Nature of the evidence. The fossil record is similar to but less numerous than that of *Au. africanus*. The dentition is well represented, some of the cranial remains are well preserved, but many of the mandibles are crushed and/or distorted. The postcranial skeleton is not well represented. Research at Drimolen was only initiated in 1992, yet already more than 80 hominin specimens have been recovered and it promises to be a rich source of evidence about *P. robustus*.

Characteristics and inferred behavior. Cranial: The brain, face, and chewing teeth of *P. robustus* are larger than those of *Au. africanus*, yet the incisor teeth are smaller. Postcranial: It has been suggested that the thumb of *P. robustus* would have been capable of the type of grip necessary for stone tool manufacture, but this claim is not accepted by all researchers. The foot retains some arboreal capability, and the pelvic morphology suggests a relatively inefficient system for mass transfer, but the finger curvature is reduced, and it has more modern humanlike limb proportions and foot morphology, evidence that *P. robustus* was a more committed biped than, say, *Au. afarensis*, or *H. habilis* (see below).

CNS-related fossil evidence. Endocranial: The *P. robustus* neurocranium is represented by at least six specimens: four from Swartkrans (SK 1585, SK 46, SK 54, and SK 859), one from Kromdraai (TM 1517), and one from Drimolen (DNH 7). The mean adult endocranial volume is 533 ($n = 4$; range 450–650). The sample mean EQ for *P. robustus* is 3.1. The evidence for modern humanlike aspects of brain morphology is much weaker than for *P. boisei*, in spite of the larger brain size of *P. robustus*. Two or three specimens (SK 1585, SK 859, and possibly SK 54) have slight left occipital petalias, but there is no indication of whether they also have right frontal petalias (Holloway *et al.*, 2004a). It is not possible to tell whether the primary visual cortex is reduced in

SK 1585, SK 54, and SK 859, since for each of these endocasts the location of the LS and the interparietal sulcus cannot be determined with certainty (Holloway, 1975; Holloway *et al.*, 2004a). One *P. robustus* individual (SK 1585) has a beak-shaped frontal lobe and a rounded temporal lobe – ape-like traits shared with other *Paranthropus*, in contrast to the morphology of *Au. africanus* and *Homo* (Falk *et al.*, 2000). Vertebral canal: There are a handful of vertebras associated with this species, none of which are thoracic vertebras, although there is one near-complete axis (SK 854).

Other endocranial morphology. Cranial venous sinuses: All three *P. robustus* specimens with the relevant anatomy preserved demonstrate an enlarged O/M sinus system. These are SK 1585, SK 46, and SK 859 (Tobias, 1967; Holloway, 1972; Kimbel, 1984). Meningeal vessels: Saban (1983) found that one *P. robustus* endocast (SK 1585) had three major branches of the middle meningeal vessels: anterior, middle, and posterior.

Taxon name. *Australopithecus garhi* Asfaw *et al.*, 1999.

Approximate time range. *c.* 2.5 Mya.

Initial discovery. GAM-VP-1/1 – left side of mandibular corpus; Gamedah, Middle Awash, Ethiopia, 1990.

Type specimen. BOU*-VP-12/130 – a cranium; Bouri, Middle Awash, Ethiopia, 1997 (*the prefix ARA was erroneously used in the text of Asfaw *et al.*, 1999).

Source(s) of the evidence. Bouri, Middle Awash, Ethiopia.

Nature of the evidence. A cranium and two partial mandibles.

Characteristics and inferred behavior. Cranial: *Au. garhi* combines a primitive cranium with large-crowned postcanine teeth. However, unlike *Paranthropus* (see above), the incisors and canines are large and the enamel lacks the extreme thickness seen in the latter taxon. Postcranial: A partial skeleton combining a long femur with a relatively long forearm was found nearby, but is not associated with the type cranium of *Au. garhi* (Asfaw *et al.*, 1999); these fossils have not been formally assigned to *Au. garhi*.

CNS-related fossil evidence. The single cranial specimen has an endocranial volume of 450 cm^3, based on water displacement of a plaster model of the reconstructed endocast (reliability A1–A2). Holloway *et al.* (2004a) described the endocast morphology, but there was no evidence of a modern humanlike brain reorganization.

Other endocranial morphology. Meningeal vessels are present, but are not diagnostic.

4.18.4.4 Transitional *Homo*

This group contains two hominin taxa (*H. habilis s.s.* and *H. rudolfensis*) that are conventionally included within *Homo*, but which some researchers (e.g., Wood and Collard, 1999) have suggested might not belong in the *Homo* clade. Until we have the means to generate sound phylogenetic hypotheses about these and other early hominin taxa, it is not clear what their alternative generic attribution should be. Thus, for the purposes of this review, these two taxa are retained within *Homo*, but are referred to as transitional hominins.

Taxon name. *Homo habilis* Leakey *et al.*, 1964.

Approximate time range. *c.* 2.4–1.6 Mya.

Initial discovery. OH 4 – fragmented mandible; Olduvai Gorge, Tanzania, 1959.

Type specimen. OH 7 – partial skull cap and hand bones; Olduvai Gorge, Tanzania, 1960.

Source(s) of the evidence. Olduvai Gorge, Tanzania; Koobi Fora, and perhaps Chemeron, Kenya; Omo (Shungura), and Hadar, Ethiopia, East Africa; perhaps also Sterkfontein, Swartkrans, Cooper's and Drimolen, South Africa.

Nature of the evidence. Mostly cranial and dental evidence with only a few postcranial bones that can be confidently assigned to *H. habilis*.

Characteristics and inferred behavior. Cranial: All the crania are wider at the base than across the vault, but the face is broadest in its upper part. The jaws and teeth are absolutely small, but when related to estimated body mass they are larger than in other later premodern *Homo* taxa. Postcranial: The curved proximal phalanges and well-developed muscle markings on the phalanges of OH 7 also indicate the hand was used for more powerful grasping (such as would be needed for arboreal activities) than is the case in any other species of *Homo*. Conclusion: The jaws and teeth of *H. habilis* are absolutely small, but they are relatively large. Also, postcranial evidence suggests that *H. habilis* was capable of traveling arboreally and bipedally.

CNS-related fossil evidence. Endocranial: The CNS-related fossil evidence for *H. habilis* comprises six crania, two from Koobi Fora (KNM-ER 1813 and KNM-ER 1805), and four from Olduvai Gorge (OH 7, OH 13, OH 16, and OH 24). Brains are larger in *H. habilis* than in *Australopithecus* and *Paranthropus*, with a mean adult endocranial volume of 609 cm^3 ($n = 6$; range 509–687 cm^3). The sample average EQ for *H. habilis* is 3.7. Most of the inferences about derived modern humanlike morphology in this species are controversial, mainly due to poor preservation of the

relevant structures. Falk (1983a) has cast doubt on the allocation of KNM-ER 1805 to *H. habilis* on the grounds that it displays ape-like frontal orbital sulci at the lateral borders of the frontal lobes. The evidence for the petalial pattern in *H. habilis* is weak. Holloway *et al.* (2004a) claim that a single specimen, KNM-ER 1805, has a slight LORF petalial pattern, but the observation of frontal petalia is not reliable because of postmortem deformation (Begun and Walker, 1993; Holloway *et al.*, 2004a). Tobias (1987) makes a case for language ability in *H. habilis*, but this is controversial as much of the derived anatomy he describes for *H. habilis* is not confirmed by other authors, who claim the fossils are too fragmented and distorted to justify these interpretations (Begun and Walker, 1993; Holloway *et al.*, 2004a). Tobias (1987) suggests that OH 24, and probably OH 16 (but there is bone missing), have right frontal petalias. According to Tobias, the morphology of the fragmented of the frontal regions of the OH 7 and OH 16 endocasts is suggestive of a modern humanlike Broca's area, but Holloway *et al.* (2004a) see too few convolutional details to confirm this. The supramarginal angular gyri of the inferior parietal region – corresponding to Brodmann areas 40 and 39, respectively – are well developed in all four Olduvai *H. habilis* specimens (i.e, OH 7, 16, 13, and 24) (Tobias, 1987). Tobias states that these gyri are included in Wernicke's area (although he allows that the area's definition is controversial). In fact, the inferior parietal lobule is probably not part of Wernicke's language comprehension area, which is restricted to the left superior temporal cortex posterior to the primary auditory cortex (i.e., posterior part of Brodmann area 22) (Wise *et al.*, 2001). However, as previously mentioned, the inferior parietal lobule is greatly expanded in humans compared to monkeys, a difference that has been correlated with the development of language and calculation abilities (Simon *et al.*, 2002). Tobias (1987) mentions that in three *H. habilis* specimens (OH 24, OH 13, and OH 7) the superior parietal lobule is well developed, a characteristic also of *Au. africanus* (Dart, 1925; Schepers, 1946). Because the development is greater on the left size, he calls the asymmetry parietopetalia, but note that none of these specimens has the typical modern humanlike LORF petalial pattern. Holloway *et al.* (2004a) do not confirm these asymmetries, and instead they note that OH 7 and OH 24 are distorted and that this makes the identification of parietal petalias questionable, and that in OH 13 inferences about a parietal petalia depend on the way the specimen is orientated. Holloway *et al.* (2004a) do not refer to the placement of the LS or to the size of the occipital lobe in *H. habilis*. However, Begun and Walker (1993) suggest that the occipital lobe in two *H. habilis* specimens (KNM-ER 1813 and KNM-ER 1805) is smaller and less projecting than in KNM-ER 1470, a cranium referred to *H. rudolfensis*. Vertebral canal: Unfortunately, no *H. habilis* thoracic vertebras are available to investigate potentially language-related aspects of spinal cord anatomy in this taxon. Behavioral interpretations: There is no evidence of modern humanlike Broca's cap morphology in *H. habilis*, nor is there any evidence for any reduction in the size of the primary visual cortex. In summary, the original suggestion that *H. habilis* is the earliest hominin with the cognitive capacity for language is no longer supported.

Other endocranial morphology. Cranial venous sinuses: Three specimens (KNM-ER 1813, KNM-ER 1805, and OH 16) demonstrate the usual modern humanlike dominant transverse-sigmoid sinus system. Meningeal vessels: *H. habilis* endocasts for which the morphology is available (KNM-ER 1805, KNM-ER 1813, OH 7) have evidence of three major branches of the middle meningeal artery (Tobias, 1991; Holloway *et al.*, 2004a).

Taxon name. *Homo rudolfensis* (Alexeev, 1986) *sensu* Wood, 1992.

Approximate time range. *c.* 1.8–1.6 Mya.

Initial discovery. KNM-ER 819 – mandible fragment; Koobi Fora, Kenya, 1971.

Type specimen. Lectotype: KNM-ER 1470 – cranium; Koobi Fora, Kenya, 1972 (Leakey, 1973).

Source(s) of the evidence. Koobi Fora, and perhaps Chemeron, Kenya; Uraha, Malawi.

Nature of the evidence. Several incomplete crania, two relatively well-preserved mandibles, and several isolated teeth.

Characteristics and inferred behavior. Cranial: *H. rudolfensis* and *H. habilis* show different mixtures of primitive and derived, or specialized, cranial features. For example, although the absolute size of the brain case is greater in *H. rudolfensis*, its face is widest in its mid-part, whereas the face of *H. habilis* is widest superiorly. The more primitive face of *H. rudolfensis* is combined with a robust mandible and mandibular postcanine teeth with larger, broader crowns and more complex premolar root systems than those of *H. habilis*. The mandible and postcanine teeth of *H. rudolfensis* are larger than one would predict for a generalized hominoid of the same estimated body mass, suggesting that its dietary niche made mechanical demands comparable to those of the archaic

hominins. Postcranial: No postcranial remains can yet be reliably linked with *H. rudolfensis*.

CNS-related fossil evidence. Endocranial – CNS-related data are preserved in three cranial specimens (KNM-ER 1470, KNM-ER 1590, and KNM-ER 3732). *H. rudolfensis* mean adult endocranial volume is 776 ($n = 3$; range 750–825), although when it is related to admittedly crude estimates of body mass (EQ = 3.2) the brain of *H. rudolfensis* is not substantially larger than that of *Paranthropus*, and is smaller than that of *H. habilis*. KNM-ER 1470 has a modern humanlike Broca's region which is expanded on the left side (Tobias, 1975; Falk, 1983a; Begun and Walker, 1993; Holloway *et al*., 2004a), a feature that KNM-ER 3732 probably shares, although distortion obscures the interpretation of the morphology of this region. The clearly delimited, modern humanlike Broca's cap is taken by Holloway (1983c) to be suggestive of both language capacity and right-handedness, although he cautions that chimpanzees may also have well-developed Broca's caps. KNM-ER 1470 has a clear, modern humanlike LORF petalial pattern. Unlike *H. habilis* (KNM-ER 1805), KNM-ER 1470 lacks an ape-like fronto-orbital sulcus on the surface of the frontal lobe (Falk, 1983a). Holloway *et al*. (2004a) found no evidence of an LS or occipital lobe reduction in *H. rudolfensis*. However, Begun and Walker (1993) mention that the occipital lobe in the *H. rudolfensis* specimen KNM-ER 1470 is larger and more projecting than in two *H. habilis* specimens (KNM-ER 1813 and KNM-ER 1805). Vertebral canal: No vertebras are known for this taxon. Behavioral interpretations: *H. rudolfensis* (in particular, KNM-ER 1470) represents the oldest undisputed evidence of modern humanlike brain anatomy and it possesses features suggestive of language ability and right-handedness (Falk, 1983a; Holloway *et al*., 2004a). In support of these interpretations, Toth (1985) has inferred that contemporaneous stone tools were produced by predominately right-handed hominins. Interestingly, there is much more evidence of modern humanlike CNS-related anatomy in *H. rudolfensis* than in *H. habilis*. Also noteworthy, the debate over particular convolutional details lessens with the appearance of *Homo*. This is in part due to the fact that the endocranial convolutions of *Homo* are less obvious than those of archaic hominins. Falk (1980a, 1980b) notes that frontal convolutions should be the focus of endocast studies as these are more visible. This visibility contrasts with the relatively poor preservation of the LS in premodern *Homo* (Falk, 1991).

Other endocranial morphology. Cranial venous sinuses: *H. rudolfensis* does not show any evidence of an enlarged O/M sinus system. Meningeal vessels: One specimen (KNM-ER 1470) has separate anterior, middle, and posterior branches of its middle meningeal artery (Holloway *et al*., 2004a).

4.18.4.5 Premodern *Homo*

The species in this category are all usually assigned to the *Homo* clade. However, at least one species, *H. ergaster*, has a brain size that overlaps with that of archaic and transitional hominins.

Taxon name. *Homo ergaster* Groves and Mázak, 1975.

Approximate time range. *c*. 1.9–1.5 Mya.

Initial discovery. KNM-ER 730 – corpus of an adult mandible with worn teeth; Koobi Fora, Kenya, 1970.

Type specimen. KNM-ER 992 – well-preserved adult mandible; Koobi Fora, Kenya, 1971.

Source(s) of the evidence. Koobi Fora, West Turkana, Kenya; Dmanisi, Republic of Georgia.

Nature of the evidence. Cranial, mandibular, and dental evidence, including a remarkably complete associated skeleton of a juvenile male individual from Nariokotome, West Turkana.

Characteristics and inferred behavior. Cranial: Two sets of features are claimed to distinguish *H. ergaster* from *H. erectus*. The first comprises features for which *H. ergaster* is more primitive than *H. erectus*, with the most compelling evidence coming from details of the mandibular premolars. The second set comprises features of the vault and base of the cranium for which *H. ergaster* is less specialized, or derived, than *H. erectus*. The small chewing teeth of *H. ergaster* imply that it was either eating different food than the australopiths, or that it was preparing the same food extraorally. This could have involved the use of stone tools, or cooking, or a combination of the two. Postcranial: Postcranial similarities to modern humans suggest these hominins were habitual bipeds. Conclusion: Overall, *H. ergaster* is the first hominin to combine modern human-sized chewing teeth with a postcranial skeleton (e.g., long legs, large femoral head, etc.) apparently committed to long-range bipedalism, and to lack morphological features associated with locomotor and postural behaviors related to arboreality.

CNS-related fossil evidence. Endocranial: Relevant fossil evidence for *H. ergaster* comprises three well-preserved crania from Africa (Koobi Fora, and West Turkana), and four crania from

Dmanisi in the Republic of Georgia. The adult mean endocranial volume of the six *H. ergaster* specimens is 762 cm^3, with an average of 851 cm^3 for the three African specimens, and a much lower average of 675 cm^3 for the three Dmanisi specimens with published endocranial volume estimates. The sample average EQ for *H. ergaster* is 2.8. When the Dmanisi specimens (for which no body mass has been estimated) are excluded, the EQ for the three African specimens of *H. ergaster* is 3.1. Convolutional details are known only for the three African specimens. Two specimens have modern humanlike LORF petalial patterns (KNM-WT 15000, KNM-ER 3883), whereas the third specimen (KNM-ER 3733) has a less pronounced asymmetry (Begun and Walker, 1993; Holloway *et al.*, 2004a). All three specimens seem to have a modern humanlike asymmetry of the Broca's cap region, with the evidence for this pattern being clearest in KNM-ER 15000, whereas in the other two East African specimens the morphology in that area is uncertain (Begun and Walker, 1993; Holloway *et al.*, 2004a). Occipital convolutional details are not preserved in any *H. ergaster* cranial specimen. Vertebral canal: The juvenile West Turkana (KNM-WT 15000) skeleton also includes a vertebral column. *H. ergaster* resembles earlier hominins and nonhuman primates in having relatively smaller thoracic vertebral canals than recent humans, suggesting that this hominin did not yet show the expanded canal that has been associated with more precise control of the muscles associated with speech (see entry for *H. neanderthalensis* for a discussion). Behavioral interpretations: Holloway *et al.* (2004a) interpret the cranial findings as clear evidence of right-handedness and language ability. In contrast, evidence from the vertebral canal suggests humanlike increased control of breathing; thus, speech most likely did not yet exist in these hominins. Great apes (Patterson, 1978; Gardner *et al.*, 1989; Shapiro and Galdikas, 1999) and human infants (Bonvillian *et al.*, 1983, 1997; Bonvillian and Patterson, 1999) lack the capacity for vocal language production, but they are able to communicate with hand signals. A study of a deaf sign-language user found that Broca's region maintains its function in nonvocal language (Corina *et al.*, 1999; Corina and McBurney, 2001). Language-related cerebral anatomy might have existed as part of a complex of preadaptations to language, along with a series of cranial modifications seen in *H. ergaster* and *H. erectus* (MacLarnon and Hewitt, 2004). These data suggest that increased nonvocal language ability preceded vocal language.

Other endocranial morphology. Cranial venous sinuses: There is no indication of enlarged O/M sinus system for this taxon. Meningeal vessels: The anterior branch of the middle meningeal artery is especially well developed in *H. ergaster* crania such as KNM-WT 15000 (Begun and Walker, 1993), and KNM-ER 3883 (Holloway *et al.*, 2004a). Anterior branches are characteristically more developed on endocasts of later African fossil *Homo*, including modern humans (Grimaud-Hervé, 1994; Holloway *et al.*, 2004a).

Taxon name. Homo floresiensis Brown *et al.*, 2004.

Approximate time range. c. <90–12 kya.

Initial fossil discovery. LB1 – partial adult skeleton; Liang Bua, Flores Indonesia, 2003 (Brown *et al.*, 2004).

Type specimen. As above.

Sources of the evidence. Liang Bua, Indonesia.

Nature of the evidence. LB1 partial adult skeleton plus cranial and postcranial evidence of a total of at least nine individuals (Morwood *et al.*, 2005).

Characteristics and inferred behavior. Cranial: Cranial morphology suggests a dwarfed *H. erectus*. Similar to *Au. afarensis* in stature and endocranial volume. Postcranial: Femur and pelvic morphologies show affinities to *H. habilis* and *Au. afarensis*. Possibly associated with oldowan-like stone artifacts. Charred bones hint at control of fire.

CNS-related fossil evidence. Endocranium: The endocranial volume estimate of LB 1 is 417 cm^3. The *H. floresiensis* EQ, assuming a body mass of 26 kg, is 3.1; this is within the *Australopithecus* range. For comparison, if one assumes the low-end body mass estimate of 16 kg, the EQ is 4.5, and assuming the given the high-end body mass estimate of 36 kg, the EQ is a mere 2.4 – the smallest of any fossil hominin. All inferences about *H. floresiensis* brain morphology were made by Falk *et al.* (2005), based on observations of both the LB1 fossil neurocranium and a virtual endocast. In endocranial shape, LB1 resembles classic Asian *H. erectus*. LB1 has a LORF petalial pattern. Details are not given, although the Broca's cap region is consistent with modern humanlike morphology (Falk *et al.*, 2005). The endocast lacks ape-like fronto-orbital sulci; these are also absent in *H. rudolfensis*, but they are retained in other (smaller-brained) early hominins such as *H. habilis* and *Au. africanus* (Falk, 1980b, 1983a). The LS is posterior to the lambdoid suture, in a derived position which suggests relative reduction of the primary visual cortex at the expense of enlargement of posterior parietal association areas (Figure 2e). There are a few features in which the

H. floresiensis brain differs from the classic *H. erectus* brain. First, it is inferred that prefrontal cortex in the region of area 10 is expanded and much more convoluted than in *H. erectus* or *Au. africanus* (Falk *et al.*, 2005). This aspect of *H. floresiensis* is significant because the prefrontal cortex is the region of the human brain that has most clearly increased in relative degree of gyrification (Zilles *et al.*, 1988; Rilling and Insel, 1998). Second, LB1 has extremely wide temporal lobes. Interestingly, the temporal lobe is predicted to have undergone more change in the modern human lineage than has any other brain component (Semendeferi and Damasio, 2000), and it is larger in modern humans than predicted for a nonhuman primate of similar brain size (Rilling and Seligman, 2002). Third, the LB1 occipital lobe does not hang over the cerebellum. An occipital lobe that overhangs the cerebellum is a derived feature of *H. erectus* (Falk *et al.*, 2005). Vertebral canal: No description available. Behavioral interpretations: *H. floresiensis* is a dwarfed hominin species in which the size reduction was more pronounced in the brain than the body. In spite of its small absolute and relative brain size, the *H. floresiensis* brain is at least as modern humanlike in its morphology as is the brain of *H. erectus*. Compared to classic *H. erectus*, *H. floresiensis* is more modern humanlike in having a well-developed temporal lobe and increased gyrification of the prefrontal region. Might the specialized brain morphology of *H. floresiensis* relate to the fact that this species has been associated with unusually sophisticated artifacts? At present, it is impossible to know. Many cognitive functions of the prefrontal and temporal regions (e.g., processing of auditory information, language production, higher-order processing of visual information, planning, and memory) are predicted to be emphasized in a species that is associated with stone tools. However, these areas are probably not the ones most involved in the act of stone toolmaking (see Stout *et al.*, 2000). An alternative hypothesis is that in this species the temporal lobe and prefrontal cortex did not become relatively larger; rather, other structures (e.g., primary visual cortex) became relatively smaller more quickly. Domesticated mammals have relatively smaller brains and sensory structures than do their wild counterparts, with the size of the primary visual cortex and eyes being the most reduced – a pattern that is also found in the fossil dwarfed bovid *Myotragus* (Köhler and Moyà-Solà, 2004).

Other endocranial morphology. Cranial venous sinuses: Present, but not diagnostic. Meningeal vessels: The configuration suggests that vessels that originate in the orbit contribute to the supply of the meninges overlying the temporal lobe; this feature is common in apes and found in some *H. erectus* (Falk, 1993; Falk *et al.*, 2005).

Taxon name. *Homo erectus* (Dubois, 1892) Weidenreich, 1940.

Approximate time range. *c.* 1.8 Mya to $<$ 200 kya.

Initial discovery. Kedung Brubus 1 – mandible fragment; Kedung Brubus, Java (now Indonesia), 1890.

Type specimen. Trinil 2 – adult calotte; Trinil, Ngawi, Java (now Indonesia), 1891.

Source(s) of the evidence. Sites in Indonesia (e.g., Trinil, Sangiran, Sambungmachan), China (e.g., Zhoukoudian, Lantian), Africa (e.g., Olduvai Gorge, Melka Kunture), and possibly India (Hathnora).

Nature of the evidence. Mainly cranial with some postcranial evidence, but little or no evidence of the hand or foot.

Characteristics and inferred behavior. Cranial: The crania belonging to *H. erectus* have a low vault, a substantial, more-or-less continuous torus above the orbits, and the occipital region is sharply angulated. The inner and outer tables of the cranial vault are thickened. The body of the mandible is less robust than that of the australopiths and in this respect it resembles *H. sapiens*, except that the symphyseal region lacks the well-marked chin that is a feature of later *Homo* and modern humans. The tooth crowns are generally larger, and the premolar roots of many specimens are more complicated than those of modern humans. All the dental and cranial evidence points to a modern humanlike diet for *H. erectus*. Postcranial: The cortical bone of the postcranial skeleton is thicker than that in modern humans. The limb bones are modern humanlike in their proportions and have robust shafts, but the shafts of the long bones of the lower limb are flattened from front to back (femur) and from side to side (tibia) relative to those of modern humans. There is no fossil evidence relevant to assessing the dexterity of *H. erectus*, but if *H. erectus* manufactured Acheulean artifacts then dexterity would be implicit. The postcranial elements are consistent with a habitually upright posture and obligate, long-range bipedalism.

CNS-related fossil evidence. Endocranial: At least 43 crania or cranial fragments preserve information about the CNS of *H. erectus*. The mean endocranial volume of *H. erectus* is 991 cm^3 ($n = 36$; range 727–1260). The EQ for *H. erectus* is 3.9, and

H.erectus shows aspects of modern humanlike brain organization similar to those described for *H. ergaster*. This taxon shows surprisingly little variability in endocast morphology. Holloway (1980), Broadfield *et al.* (2001), and Holloway *et al.* (2004a) drew attention to the very clear pattern of LORF petalial patterns and modern humanlike asymmetry of the Broca's cap region. There is some suggestion of a right-hander's petalial configuration from all sites with scored fossils (i.e., Olduvai, Ngandong, Sambungmacan, Sangiran, Trinil, Zhoukoudian) (Holloway *et al.*, 2004a). The asymmetries range from slight to strong, and in many specimens the evidence exists for both the left occipital and the right frontal (Ngandong 1, 6, 17, 14; Sangiran 2, 17; Trinil 2, Zhoukoudian III E, III L), although in other specimens there are missing data or there is distortion in either the left occipital or right frontal regions (Ngandong 13; OH 9, 12; Sangiran 4, 10, 12; Zhoukoudian I L; Holloway *et al.*, 2004a). However, Begun and Walker (1993) took a different position with respect to the existence of petalias in the Zhoukodian specimens that they examined. They state that no frontal petalias are evident for Zhoukoudian I L, II, III E, and III L, and no occipital petalias are evident for Zhoukoudian II and III E, although they do allow that occipital petalias are evident for Zhoukoudian I L and III L. Holloway *et al.* (2004a) do not describe the morphology of Zhoukoudian II. Most authors agree that the frontal lobe of *H. erectus* is derived toward the modern humanlike condition. Convolutional details of the frontal lobe are more modern humanlike than ape-like for Sangiran 2 (Ariens Kappers and Bouman, 1939; Weidenreich, 1943; Connolly, 1950). Broca's region is more modern humanlike in *H. erectus* than in earlier hominins. This was first mentioned by Dubois (1897), who noted that the type specimen (Trinil 2) had a fairly well-developed third (=inferior) frontal convolution, the gyrus associated with Broca's area. In fact, the Broca's cap region is enlarged, and/or there is an asymmetry in which the left side is larger and better defined or protrudes more laterally in all scorable specimens (i.e., Ngandong 6 and 14, Sambungmacan 3, Sangiran 2, 3, 10 and 17, Trinil 2, and Zhoukoudian III E) (Holloway, 1980; Grimaud-Hervé, 1994; Holloway *et al.*, 2004a). Holloway *et al.* (2004a) infer that *H. erectus* was capable of rudimentary language and was predominantly right-handed. In the occipital lobe, the LS has been identified in a few specimens (Sangiran 10, OH 12, Trinil 2) and in all it is in a posterior position. A possible exception is Sangiran 2 in which the identification of the LS is uncertain, and the sulcus referred to may actually be a lateral calcarine sulcus in a position that would be unusual in modern humans. The two fossils from Olduvai (OH 9 and OH 12) resemble the Asian specimens in aspects of brain morphology. OH 9 has a strong left occipital petalia like other *H. erectus* and *H. ergaster* (due to missing data, whether or not there was a right frontal petalia cannot be determined). OH 12 has a posterior LS like other *H. erectus* (convolutional details in this region are not preserved in any *H. ergaster* specimen). However, whereas OH 9 is in the upper 50% of *H. erectus* endocranial volume, OH 12 has the smallest *H. erectus* endocranial volume and fits better with *H. ergaster*. The development of three cranial features (the tympanic plate, the bregmatic area of the cranial vault, and the subarcuate fossa) and a comparison of adult *H. erectus* endocranial volumes with that of the Mojokerto child, reveal an ape-like pattern of brain ontogeny for this specimen (Coqueugniot *et al.*, 2004).

Other endocranial morphology. Cranial venous sinuses: Three specimens (Zhoukoudian II, III E, and III L) have dominant transverse-sigmoid sinus systems, and one specimen (Trinil 2) has an O/M sinus system on the left and an enlarged transverse sinus on the right (Falk, 1986). Meningeal vessels: Weidenreich (1938) used a modern human-based system of classification in his description of the Zhoukoudian fossils which overlooked similarities between the *H. erectus* meningeal patterns and that of the apes; but he did note that *H. erectus* was primitive in having fewer ramifications than modern humans. In Falk's re-evaluation of Weidenreich's description of the meningeal vessels (Falk, 1993), she concludes that they are ape-like to the extent that there is a high frequency of the meningeal arteries originating from the orbit rather than the floor. In comparison to modern humans, in Asian *H. erectus* specimens the obelic branch of the middle meningeal artery is more developed, and may have contributions from both the anterior and the posterior branches. Grimaud-Hervé describes three regional patterns. First, it is common for the obelic branch to arise independently as a third major branch in Zhoukoudian endocasts (e.g., Zhoukoudian I L, III E). Second, the obelic branch bifurcates near the origin of middle branch in Trinil 2 and several Sangiran endocasts (e.g., Sangiran 17). Third, in most Ngandong endocasts, the obelic branch derives from the anterior branch but also has a contribution from the posterior branch; the anterior branch is often better developed than the posterior (e.g., Ngandong 3, 7). In the North African *H. erectus* Ternifine 4, a very ramified obelic branch stems from the anterior branch of the

middle meningeal artery (Saban, 1984; Holloway *et al.*, 2004a). The developed anterior branch is said to be typical of African specimens of later taxa, but, as mentioned above, the developed obelic branch is also typical of *H. erectus*. The principal vessel of the anterior branch has posterior ramifications that run parallel to it in *H. erectus*, in contrast to their more oblique orientation in modern humans (Grimaud-Hervé, 1994; see The Evolution of Parallel Visual Pathways in the Brains of Primates).

Taxon name. Homo antecessor Bermúdez de Castro *et al.*, 1997.

Approximate time range. c. 700–500 kya.

Initial discovery. ATD6-1 – left mandibular canine; Level 6, Gran Dolina, Spain, 1994.

Type specimen. ATD6-5 – mandible and associated teeth; Level 6, Gran Dolina, Spain, 1994.

Source(s) of the evidence. Gran Dolina, Atapuerca, Spain.

Nature of the evidence. The partial cranium of a juvenile, parts of mandibles and maxillae, and isolated teeth.

Characteristics and inferred behavior. Cranial: Researchers who found the remains claim the combination of a modern humanlike facial morphology with large and relatively primitive tooth crowns and roots is not seen in *H. heidelbergensis* (see below). The Gran Dolina remains also show no sign of any derived *H. neanderthalensis* traits. Its discoverers suggest *H. antecessor* is the last common ancestor of Neanderthals and *H. sapiens*.

CNS-related fossil evidence. The only *H. antecessor* cranium (ATD-15) has an estimated endocranial volume of 1000 cm^3 (Bermudez de Castro *et al.*, 1997).

Taxon name. Homo heidelbergensis Schoetensack, 1908.

Approximate time range. c. 600–100 kya.

Initial discovery. Mauer 1 – adult mandible; Mauer, Heidelberg, Germany, 1907.

Type specimen. As above.

Source(s) of the evidence. Sites in Europe (e.g., Mauer, Petralona); Near East (e.g., Zuttiyeh); Africa (e.g., Kabwe, Bodo); and China (e.g., Dali, Jinniushan, Xujiayao, Yunxian). Researchers who see distinctions between the African and non-African components of the hypodigm refer to the former as *H. rhodesiensis*.

Nature of the evidence. Many crania but relatively little mandibular and postcranial evidence.

Characteristics and inferred behavior. Cranial: What sets this material apart from *H. sapiens* and *H. neanderthalensis* (see below) is the morphology of the cranium and the robusticity of the postcranial skeleton. Some brain cases are as large as those of modern humans, but they are always more robustly built with a thickened occipital region, an evenly projecting face with large separate ridges above the orbits, unlike the more continuous brow ridge of *H. erectus*. Compared with *H. erectus* (see above), the parietals are expanded, the occipital is more rounded and the frontal bone is broader. The crania of *H. heidelbergensis* lack the specialized features of *H. neanderthalensis* such as the anteriorly projecting midface and the distinctive swelling of the occipital region. *H. heidelbergensis* is the earliest hominin to have a brain as large as anatomically modern *H. sapiens*. Postcranial: the postcranial skeleton of *H. heidelbergensis* suggests that its robust long bones and large lower limb joints were well suited to long-distance bipedal walking.

CNS-related fossil evidence. Endocranial: *H. heidelbergensis* is represented by at least 22 cranial fossils that preserve evidence of the CNS. The mean adult endocranial volume for *H. heidelbergensis* is 1242 cm^3 ($n = 21$; range 880–1450 cm^3). There is an increase in mean endocranial volume compared to *H. erectus* and *H. ergaster*. The EQ for *H. heidelbergensis* is 4.2. For all the specimens, the LS is in the modern humanlike posterior position, according to Holloway *et al.* (2004a). Broken Hill 1 was described by Smith (1928) to have an ape-like LS, but Holloway *et al.* (2004a) disagree with this interpretation. Holloway *et al.* (2004a) describe LORF petalial patterns in most specimens, and very pronounced asymmetrical Broca's regions in some specimens. This is most pronounced in Arago, which has very protruding Broca's caps on both sides. Unfortunately, several specimens are missing entire frontal lobes, and there is no specimen for which all the relevant language/handedness morphology is preserved.

Other endocranial morphology. Cranial venous sinuses: Arago, Broken Hill, and Salé lack an O/M sinus system, although Guomde has an O/M sinus system (on both sides) and Swanscombe has an O/M sinus system on the right, not on the left (Falk, 1986). Meningeal vessels: The anterior branch of the middle meningeal artery is especially developed in African *H. heidelbergensis*. The anterior branch has a few anastomoses in Salé, and more numerous anastomoses in Broken Hill 1. In some European *H. heidelbergensis* (e.g., Swanscombe, Ehringsdorf 9), the anterior branch is more prominent and is the source of the obelic branch (Falk, 1986). In contrast, in one European *H. heidelbergensis* the posterior ramus is just as prominent as the anterior ramus (Falk, 1986).

Taxon name. Homo neanderthalensis King, 1864.

Approximate time range. *c.* >400 or 200–30 kya.

Initial discovery. Engis 1 – a child's cranium; Engis, Belgium, 1829.

Type specimen. Neanderthal 1 – adult calotte and partial skeleton; Feldhofer Cave, Mettmann, Germany, 1856.

Source(s) of the evidence. Fossil evidence for *H. neanderthalensis* has been found throughout Europe, with the exception of Scandinavia, as well as in the Near East, the Levant and Western Asia. Taxonomic note: The scope of the hypodigm of *H. neanderthalensis* depends on how inclusively the taxon is defined. For some researchers the taxon is restricted to fossils from Europe and the Near East that used to be referred to as classic Neanderthals. Others interpret the taxon more inclusively and include within the hypodigm fossil evidence that is generally earlier and less derived (e.g., Steinheim, Swanscombe and Atapuerca (Sima de los Huesos)).

Nature of the evidence. Many specimens are burials and so all anatomical regions are represented in the fossil record.

Characteristics and inferred behavior. Cranial: The distinctive features of the cranium of *H. neanderthalensis* include thick, double-arched brow ridges, a face that projects anteriorly in the midline, a large nose, laterally projecting and rounded parietal bones, and a rounded, posteriorly projecting occipital bone (i.e., an occipital bun). The size and wear on the incisors suggest that the Neanderthals regularly used their anterior teeth as tools, either for food preparation or to grip hide or similar material. Postcranial: Neanderthals were stout with a broad rib cage, a long clavicle, a wide pelvis, and limb bones that are generally robust with well-developed muscle insertions. The distal extremities tend to be short compared to most *H. sapiens*, but Neanderthals were evidently obligate bipeds. The generally well-marked muscle attachments and the relative thickness of long bone shafts point to a strenuous lifestyle.

CNS-related fossil evidence. Endocranial: There are at least 27 neurocranial fossils preserving evidence of the *H. neanderthalensis* CNS. The mean adult endocranial volume for *H. neanderthalensis* is 1404 cm^3 ($n = 27$; range 1172–1740 cm^3). The EQ for *H. neanderthalensis* is 4.7, smaller than that of *H. sapiens*. Neanderthals were often considered to have larger cranial capacities than *H. sapiens*, but this has recently been reconsidered in light of new estimates (Holloway *et al.*, 2004a, pp. 301, 304–305). Holloway *et al.* (2004a) suggest that Neanderthals have an overall modern humanlike brain shape, although there is occipital bunning in many specimens. Similarities with modern human brain shape include broad, round, vertical prefrontal lobes, and wide, full parietal lobes. In contrast, Bruner *et al.* (2003) argue that brain shape in Neanderthals follows archaic (i.e., *H. erectus* and *H. heidelbergensis*) allometric trends for brain size expansion. In contrast, they suggest that modern humans show a different pattern of brain growth that emphasizes expansion of the parietal lobes. Distinct petalias are the norm for Neanderthals. Many have very strong petalias, although not all specimens display the LORF pattern. One of the crania with an atypical petalia (La Ferrassie 1) also has a larger Broca's area on the right than on the left, and in another (La Chapelle-aux-Saints), a usual LORF petalial pattern is combined with a right dominant Broca's area. However, most have a modern humanlike pattern of Broca's area asymmetry.

Holloway *et al.* (2004a) suggest that, in all the Neanderthals they looked at, the LS is posterior to the lambdoid suture, as it is in modern humans. They are most certain about the position of the LS in Monte Circeo I, where it is far posterior to the lambdoid suture. They are uncertain about the identification of, or make indirect inferences about, the location of the LS in Le Ferrassie 1, La Quina 5, Neanderthal, Spy I, and Spy II. In La Chapelle-aux-Saints, Boule and Anthony (1911) had originally suggested the LS was anterior to the lambdoid suture. This was disputed (Symington, 1916; Le Gros Clark *et al.*, 1936), and Holloway *et al.* (2004a) point out that Boule and Anthony (1911) misidentified the LS, and that the real LS is posterior to the lambdoid suture. Holloway *et al.* did not look at the Krapina endocasts in detail, but they suggest that the Krapina specimens have more derived and modern features than Western European Neanderthals. Holloway *et al.* (2004a, p. 235) consider that, in terms of their CNS morphology, *H. sapiens* and *H. neanderthalensis* should only be separated at the subspecific level. Vertebral canal: At least four (Kebara, La Chapelle-aux-Saints, Shanidar 2, Shanidar 3) sets of Neanderthal fossil vertebras provide information about the size of the vertebral canal that transmits the spinal cord. Neanderthals resemble modern humans (and are different from all earlier hominins) in having enlarged thoracic vertebral canals relative to nonhuman primates. Increased muscular control associated with speech may explain the increase in spinal cord size in the Late Pleistocene hominins (MacLarnon and Hewitt, 1999, 2004).

Behavioral interpretations. *H. neanderthalensis* is modern humanlike in terms of its endocranial volume, inferred spinal cord dimensions, and

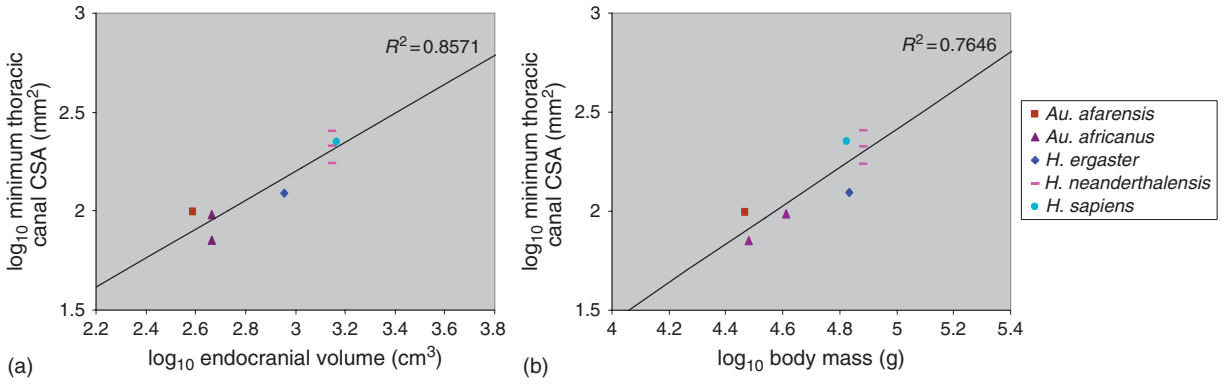

Figure 3 Log–log plots of minimum thoracic vertebral canal cross-sectional area (CSA) versus endocranial volume (a) and body mass (b) for fossil hominin specimens. Fossil hominin endocranial volume sources are available from the authors, by request. Thoracic CSA and body mass data are from MacLarnon and Hewitt (1999) and references therein.

brain organization. This, taken together with anatomy consistent with spoken language, and archeological evidence of complex, possibly symbolic burial behavior, suggests that Neanderthals shared with their early modern human contemporaries many characteristics of modern human cognition. However, no Neanderthal remains are associated with artifacts that can unequivocally be interpreted as art, which would serve as undisputed evidence of symbolic behavior. In contrast, contemporaneous early modern humans are associated with art throughout Europe by the time of Neanderthals last appearance *c*. 28 kya, suggesting that these two hominin taxa differed in terms of culture and cognition (Klein, 1999). The increase in thoracic vertebral canal cross-sectional area from early to late hominins might be explained by the corresponding increase in endocranial volume. The only Neanderthal for which both measures are available, La Chapelle-aux-Saints, has the largest minimum thoracic vertebral cross-sectional area of any primate specimen listed (253 mm^2 at T6) and a very large endocranial volume (1625 cm^3) (MacLarnon and Hewitt, 1999, 2004, p. 188). MacLarnon and Hewitt's conclusions are based on hominin values compared with a thoracic vertebral canal to body size scaling relationship for extant primates. But different taxa have different brain mass to body mass scaling relationships (Holloway and Post, 1982), and the relationship between brain size and spinal cord size is even more variable within large taxonomic groups (MacLarnon, 1995). A within-hominin spinal cord to brain size scaling relationship (or CNS scaling law) could account for the observed variation in spinal cord size (see Figure 3a). Further, the pivotal specimen for the argument of MacLarnon and Hewitt is a single *H. ergaster* specimen – a juvenile for which the growth trajectory is uncertain (see Coqueugniot *et al.*, 2004, and references therein) and for which the small vertebral canal may be pathological (Ohman *et al.*, 2002). This specimen sits on the regression line, and most hominin fossils plot above it (see MacLarnon and Hewitt, 1999; Figure 3b). A best-fit line for thoracic vertebral canal cross-sectional area against body mass for the fossil hominin specimens is much steeper than that for all primates (Figure 3b). Therefore, Neanderthals and modern humans have thoracic vertebral canal cross-sectional areas that fall within the range predicted for a hominin of similar brain size and body mass, although *H. ergaster* has a smaller thoracic vertebral canal cross-sectional area than expected for its body mass.

Other endocranial morphology. Cranial venous sinuses: There is no evidence of an O/M sinus system in any Neanderthal specimen (Falk, 1986). Meningeal vessels: In some Neanderthal crania (Le Moustier, Neanderthal, La-Chapelle-aux-Saints), the posterior branch of the middle meningeal artery is as prominent as the anterior branch (Holloway *et al.*, 2004a). In other Neanderthals (Gibraltar 1 and 2, Teshik Tash 1, Engis 2, La Ferrassie 1, La Quina H 5), the anterior branch is more prominent, and is the source of the obelic branch, as is common in modern humans. Both configurations found in Neanderthals were also found in European *H. heidelbergensis* (Holloway *et al.*, 2004a).

4.18.4.6 Anatomically Modern *Homo*

This category includes all those specimens that lack the autapomorphies of premodern *Homo* taxa, or which cannot be distinguished from living *H. sapiens*.

Taxon name. *Homo sapiens* Linnaeus, 1758.

Approximate time range. *c*. 190 kya to the present day.

Initial fossil discovery. With hindsight, the first recorded evidence to be recovered was the Red Lady of Paviland, found in Wales in 1822–23.

Type specimen. Linnaeus did not designate a type specimen.

Source(s) of the evidence. Fossil evidence of *H. sapiens* has been recovered from sites on all continents except the Antarctic. The earliest absolutely dated remains are from Omo Kibish (McDougall *et al*., 2005) and Herto (White *et al*., 2003) in Ethiopia. Taxonomic note: Researchers who wish to make a taxonomic distinction between fossils such as Florisbad, Omo 2, and Laetoli 18, and subrecent and living modern humans refer the African subset to *H.* (*Africanthropus*) *helmei* (Dreyer, 1935).

Nature of the evidence. Many are burials so the fossil evidence is good, but in some regions of the world (e.g., West Africa) remains are few and far between.

Characteristics and inferred behavior. Cranial: The earliest evidence of anatomically modern human cranial morphology in the fossil record comes from sites in Africa and the Near East. It is also in Africa that there is evidence for a likely morphological precursor of anatomically modern human morphology. This takes the form of crania that are generally more robust and archaic-looking than those of anatomically modern humans yet which are not archaic enough to justify their allocation to *H. heidelbergensis* or derived enough to be *H. neanderthalensis* (see above). Specimens in this category include Jebel Irhoud from North Africa, Omo 2 and Laetoli 18 from East Africa, and Florisbad and Cave of Hearths in southern Africa. There is undoubtedly a gradation in morphology that makes it difficult to set the boundary between anatomically modern humans and *H. heidelbergensis*, but unless at least one other taxon (e.g., *H. neanderthalensis*) is recognized the variation in the later *Homo* fossil record is too great to be accommodated in a single taxon. Postcranial: There are relatively few early *H. sapiens* postcranial fossils.

CNS-related fossil evidence. Endocranial: Early modern *H. sapiens* have a mean endocranial volume of 1463 cm^3 ($n = 79$, range 1090–1880 cm^3). The EQ for early *H. sapiens* is 5.3. The mean brain mass and EQ are within the range of recent modern human values. However, the extreme fossil endocranial volumes (when converted to brain masses, 1133–1799 g) fall outside of the range for a sample ($n > 227$) of recent modern humans aged 20–30 years (1239–1526 g).

Fossil modern humans are essentially modern humanlike in shape and endocranial volume. The Jebel Irhoud crania (Jebel Irhoud 1 and 2) are also considered to be *H. sapiens* but are said to be Neanderthal-like in overall shape; for example, Jebel Irhoud 2 has some occipital bunning, and the Jebel Irhoud 1 cranium is low and broad. The two Jebel Irhoud endocrania show evidence of modern humanlike brain morphology, including a LORF petalial pattern, an asymmetrically enlarged left Broca's area, and a reduced primary visual cortex, plus taxonomic indicators not related to the CNS (mentioned below). As is the case of recent modern humans, the manifestation of the LORF petalia pattern is variable. Cro-Magnon III, Dolni Vestonice 3 – and probably also Combe Capelle, and Brno 3 – have typical LORF petalias. Predmosti 10 has one of the most extreme cases of the right-hander's LORF petalial pattern, but there is no clear evidence of this pattern from the other specimens from the site (Predmosti 3, 4, and 9). Brno II has a reversed, left-hander's pattern of right-occipital and left-frontal petalias. Jebel Irhoud 1 and 2 provide the only evidence of asymmetrically enlarged left Broca's cap regions. The right side of Cro-Magnon III Broca's area is enlarged but the left is not preserved. Fossil and modern *H. sapiens* neurocrania are uniquely globular in shape (Lieberman *et al*., 2002), apparently having overcome constraints which cause *H. neanderthalensis* and archaic (i.e., *H. erectus* and *H. heidelbergensis*) neurocranial shape to plot along the same allometric trajectory (Bruner *et al*., 2003). Expanded temporal and possibly frontal lobes in modern humans possibly contribute to this morphological change (Lieberman *et al*., 2002). More recently, it has been suggested that volumetrically expanded parietal lobes have consequences for overall brain shape which contribute to the characteristic globularity of the human brain (Bruner *et al*., 2003). In comparison to the brains of earlier *Homo*, the modern human brain is shaped such that the anterior and posterior ends seem to approach each other from below. This is related to a decrease in relative endocranial length, a relative shortening of the frontal and occipital poles, and displacement of the cerebellum to a more inferior position (Bruner, 2004). Weaver (2005) suggests that relative cerebellum size differs between fossil modern humans and recent modern humans. The Early to Middle Pleistocene group (*Au. africanus*, *P. boisei*, *H. habilis*, *H. rudolfensis*, and *H. erectus*, *H. heidelbergensis*) does not differ significantly from recent modern humans or from the great apes with respect to CQ values (Weaver, 2005). However, the very low CQ values of the Late Pleistocene group (*H. neanderthalensis* and

H. sapiens) are significantly different from those of the Early to Middle Pleistocene group, the great apes, and from recent humans (Weaver, 2005). The shift toward larger CQ values in recent humans is said to be due to expansion of relative cerebellar size during a time of stasis in encephalization. Weaver suggests the cerebellum became expanded in recent humans to better manage the complex cognitive functions. However, this interpretation assumes that the Late Pleistocene group represents the condition directly ancestral to recent humans. The group with the lowest CQ values are Late Pleistocene hominins, which are all European, and the lowest value in the entire sample is from the Swanscombe specimen (CQ = 0.60). The decrease to the CQ values characteristic of the European Middle–Late Pleistocene group may be due to shared ancestry or geographical convergence, and it may not necessarily indicate the condition which precedes that of recent humans.

Other endocranial morphology. Cranial venous sinuses: Jebel Irhoud 1 and 2, Kanjera 1, and Predmost II and X have evident dominant transverse sinuses and no O/M system (Holloway *et al.*, 2004a). Brno III has an enlarged O/M sinus on the right (its left side is strongly deformed), and Predmost IV has evidence of an O/M sinus system on the right but not on the left. Skhul I has an O/M system on both sides. Meningeal vessels: The number of ramifications and anastomoses is increased from the ape-like level seen in *H. erectus* (Weidenreich, 1943, p. 13) to the characteristic modern human condition (Saban, 1984; Grimaud-Hervé, 1994). The principal vessel of the anterior ramus has posterior ramifications which run parallel to it in *H. erectus*, but which become more oblique inferoposteriorly relative to the more rounded occipital of recent humans (Grimaud-Hervé, 1994). In modern humans, the anterior and obelic branches are more developed, and the posterior branch is less developed – a pattern also seen in KNM-WT 15000. The anterior branch of the middle meningeal artery is well developed in fossil African *H. sapiens*, with numerous anastomoses in Jebel Irhoud (1 and 2) and Omo 2. In some fossil European *H. sapiens*, the obelic branch takes origin from both the anterior and posterior branches (Predmost 3, Predmost 4 left side, Dolni Vestonice 1, 2).

4.18.5 Trends in Hominin CNS Evolution

4.18.5.1 Primitive Brain Morphology

In order to determine whether a morphological feature is primitive or derived within the hominin clade, it is necessary to consider the brain morphology of the most recent hypothetical common ancestor of modern humans and living chimpanzees. The principle of parsimony suggests the panin–hominin ancestor possessed all shared derived features of extant humans and chimpanzees, but it would lack those features acquired solely along either the panin or the hominin lineages. It is difficult to reconstruct the panin–hominin ancestor with certainty with respect to well-represented regions of the hard tissue fossil record, and it is particularly difficult to do so for CNS-related morphology for which the extant and fossil evidence is both sparser and more difficult to interpret. For simplicity, we will assume that the chimpanzee brain is equivalent morphologically to the primitive hominin brain. There is no significant evidence for derived chimpanzee brain morphology which is not also shared with modern humans, although chimpanzees are likely to have evolved CNS autoapomorphies related to species-specific behaviors, which may be brought to light by future hominoid comparative neuroanatomical studies.

4.18.5.2 Modern Human or Hominin Lineage

4.18.5.2.1 Earliest appearance of derived modern human morphology The data suggest that, whereas fully modern human brain morphology only occurs in recent humans, some aspects of modern human brain morphology are present in earlier forms (see Table 3).

The aspect of modern human brain morphology that may have appeared earliest is the reduction of the primary visual cortex, as evidenced by the position of the LS. A posterior LS has been reported for some *Au. afarensis* specimens, but at the least this feature is variable within the taxon. Given the small sample it is difficult to tell whether the *Au. afarensis* brain really is derived in the direction of the modern human brain, or whether it expresses variability similar to that seen in chimpanzees. In contrast, there are several aspects of endocast anatomy derived in the direction of modern humanlike brain reorganization in *Au. africanus*, which has better evidence for a reduced primary visual cortex. In addition, *Au. africanus* shows evidence of: (1) a somewhat expanded, blunt orbitofrontal cortex, (2) anteriorly expanded, laterally pointed temporal poles, (3) an incipient LORF petalial pattern, and (4) a modern humanlike Broca's cap region. Although these features are not as pronounced as in modern humans, they can be interpreted as being derived in the direction of modern humans. The LORF petalial pattern and Broca's cap region

Table 3 Aspects of endocranial morphology and/or inferred CNS morphology

Taxon	*FAD (Mya)*	*Mean endocranial volume (cm^3)*	*EQ*	*LORF petalial pattern*[a]	*Fronto-orbital sulcus*[b]	*Orbital surface of the frontal lobe*[c]	*Broca's cap region*[d]	*Neurocranial globularity*[e]	*Temporal pole morphology*[f]	*Lunate sulcus position*[g]	*Relative size of cerebellum (CQ)*[h]	*Thoracic vertebral canal*[i]
Pan troglodytes (M)			1.6									
Pan troglodytes (F)			1.9	**P**	**P**	**P**	**P**	**P**	**P**	**P**	**1.2**	**P**
Recent *H. sapiens* (M)			5.1									
Recent *H. sapiens* (F)			5.4	**M**	**M**	**M**	**M**	**M**	**M**	**M**	**1**	**M**
S. tchadensis	7	365		–	–	–	–	–	–	–	–	–
O. tugenensis	6			–	–	–	–	–	–	–	–	–
Ar. kadabba	5.8			–	–	–	–	–	–	–	–	–
Ar. ramidus	4.5			–	–	–	–	–	–	–	–	–
Au. anamensis	4.2			–	–	–	–	–	–	–	–	–
Au. afarensis	3.9	446	2.5	**I**	–	–	**P**	–	–	**P/M**	–	**P**
K. platyops	3.5			–	–	–	–	–	–	–	–	–
Au. bahrelghazali	3.5			–	–	–	–	–	–	–	–	–
Au. africanus	3	460	2.8	**m**	**P**	**m**	**m**	–	**m**	**M**	**0.8**	**P**
Au. garhi	2.5	450		–	–	–	–	–	–	–	–	–
P. aethiopicus	2.5	410		**m**	–	**P**	–	–	**P**	–	–	–
P. boisei s.s.	2.3	488	2.5	**m**	–	**P**	–	–	**P**	**M**	**1**	–
P. robustus	2	533	3.1	**I**	–	**P**	–	–	**P**	**M**	–	–
H. habilis s.s.	2.4	609	3.7	**P**	**P**	–	**I**	–	–	–	**1**	–
H. rudolfensis	1.8	776	3.2	**M**	**M**	–	**M**	–	–	–	**0.9**	–
H. ergaster	1.9	763	2.8	**M**	–	–	**I**	–	–	–	**0.9**	**P**
H. erectus s.s.	1.8	991	3.9	**M**	–	–	**M**	**P**	–	**M**	**0.9**	–
H. antecessor	0.7			–	–	–	–	–	–	–	–	–
H. heidelbergensis	0.6	1242	4.2	**M**	–	–	**M**	**P**	–	**M**	**0.8**	–
H. neanderthalensis	0.2	1404	4.7	**M**	–	–	**M**	**P**	–	**M**	**0.7**	**M**
H. sapiens s.s.	0.19	1463	5.3	**M**	–	–	**M**	**M**	–	**M**	**0.7**	**M**
H. floresiensis	0.090	417	3.1	**M**	**M**	**M**	**I**	–		**M**	–	–

[a]LORF (left occipital right frontal) petalial pattern (P) infrequent, rarely involves both frontal and occipital lobes; (M) usual.
[b]Fronto-orbital sulcus (P) present; (M) absent.
[c]Orbitofrontal region (P) beak-shaped; (M) blunt and expanded.
[d]Asymmetrical Broca's area (P) not asymmetrically enlarged; (M) L > R asymmetry.
[e]Endocast shape (P) archaic; (M) globular, suggests expanded parietal.
[f]Temporal pole morphology (P) rounded; (M) anteriorly expanded, laterally pointed.
[g]Lunate sulcus position (P) anterior (some variability); (M) more posterior.
[h]Taxon mean EQ (encephalization quotient) values, calculated from specimen CQ (cerebellar quotient) values (LSR-05 in Weaver, 2001).
[i]Thoracic vertebral canal cross-sectional area (P) size expected for a primate of similar body mass; (M) larger than expected for a primate of similar body mass.
–, No relevant evidence; **I**, insufficient evidence; **M**, modern humanlike morphology either described or inferred; **m**, incipient modern human morphology either described or inferred; **P**, *Pan*-like morphology either described or inferred.
Pan-like (P) and modern humanlike (M) morphology (please refer to text for a more detailed explanation).

become even more modern in *H. rudolfensis*, the earliest taxon for which there is evidence for humanlike brain organization (there are insufficient data for the other three aforementioned features until later *Homo*). In addition, *H. rudolfensis* is the earliest taxon not to have an orbitofrontal sulcus. Interestingly, there is no good evidence for a modern humanlike LORF petalial pattern and a Broca's cap region in *H. habilis*; indeed, there is evidence of an African ape-like orbitofrontral sulcus. Where data exist, *H. erectus* and *H. ergaster* endocasts tend to share the modern humanlike features that are found in *H. rudolfensis*. *H. neanderthalensis* is the earliest taxon known to have an expanded thoracic vertebral canal. A globular brain due to parietal lobe expansion has been proposed as an autoapomorphy of modern humans (Bruner *et al.*, 2003). An increase in relative cerebellum size from fossil to recent anatomically modern humans might be a final refinement within this species.

4.18.5.2.2 Earliest appearance of increase in absolute and relative brain size Modern human mean brain mass for adults 21–39 years old is 1450 g for males, and 1290 g for females (Figure 4, Table 4) (Dekaban and Sadowsky, 1978). The chimpanzee mean brain weight for adolescents and young adults (7–30 years) is 406 g for males and 368 g for females (Herndon *et al.*, 1999). In both species, average brain mass decreases in older individuals; for example, Dekaban and Sadowsky (1978) reported a 7.4% decrease (approximately 100 g) in modern human brain mass between 20–30 years and 70–80 years. In fact, the mean endocranial volumes from a more typical modern human autopsy data set (average age 65 years) are dramatically different (male = 1308 g, female = 1179 g) (Zilles, 1972). Sex is also an important consideration in brain size comparisons because male and female samples of hominoid taxa have significantly different brain sizes. It is not possible to know the sex of fossil specimens, and statistical methods of sexing are not possible for the small early hominin cranial samples. Therefore, fossil taxa are not assigned to sex, but are compared as whole taxon samples to samples of both sexes of extant taxa. Previously, absolute brain size has been used to determine a cerebral Rubicon criterion for inclusion in the genus *Homo*, variably set between 600 and 800 cm^3 (Leakey *et al.*, 1964). Currently, absolute brain size is thought to lack biological significance, since it does not give an indication of degree of encephalization, or the number of extra neurons (Jerison, 1973; Martin, 1990). However, aspects of brain morphology such as brain component volumes and degree of gyrification scale to absolute brain size (Zilles *et al.*, 1988, 1989; Semendeferi and Damasio, 2000; Semendeferi *et al.*,

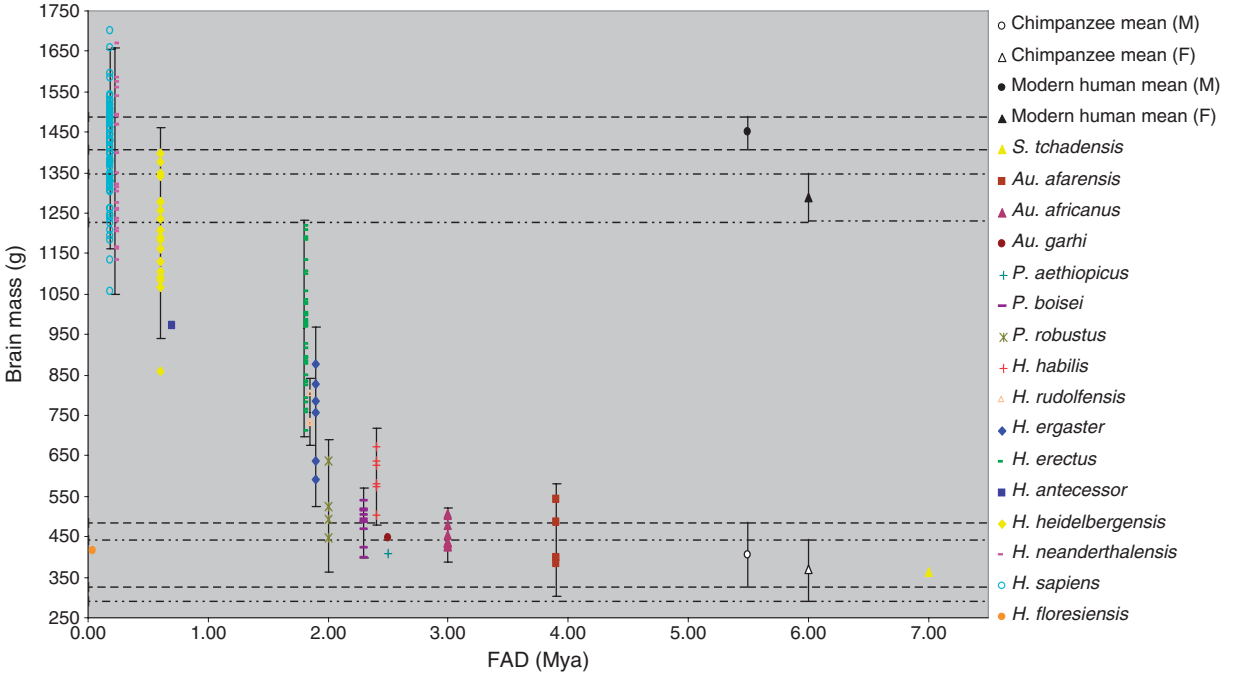

Figure 4 Chimpanzee and recent modern human male and female brain mass means are plotted, with *y*-axis bars and dashed lines showing ranges within two standard deviations. Fossil hominin brain mass individual specimen values are plotted with *y*-axis bars showing range within two standard deviations from the mean. FAD, first-appearance datum. For more information, see Table 4.

Table 4 Absolute and relative brain sizes for fossil and extant panin and hominin taxa

Taxon[a]	*FAD (Mya)*	*Number in sample*	*Mean endocranial volume*	*Minimun endocranial volume*	*Maximum endocranial volume*	*Mean brain mass*[b]	*Minimum brain mass*	*Maximum brain mass*	*Brain mass SD*	*Mean body mass*	*EQ*[c]
Pan troglodytes (M)		17				406	347	530	39	58	1.6
Pan troglodytes (F)		17				368	308	458	37	43	1.9
Recent *H. sapiens* (M)		351				1450	1343	1526	20	70	5.1
Recent *H. sapiens* (F)		201				1290	1239	1366	30	57	5.4
S. tchadensis	7	1	365			363					
Au. afarensis	3.9	5	446	387	550	442	385	542	69	38	2.5
Au. afarensis (M?)		2	521	492	550	514	486	542	40	45	2.6
Au. afarensis (F?)		3	396	387	400	393	385	397	7	29	2.7
Au. africanus	3	9	460	428	515	455	424	508	33	34	2.8
Au. garhi	2.5	1	450			446					
P. aethiopicus	2.5	1	410			407					
P. boisei	2.3	10	488	400	545	483	397	537	43	41	2.5
P. robustus	2	4	533	450	650	525	446	638	82	36	3.1
H. habilis	2.4	6	609	509	687	599	503	674	60	33	3.7
H. rudolfensis	1.8	3	776	750	825	758	734	805	41	55	3.2
H. ergaster	1.9	6	763	600	900	746	590	877	111	64	2.8
H. ergaster (Africa)		3	851	804	900	830	785	877	46	64	3.1
H. ergaster (Dmanisi)		3	675	600	775	662	590	758	86		
H. erectus	1.8	36	991	727	1260	963	712	1218	134	58	3.9
H. antecessor	0.7	1	1000			972					
H. heidelbergensis	0.6	21	1242	880	1450	1200	858	1397	131	71	4.2
H. neanderthalensis	0.2	27	1404	1172	1740	1353	1135	1669	153	72	4.7
H. sapiens	0.19	79	1463	1090	1880	1408	1057	1799	124	64	5.3
H. floresiensis	0.090	1	417			414				26	3.1

[a]Sources: Chimpanzee brain and body mass data for individuals 7–30 years from Herndon *et al.* (1999). Recent modern human brain and body mass data for adults 21–39 years (except minimum and maximum brain mass, which are for 20–30 years) from Dekaban and Sadowsky (1978). In both data sets, brain mass is taken from fresh autopsy specimens and includes brain tissue as well as leptomeninges and CSF. Fossil hominin endocranial volume raw data and sources are available from the authors, by request.

[b]Fossil endocranial volumes were converted into brain masses after Ruff *et al.* (1997).

[c]EQ (encephalization quotient) values after Martin (1981), and Ruff *et al.* (1997). Extant taxon EQ values are means of individual EQ values. Fossil taxon sample mean EQ values are obtained from each taxon's mean brain mass and mean body mass estimates (SD, standard deviation). EQ values obtained by either method are very similar and have been used interchangeably (e.g., Ruff *et al.*, 1997).

2002; Weaver, 2005), an important consideration when investigating the evolution of the brain of modern humans.

The smallest adult hominin brain belongs to the single cranial specimen of *Sahelanthropus*, the earliest possible hominin, and its endocranial volume falls slightly below the female chimpanzee mean. Single specimens of *P. aethiopicus*, *Au. garhi*, and *H. floresiensis* plot around the male chimpanzee mean. The *Au. afarensis* sample is not significantly different from the combined sex sample of chimpanzees ($p = 0.093$), nor from the male chimpanzee sample ($p = 0.456$), although it is significantly larger than the female chimpanzee sample ($p = 0.011$) (all statistical comparisons are derived from a Kruskal–Wallis test of significance). The *Au. africanus* sample is significantly different from the combined sex sample ($p < 0.001$), and the male ($p = 0.001$) and female ($p < 0.001$) subsamples of chimpanzees. However, this does not suggest that the brain size of *Au. africanus* is significantly increased relative to chimpanzees and that of *Au. afarensis* is not; these two groups do not differ significantly from each other ($p = 0.385$). Although the *Au. africanus* mean value (455 g) is only slightly larger than that for *Au. afarensis* (442 g), the range for *Au. africanus* is much smaller than that for the sexual dimorphic *Au. afarensis* (but see Reno *et al.*, 2005 for an alternative interpretation that suggests only modest levels of sexual dimorphism in *Au. afarensis*). *Au. afarensis* attains higher individual brain mass estimates than *Au. africanus*.

Early hominin fossil crania for which endocranial volume and body mass have been reliably estimated are extremely rare, making it impossible to do comparative statistical tests of EQs. However, given that early fossil hominins (e.g., *Australopithecus* and *Paranthropus*) have smaller estimated body masses than chimpanzees (mean body mass 58 kg for males, 43 kg for females; Herndon *et al.*, 1999), any significant increase in relation to chimpanzee brain volume can be assumed to be an increase in both absolute and relative brain size (Table 4). Thus, the increase from the brain size of a chimpanzee-like hypothetical common ancestor to brains the size of those belonging to *Au. afarensis* and *Au. africanus* is evidence of an increase in relative brain size. This finding is further evidenced by the EQ values of *Au. afarensis* (2.5) and *Au. africanus* (2.8), which are well above those for chimpanzees (male EQ = 1.7, female EQ = 1.9), overlapping with those of *Paranthropus* (*P. boisei* EQ = 2.5; *P. robustus* EQ = 3.1), and approximating that of *H. ergaster* (2.8).

By the appearance of *H. rudolfensis* and *H. habilis*, both absolute and relative brain size have clearly departed from the *Pan*-like condition. *H. habilis* is the smallest-brained hominin for which all the specimen values fall outside of two standard deviations of the male chimpanzee mean. *H. habilis* and *H. rudolfensis* are significantly different in brain mass ($p = 0.02$), and the entire range of *H. rudolfensis* values plot above the range of *H. habilis* values. However, when the brain mass data are seen in the light of body mass data, *H. habilis* (EQ = 3.7) is more encephalized than *H. rudolfensis* (EQ = 3.2). Relative brain mass in both *H. habilis* and *H. rudolfensis* is greater than that in *Australopithecus* and *Paranthropus,* and it approaches the values for *H. erectus* (EQ = 3.9).

In summary, although encephalization in the hominin lineage might have begun as early as *Au. afarensis*, it was evident in *Au. africanus* (in parallel to the encephalization of *Paranthropus*, see Section 4.18.5.3) and definitely by the time of the appearance of *H. habilis* and *H. rudolfensis*.

4.18.5.2.3 Appearance of derived modern human CNS morphology in relation to brain size Although there are hints of a trend toward a modern humanlike relative brain size and brain morphology in *Au. afarensis*, there is a lot of variability in the size and morphology of this taxon. Given small samples, one cannot be certain whether or not this variation is different from the variation seen in chimpanzees. Further, the functional and adaptive significance of these features in the early taxa is questionable. Modern humanlike endocranial anatomy in *Au. afarensis* might be a pre-adaptation which only acquires its modern functions in *Au. africanus*, *H. rudolfensis,* or in even later hominins.

Most aspects of modern humanlike endocast morphology make an appearance in *Au. africanus*, but they do not yet show the fully modern form. The reason for their occurrence in this taxon is uncertain, but might be influenced by brain size increase, and it is quite possibly related to exceptional preservation of brain morphology in this taxon. The appearance of several aspects of modern brain morphology in *Au. africanus* complement the fact that this taxon is the first to have a brain size significantly different from chimpanzees. However, as a whole, the *Au. africanus* brain still differs considerably from the modern human brain, and any similarities are not considered sufficient to suggest a modern humanlike cognitive capacity or behavior for *Au. africanus*.

This is in contrast to the more modern humanlike brain morphology of *H. rudolfensis* which is generally taken as evidence of more modern humanlike

cognitive capacities. Most notably, these features are suggestive of language ability and right-handedness, coincident with the first stone tools which apparently were made by right-handed hominins. This is associated with the earliest brain masses outside of what is expected for a chimpanzee, and an EQ higher than that of earlier taxa. However, *H. habilis* has a higher EQ than *H. rudolfensis*, and this later appearing hominin also has brain mass values outside of what is expected for a chimpanzee. It is not yet possible to tell whether the more modern humanlike brain morphology of *H. rudolfensis*, compared to *H. habilis*, is, or is not, size-related.

4.18.5.3 Brain Evolution in Other Lineages

The *P. boisei* mean value (483 g) for estimated brain mass is larger than the value for *P. aethiopicus* (407 g) and somewhat larger than the means for *Au. africanus* (455 g) and *Au. afarensis* (442 g). Further, the majority of the *P. boisei* specimens fall outside of two standard deviations of the male chimpanzee mean. Therefore, it is inferred that *P. boisei* has increased its absolute brain size relative to the primitive condition. The *P. boisei* sample mean is not significantly different ($p = 0.357$) from that of the later occurring *P. robustus* sample, even though the latter attains a much higher maximum value (638 g) and has a much higher mean (565 g). *P. boisei* and *P. robustus* have EQs that are higher than those for male and female chimpanzees. However, the *P. boisei* EQ is smaller than that of *Au. africanus* and is similar to the *Au. afarensis* value. Given the lack of postcranial evidence, one cannot be certain that EQ has increased from *P. aethiopicus* to later *Paranthropus* taxa. These data are, however, consistent with the suggestion of a temporal trend for brain size increase within the *Paranthropus* lineage (Elton *et al.*, 2001).

There is little evidence to suggest modern humanlike reorganization of the *Paranthropus* brain. In particular, slight LORF petalial patterns are found in *P. aethiopicus* and *P. boisei*, and a posteriorly positioned LS has been identified in *P. boisei*. The evidence does not suggest that the *Paranthropus* brain becomes increasingly modern humanlike over time, as is the case for *Homo*. Further, *Paranthropus* retains ape-like beak-shaped orbital surface of frontal lobe and rounded temporal poles, differentiating it from *Au. afarensis* and *H. sapiens*. The modern humanlike endocranial features seen in *Paranthropus* most likely reflect a shared ancestry with the modern human lineage. Similarly, brain size increase in *Paranthropus* is probably the continuation of a trend beginning in a common ancestor of *Paranthropus* and modern humans.

There is presumed to be a decrease in absolute and relative brain size in *H. floresiensis* (Brown *et al.*, 2004), but this trend might also apply to other fossil hominin taxa. *H. floresiensis* had a tiny brain (414 g), with an EQ (3.0) much lower than that of its presumed closest fossil relative, *H. erectus*. Interestingly, its EQ is higher than the one listed here for *H. ergaster* (2.8, includes Dmanisi) and only slightly lower than the EQ for African *H. ergaster* (3.1). Body mass estimates obtained from Dmanisi postcranial remains will refine the *H. ergaster* EQ. Given that *H. ergaster* is thought to have expanded outside of Africa, evidence from the relative brain size alone suggests that it, rather than *H. erectus*, may be the sister taxon of *H. floresiensis*. If so, this would indicate that EQ did not actually decrease in *H. floresiensis*, thereby solving one of the major puzzles of this taxon (Brown *et al.*, 2004). It is noted that *H. floresiensis* possesses much morphology that is derived from the primitive ape-like condition. Several of these features are thought to be found in the most recent *H. floresiensis*–modern human common ancestor (which may also be the most recent *H. erectus*–modern human common ancestor).

Brain size increase and the appearance of some aspects of modern humanlike brain morphology occur in at least two hominin lineages. Both *Paranthropus* and *Homo* have absolutely and relatively larger brains than *Australopithecus*. However, only in *Homo* does brain size increase occur in parallel with the acquisition of modern humanlike brain morphology. Interestingly, *H. floresiensis* provides striking evidence that, even within *Homo*, estimated brain mass and inferred brain morphology can become disassociated.

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4.19 The Evolution of Human Brain and Body Growth Patterns

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Glossary

adolescence A stage in the human life cycle, covering the 5–8 years after the onset of puberty. The adolescent phase is characterized by a growth spurt in height and weight, virtual completion of permanent tooth eruption, development of secondary sexual characteristics, sociosexual maturation, intensification of interest and practice of adult social, economic, and sexual activities.

adolescent growth spurt The rapid and intense increase and then decrease in the rate of growth in height and weight that occurs during the adolescent stage of the human life cycle.

adrenarche A stage of maturation of the cortex of the human adrenal glands. It typically occurs between ages 6 and 10 years and involves a progressive increase in the production of androgens.

adulthood The stage of the human life cycle that begins at about age 20 years. The prime of adulthood lasts until the end of child-bearing years (late forties) and is a time of homeostasis in physiology, behavior, and cognition. Old age and senescence mark the period of decline in the function of many body tissues or systems during adulthood. This later phase lasts from about the end of child-bearing years to death.

childhood A stage in the human life cycle that occurs between the end of infancy and the start of the juvenile growth period (about the ages 3.0–6.9 years). Children are weaned from breast-feeding (or bottle feeding) but must be provided specially prepared foods and require intensive care by older individuals. Relatively rapid neurological development and slow physical growth and development characterize childhood.

cooperative breeding Also called communal breeding; occurs when nonbreeding helpers raise young produced by dominant breeders. The helpers may be genetic kin of the breeders.

infancy A stage in the life cycle of all mammals when feeding is done by lactation. For human beings, infancy lasts from birth to the end of lactation, usually by age 36 months. Human infancy is characterized by: (1) rapid growth velocity with a steep deceleration in velocity with time, (2) feeding by lactation, (3) deciduous tooth eruption, and (4) many developmental milestones in physiology, behavior, and cognition.

gonadarche Maturation of neuroendocrine system at puberty resulting in a change from inhibition to stimulation of gonadal steroid secretion. This event is also called puberty.

juvenile A stage in the life cycle of most social mammals, including all of the higher primates. The juvenile stage is defined as the time of life when an individual is no longer dependent on adults (parents) for survival, and prior to that individual's sexual maturation. For human beings, the juvenile stage begins after childhood at about 7 years and ends at puberty (gonadarche) at about 9 years for girls and 11 years for boys.

life history theory A field of biology concerned with the strategy evolved by natural selection that an organism uses to allocate its energy toward growth, maintenance, reproduction, raising offspring to independence, and avoiding death. For a mammal, it is the strategy of when to be born, when to be weaned, how many and what type of prereproductive stages of development to pass through, when to reproduce, and when to die.

plasticity The concept that the development of the phenotype is responsive to variations in the quality and quantity of environmental factors required for life. Such variations produce many of the differences in growth, physiology, and behavior observed between individuals or groups of people.

puberty An event of short duration (days or a few weeks) that marks the reactivation of the central nervous system regulation of sexual development. The onset of puberty is accompanied by a dramatic increase in the gonadal secretion of sex hormones. In social mammals, including humans, puberty occurs at end of the juvenile stage.

trade-offs Life history strategies used when competition between two biological or behavioral traits requires a partial allocation of energy or materials to each trait, or complete allocation to only one of several competing traits.

weaning The termination of breast-feeding.

4.19.1 Human Brain and Body Growth

Shown in Figure 1 is the size of the adult brain relative to that of adult body weight for 61 species of the order Primates (data for Old World monkeys, apes, and humans). The curve drawn through the data points is a logarithmic regression fit to the data for all species. All of the monkeys and the three great apes, *Pan* (chimpanzee), *Pongo* (orangutan), and *Gorilla*, lie close to the predicted curve for brain weight/body weight. Human (*Homo*) brain size departs significantly from the predicted curve. It is known that virtually all parts of the human brain enlarged during evolution, especially the size of the neocortex, which comprises about 80% of the mass of the modern human brain (see Brain Size in Primates as a Function of Behavioral Innovation).

At all stages of life after birth, human beings have brains that are significantly larger than expected given the human body size. Human beings grow a large brain by maintaining the rapid rate of fetal brain growth into the postnatal period and extending the time for rapid brain growth to about age 7 years (Figures 2 and 3). In contrast, chimpanzee brain growth slows down greatly after birth and ends by 5 years of age (Vrba, 1998). Human body growth reaches a zenith in the second trimester of pregnancy and then slows precipitously through the third trimester (Figure 4). The rate of human body growth continues to decelerate for the first 3 years after birth, levels off at about 3 years of age, and remains relatively slow until puberty and adolescence when all healthy humans experience a growth spurt in height and weight (Figure 5). No other species of primate or mammal follows this pattern of brain–body growth (Figure 6).

4.19.2 Human Life History

Life history can be defined as the strategy of when to be born, when to be weaned, how many and what

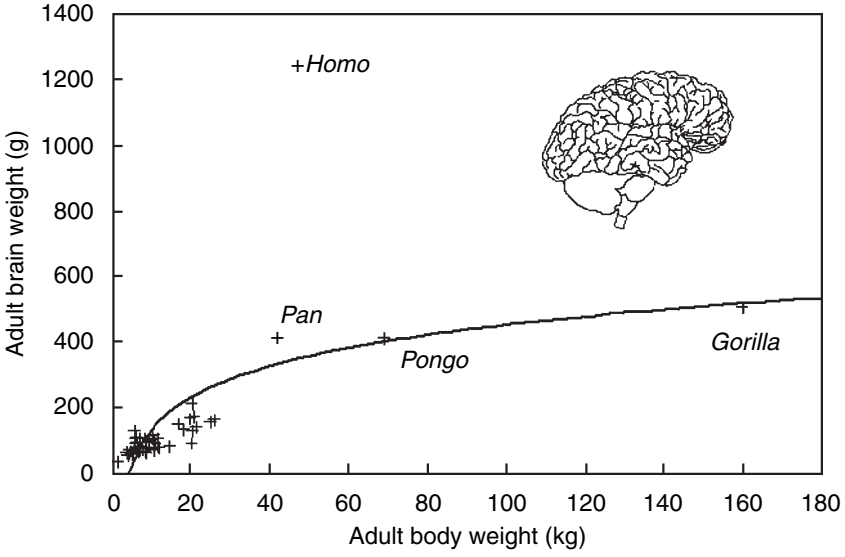

Figure 1 Adult body weight and brain weight plotted for 61 species of Cercopithcidae (Old World monkeys, apes, and people). The curve is logarithmic regression fit to the data for all species. The image is a lateral view of the human brain. Data from Harvey, P., Martin, R. D., and Cluton-Brock, T. H. 1987. Life histories in comparative perspective. In: Primate Societies (*eds.* B. Smuts, D. L. Chency, R. M. Seyfarth, R. W. Wrangham, and T. T. Struhsaker), pp. 181–196. University of Chicago Press.

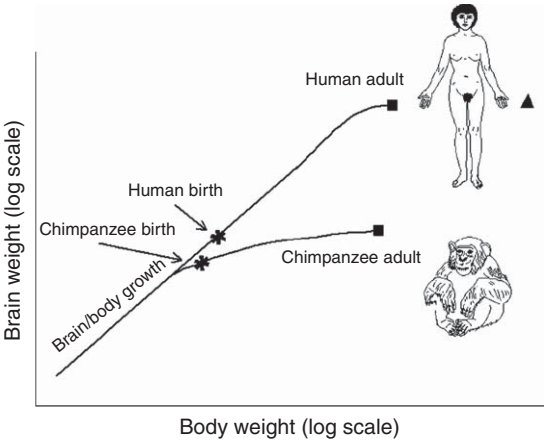

Figure 2 Growth curve for human brain and body compared with the chimpanzee. The length of the human fetal phase, in which brain and body grow at the same rate for both species, is extended for humans. Chimpanzee brain growth slows after birth, but humans maintain the high rate of brain growth during the postnatal phase. In contrast, the rate of human body growth slows after birth. If human brain/body growth rate were equal to the chimpanzee rate, then adult humans would weigh 454 kg and stand nearly 3.1 m tall, indicated by the ▲ symbol. From Bogin (1999) based on the data and concepts of Martin (1983).

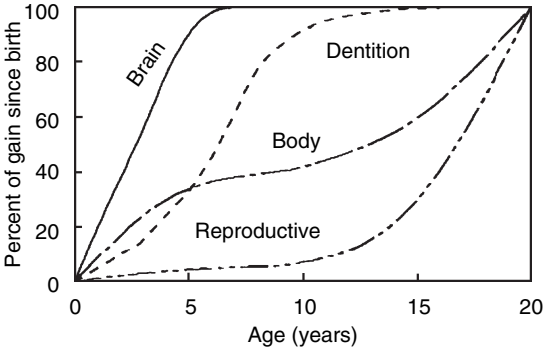

Figure 3 Growth curves for different body tissues (Bogin, 1999). The brain curve is for total weight of the brain (Cabana *et al.*, 1993). The dentition curve is the median maturity score for girls based on the seven left mandibular teeth (I1, I2, C, PM1, PM2, M1, M2) using the reference data of Demirjian (1986). The body curve represents growth in stature or total body weight and the reproductive curve represents the weight of the gonads and primary reproductive organs (Scammon, 1930).

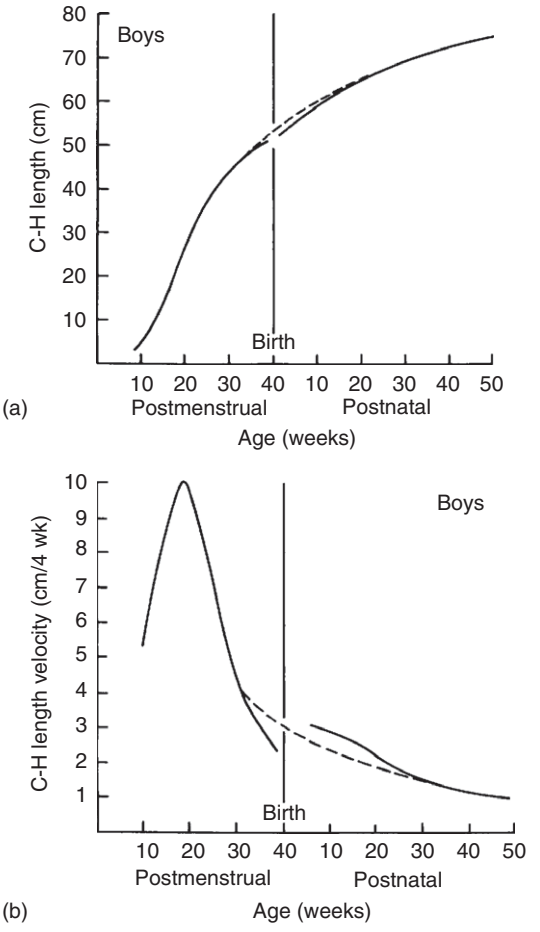

Figure 4 Distance curve (a) and velocity curve (b) for growth in body length (measured as crown-heel (C-H) length) during human prenatal and postnatal life. The figure is diagrammatic, as it is based on several sources of data. The dashed lines depict the predicted curve of growth if no uterine restriction takes place. In fact, such restrictions do take place toward the end of pregnancy and this may impede the flow of oxygen or nutrients to the fetus. Consequently, growth rate slows but rebounds after birth and returns the infant to the size she or he would be without any restriction (Tanner, 1990). Reprinted by permission of the publisher from Fetus into Man: Physical Growth from Conception to Maturity by J. M. Tanner, p. 39, Cambridge, MA: Harvard University Press, Copyright © 1978, 1989, by J. M. Tanner and Cartlemead Publishers.

type of prereproductive stages of development to pass through, when to reproduce and when to die. For a mammal, it is the strategy of when to be born, when to be weaned, how many and what type of prereproductive stages of development to pass through, when to reproduce, and when to die. Living things on earth have greatly different life history strategies, and understanding what shapes these histories is one of the most active areas of research in whole-organism biology.

The majority of mammals have two postnatal life history stages: infancy and adulthood. Highly social mammals such as wolves, wild dogs, lions, elephants, and the primates postpone adulthood by inserting a period of juvenile growth and behavior between infancy and adulthood. Most mammalian biologists define infancy as the stage of life when the offspring are fed by nursing. Adulthood is the stage of life when the individual is capable of reproduction. Juveniles may be defined as "prepubertal individuals that are no longer dependent on their mothers (parents) for survival" (Pereira and Altmann, 1985, p. 236).

Human beings add two new stages of development between birth and adulthood: childhood and adolescence. As can be seen in Figure 5, changes in growth

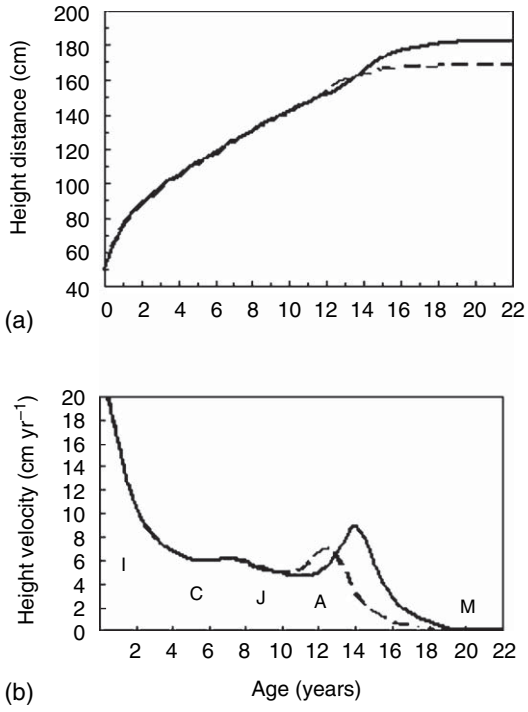

Figure 5 Average distance (a) and velocity (b) curves of growth in height for healthy girls (dashed lines) and boys (solid lines) showing the postnatal stages of the pattern of human growth. The stages of postnatal growth are abbreviated as follows: I, infancy; C, childhood; J, juvenile; A, adolescence; M, mature adult. Data used to construct the curves come from Prader, A 1984. Biomedical and endocrinological aspects of normal growth and development. In: Human Growth and Development (*eds.* J. Borms, R. R. Hauspie, A. Sand, C. Susanne, and M. Hebbelinck), pp. 1–22. Plenum; Bock, R. D. and Thissen, D. 1980. Statistical problems of fitting individual growth curves. In: Human Physical Growth and Maturation, Methodologies and Factors (*eds.* F. E. Johnston, A. F. Roche, C. Susanne), pp. 265–290. Plenum.

rate are associated with each stage of development. Each stage may also be defined by characteristics of the dentition, changes related to methods of feeding, physical and mental competencies, or maturation of the reproductive system and sexual behavior (Bogin, 1999, provides detailed discussion for the following material; more recent references are provided herein).

As for all mammals, human infancy is the period when the mother provides all or some nourishment to her offspring via lactation or some culturally derived imitation of lactation (e.g., formula feeding). During infancy, the deciduous dentition (the so-called milk teeth) erupts through the gums. Motor skills (i.e., what a baby can do physically) develop rapidly during infancy. There is a similar progression of changes in the problem solving, or cognitive, abilities of the infant. Human infancy ends when the child is weaned from the breast, which in preindustrialized societies occurs between 30 and 36 months of age.

The childhood stage encompasses the ages of about 3–7 years. At the beginning of childhood, the body growth rate levels off at about 5–6 cm per year. Brain growth continues at a relatively fast pace (Figure 3). The leveling-off in body growth rate is unusual for mammals, because almost all other species either stop growing or, in the case of social mammals, continue a pattern of deceleration during the juvenile stage (Figure 6).

A suite of additional features defines the childhood stage. These are:

- slow and steady rate of body growth and relatively small body size;
- a large, fast-growing brain;

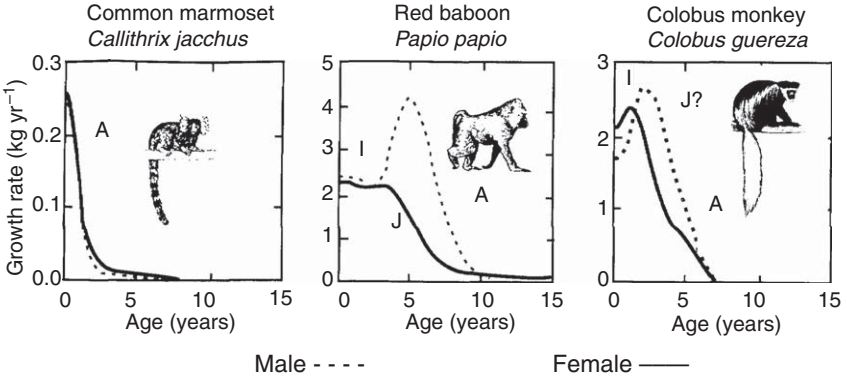

Figure 6 Three nonhuman primate species showing different patterns of postnatal growth in weight. The human pattern of growth differs significantly from each of these species. Marmosets are weaned at 63 days and can breed at 1 year of age (Harvey *et al.*, 1987). Marmoset growth rate shows no postnatal growth spurt and no change in growth rate typical of a juvenile growth stage. Baboons are weaned by 18 months and begin puberty at about 3.5 years for females and 4.5 years for males (Harvey *et al.*, 1987). Baboons have a juvenile growth stage for both males and females, but only males have clear weight growth spurt. Colobus monkeys are weaned at about 13 months and females have their first birth at about 4.6 years of age. Colobus monkeys show a postnatal spurt for both sexes, but it is not clear if there is a juvenile growth stage. The velocity curves are fit to cross-sectional data collected from captive animals. The curves are fit using the statistical estimates based on lowess regression (Leigh, 1994). I, infancy; J, juvenile; A, adult. Reproduced from Leigh, S. R. 1994. Growing up to be a Primate (book review). *Evol. Anthropol.* 3, 106–108, with permission from John Wiley & Sons, Inc.

- higher resting metabolic rate (RMR) of brain relative to body than any other mammalian species;
- immature dentition;
- motor immaturity;
- cognitive immaturity; and
- adrenarche and the midgrowth spurt.

No other mammalian species has this entire suite of features.

The large and fast-growing brain of infants and children is an especially important feature of human biology. The newborn uses 87% of its RMR for brain growth and function. By the age of 5 years, the percent RMR usage is still high at 44%, whereas in the adult human, the figure is between 20% and 25% of RMR. At comparable stages of development, the RMR values for the chimpanzee are about 45%, 20%, and 9%, respectively (Leonard and Robertson, 1994).

The human juvenile stage begins at about 7 years of age. In girls, the juvenile period ends, on average, at about the age of 10, 2 years before it usually ends in boys, the difference reflecting the earlier onset of adolescence in girls. The beginning of juvenility is marked by adrenarche and, in some children, a midgrowth spurt in height (Figure 1). Adrenarche is the progressive increase in the secretion of adrenal androgen hormones (Auchus and Rainey, 2004). Adrenal androgens may launch the growth of axillary and pubic hair. The physical changes induced by adrenarche are accompanied by cognitive and social advances, and there are sexually dimorphic changes in behavior and voice during this stage as well (Wuyts *et al.*, 2003).

By the beginning of the juvenile stage (about age 7 years), the growth in weight of the brain is virtually complete, but much biological and cognitive development of the brain must still take place. (A small increase in brain mass occurs at puberty (Durston *et al.*, 2001; Sowell *et al.*, 2001); myelination and some nerve proliferation continue into adulthood (Bjorklund and Pellegrinni, 2002; Taupin and Gage, 2002).) The energy requirements for brain growth are about 38% of RMR, which is still large as juvenile body weight is relatively small, at about 31% of adult body weight. Energy investment in the juvenile brain may be related to the onset of new behavioral and cognitive competencies. A great deal of learning, physical activity play, and social competition takes place for all juvenile primates (Bogin, 2002). Language and symbolic thinking skills mature rapidly (Locke and Bogin, in press; see The Evolution of Language Systems in the Human Brain) and the 7-year-old individual can perform many basic tasks, including food preparation with little or no supervision. Juveniles can also conceptualize themselves as independent of older people. Energy invested in brain and behavior takes away from energy available for body growth. Indeed, the juvenile stage is characterized by the slowest rate of growth since birth. No energy is used for reproduction, as juveniles are prefertile and immature in terms of both their primary and secondary sexual characteristics.

Human adolescence is the stage of life when social and sexual maturation takes place. Adolescence begins with puberty, or more technically with gonadarche, which is an event of the neuroendocrine system. The current understanding of the control of gonadarche is that one, or perhaps a few, centers of the brain change their pattern of neurological activity and their influence on the hypothalamus (Plant and Barker-Gibb, 2004). The hypothalamus, which has been basically inactive in terms of sexual development since about age 3 years, is again stimulated to produce GnRH (gonadotrophin-releasing hormone). It is not known exactly how this change takes place. As stated above, the production of GnRH by the hypothalamus becomes inhibited by the age of about 2 years. The inhibitor has not been identified but likely is located in the brain and certainly not in the gonads. Human children born without gonads as well as rhesus monkeys and other primates whose gonads have been surgically removed still undergo both hypothalamus inhibition in infancy and hypothalamus reactivation at puberty.

None of these hormonal changes can be seen without sophisticated technology, but the effects of gonadarche can be noted easily as visible and audible signs of sexual maturation. One such sign is a sudden increase in the density of pubic hair. In boys, the deepening of the voice is another sign of puberty. In girls, a visible sign is the development of the breast bud, the first stage of breast development. The pubescent boy or girl, his or her parents, and relatives, friends, and sometimes everyone else in the social group can observe these signs of early adolescence. The adolescent stage also includes sexual dimorphism in body size and composition and the onset of greater interest and practice of adult patterns of sociosexual and economic behavior. These physical and behavioral changes occur with puberty in many species of social mammals. What makes human adolescence unusual among the primates are two important differences. The first is the length of time between age at puberty and age at first birth. Humans take, on average, at least 10 years for this transition. Monkeys and apes take less than 3 years to make the transition from puberty to parenthood. The second human difference is that during this life stage, both boys and girls experience a rapid acceleration in the growth velocity of almost all skeletal

tissue – the adolescent growth spurt. Other primate species may show a rapid acceleration in soft tissue growth, such as muscle mass in many male monkeys and apes. However, unlike humans, other primate species either have no acceleration in skeletal growth or a very small increase in growth rate. The human skeletal growth spurt is unequaled in amount and duration by other species, and when viewed graphically, the growth spurt is a defining feature of human adolescence (Figure 5b).

Adolescence ends and early adulthood begins with the completion of the growth spurt, the attainment of adult stature, and the achievement of reproductive maturity, meaning both the physical and psychosocial maturity needed for successful reproduction. All of these developments coincide, on average, by about age 19 in women and 21–25 years of age in men.

4.19.3 Life History Trade-Offs between the Growth of Brain versus Body Tissues

Perhaps it is best to conceptualize the pattern of life history of a species as a series of trade-offs, or compromises, that an organism makes between principal biological or behavioral traits. Some mammalian life history traits and trade-offs are listed in Table 1. Principal traits for mammals, including human beings, include the timing of birth versus continued fetal development, the length of the lactation stage versus weaning (the cessation of lactation), the size of the brain, the age at first reproduction, and the age at death. A trade-off may be thought of as the competition between two biological or behavioral traits. Stearns says that ". . . trade-offs occur when two traits compete for materials and energy within a single organism . . ." or ". . . when selection for one trait decreases the value of a second trait" (Stearns, 1992, p. 223). An example of the first type of trade-off is competition between organs or tissues of the body during growth. For example, should energy and materials be devoted to growing a large set of muscles and gut or a larger brain? An example of the second type of trade-off is the choice of producing one large offspring or many, smaller offspring.

During human evolution, and in comparison to the evolution of the nonhuman apes, several trade-offs were made. At least four trade-offs are most important to the evolution of brains and bodies. First, grow a large brain early in life, but reduce the rate of body growth after birth. Second, wean infants before the eruption of their permanent teeth, enabling the mother to reproduce more rapidly, but pass-on child care responsibilities to other group members. Third, reduce the size of the gut (stomach and intestines), but divert energy to the growth and maintenance of the brain. Fourth, prolong the total duration of body growth, delay the onset of adult reproduction, but allow for greater plasticity and adaptability in physical development and learning.

Table 1 Life history principal traits and trade-offs

Principals	*Trade-offs*
1. Size at birth	1. Current reproduction vs. future reproduction
2. Brain size	2. Current reproduction vs. survival
3. Growth patterns • Number of life cycle stages • Duration of each stage	3. Number vs. size offspring 4. Parental reproduction vs. growth 5. Brain size vs. body size
4. Age at eruption of first permanent molar	6. Parental health vs. offspring growth
5. Rate of maturation • Age at first reproduction • Age of last reproduction	7. Parental vs. offspring reproduction
6. Size at maturity	
7. Number and sex ratio of offspring	
8. Reproductive investment in each offspring	
9. Length of life • Rate of aging/senescence • Age at death	

This is a partial list of the most important traits. The list is based on the discussion in Cole (1954) and Stearns (1992), who provide additional traits.

4.19.4 Implications for Behavioral and Cognitive Maturation of Human Children

Trade-offs between brain and body growth, combined with early weaning and the short duration of human infancy have several implications. After weaning, the infant enters the childhood stage. Human children require specially prepared foods because of the immaturity of their dentition, the small size of their stomachs and intestines, and the rapid growth and relatively high RMR of their brain. The human constraints of immature dentition and small digestive system necessitate a childhood diet that is easy to chew and swallow and low in total volume. The child's relatively large and active brain, almost twice the size of an adult chimpanzee's brain, requires that the low-volume diet be dense in energy, lipids, and proteins. Children do not yet have the motor and cognitive skills to prepare such a diet for themselves.

Children are also especially vulnerable to predation and disease and thus require protection. Children will not survive in any society if deprived of the care provided by older individuals. So-called wolf children and even street children, who are sometimes alleged to have lived on their own, are either myths or not children at all. My search of the literature finds no case of a child (i.e., a youngster under the age of 6 years) living alone, either in the wild or on urban streets. The dependence of children on older members of the social group creates the need for cooperative breeding, a style of reproduction found in several groups of social insects and mammals (Bogin, in press).

Two of the important physical developmental milestones of childhood are the replacement of the deciduous teeth with the eruption of the first permanent teeth and completion of brain growth in weight (Cabana *et al.*, 1993). First molar eruption takes place, on average, between the ages of 5.5 and 6.5 years in most human populations. Eruption of the central incisor quickly follows, or sometimes precedes, the eruption of the first molar. By the end of childhood, usually at the age of 7 years, most children have erupted the four first molars, and permanent incisors have begun to replace milk incisors. Along with growth in size and strength of the jaws and the muscles for chewing, these new teeth provide sufficient capabilities to eat a diet similar to that of adults. At this stage of development, not only is the child capable dentally of processing an adult-type diet; the nutrient requirements for brain growth also diminish. Moreover, cognitive and emotional capacities mature to new levels of self-sufficiency. Language and symbolic thinking skills mature rapidly (Locke and Bogin, in press), social interaction in play and learning become common, and the 7-year-old individual can perform many basic tasks, including food preparation with little or no supervision (Bock and Sellen, 2002).

4.19.5 Evolution of Human Brains and Life History

Figure 7 is an attempt to represent the evolution of human development, though at present, the only reliable data are associated with *Pan* (chimpanzee) and *Homo sapiens*. Known or estimated adult brain sizes are given at the top of each bar. Smith and Tompkins (1995) calculated estimated ages for the first permanent molar (M1) in other species. Age of eruption of M1 is an important life history event that correlates very highly with other life history events. The fossil taxa named in Figure 7 are meant to represent grades of hominin evolution.

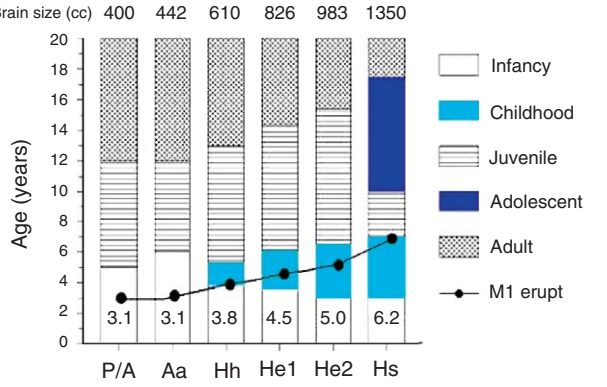

Figure 7 The evolution of hominid life history during the first 20 years of life. P/A, *Pan* and *Australopithecus afarensis*; Aa, *Australopithecus africanus*; Hh, *Homo habilis*; He1, early *Homo erectus*; He2, late *Homo erectus*; Hs, *Homo sapiens*. Mean brain sizes are given at the top of each histogram. Mean age at eruption of the M1 is graphed across the histograms, and identified numerically at the base.

Appearing about 3.9 Mya, *Australopithecus afarensis* shares many anatomical features with nonhominid pongid (ape) species, including an adult brain size of about 400 cc and a pattern of dental development indistinguishable from extant chimpanzees. Therefore, the chimpanzee and *A. afarensis* are depicted as sharing the typical tripartite stages of postnatal growth of social mammals: infancy, juvenility, and adulthood. The duration of each stage and the age at which each stage ends are based on empirical data for chimpanzees. A probable descendent of *A. afarensis* is the fossil species *A. africanus*, dating from about 3 Mya. Achievement of the larger adult brain size of *A. africanus* (average of 442 cc) may have required an addition to the length of the fetal and/or infancy periods. Figure 3 depicts a 1 year extension of infancy.

As the figure indicates, M1 of the chimpanzee erupts at 3.1 years, even though infancy continues for nearly 2 more years. Before 5 years, young chimpanzees are dependent on the mother and will not survive if she dies or becomes unable to provide care and food. After the eruption of M1, they may be able to manage an adult diet, but still must learn how to find and process foods, and it takes time to learn how to open shelled fruits and extract insects from nests. This may be why chimpanzees extend infancy beyond the eruption of M1. It is likely that early hominids, such as *A. afarensis* and *A. africanus*, followed a pattern of growth and development very similar to chimpanzees and also extended infancy for at least 1 year beyond the age of M1 eruption. Analyses of postcranial anatomy and archaeological records suggest a similarity between the behavioral capacities of hominids and extant chimpanzees.

The *H. habilis* grade of hominin evolution follows. At this grade there are delays in dental development and a pattern of femur growth that departed from that of the australopithecines, but resembled the pattern seen in later hominids (Tardieu, 1998). Along with larger adult brain size, all of this indicates a probable childhood stage of growth. This stage may have started after the eruption of M1 and lasted for about 1 year. During childhood, the *H. habilis* youngsters would need to be supplied with special weaning foods. There is archaeological evidence for just such a scenario. *H. habilis* seems to have intensified its production and dependence on stone tools. There is considerable evidence that some of these tools were used to scavenge animal carcasses, especially to break open long bones and extract bone marrow (Potts, 1988). This behavior may be interpreted as a strategy to feed children. Such scavenging may have been needed to provide the essential amino acids, some of the minerals, and especially the fat (as both essential lipids and a dense source of energy) that children require for growth of the brain and body.

A further increase in brain size occurred during the time of *H. erectus*, which began about 1.6 Mya. The earliest adult specimens have mean brain sizes of 826 cc, but many individual adults had brain sizes between 850 and 900 cc. Insertion or expansion of childhood would have provided the time needed for a rapid, humanlike pattern of brain growth. In the time of *H. habilis* and early *H. erectus*, the transition from infancy to childhood took place before M1 eruption. If the new childhood was stolen from infancy, or reduced its length, then *H. erectus* would have enjoyed a greater reproductive advantage than any previous hominid. This seems to be the case, since by the time of *H. erectus* hominin populations increased in size and began to spread throughout Africa and other regions of the world.

Figure 7 also shows later *H. erectus*, with an average adult brain size of 983 cc and a further expansion of the childhood stage. In addition to larger brains (some measuring 1100 cc), the archaeological record for later *H. erectus* shows increased complexity of technology (tools, fire, and shelter) and social organization. These technosocial advances, and a parallel increase in learning, were grafted onto the reproductive value of childhood. By the time of modern *H. sapiens*, the selective value of reproductive efficiency and learning expanded the length of the childhood stage to its current length.

As for when adolescence evolved, it is possible that this stage first appeared in *H. erectus* (Antón and Leigh, 2003; Smith, 2004). Other research indicates that *H. erectus*, later hominids such as *Homo antesesor* (800 000 BP) and *H. heidelbergensis* (400 000–500 000 BP), and even the Neanderthals of 40 000 BP grew up too quickly to have adolescence (Dean *et al.*, 2001; Ramirez-Rossi and Bermudez de Castro, 2004). If so, then adolescence evolved with archaic or modern *H. sapiens* (as depicted in Figure 7). Adolescence may be a product of contemporary brain size and brain function that creates the human world of complex technological, social, and ideological capacities and cultures.

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4.20 Constraints on Brain Size: The Radiator Hypothesis

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Glossary

Australopithecus	A genus of early hominin with many species, one of which probably gave rise to the human lineage; sometimes called gracile australopithecines.
emissary veins	Veins that pass through apertures (foramina) in the cranial wall and establish communication between the sinuses and veins inside the braincase and the veins external to it.
hominin	Humans and their early bipedal ancestors (excludes apes); what 'hominid' used to mean.
occipital/marginal (O/M) sinus	Route that delivers venous blood to the vertebral plexus of veins and causes a groove on the occipital (O) bone and along the margin (M) of the foramen magnum in some hominins.
Paranthropus	A rugged-looking genus of early hominin that was not ancestral to humans, also called robust australopithecines.
selective brain cooling	A natural mechanism that enables mammals to maintain brain temperature below that of the rest of the body during states of hyperthermia.
transverse/sigmoid sinuses	Cranial venous sinuses that drain blood from the back of the cranium to the jugular veins in most humans.
vertebral plexus of veins	Network of veins surrounding the spinal column that drains significantly more cranial venous blood from upright than supine humans.

4.20.1 Synopsis of Radiator Hypothesis

4.20.1.1 Temperature Sensitivity and Selective Brain Cooling in Humans

The human brain is an exquisitely heat-sensitive organ. According to the noted vascular physiologist, Mary Ann Baker (1979):

> A rise of only four or five degrees C above normal begins to disturb brain functions. For example, high fevers in children are sometimes accompanied by convulsions; these are manifestations of the abnormal functioning of the nerve cells of the overheated brain. Indeed, it may be that the temperature of the brain is the single most important factor limiting the survival of man and other animals in hot environments.

Humans lack the special network of arteries and veins (*rete mirabile*) that helps regulate brain temperature in numerous carnivores and ungulates. The differentially enlarged human brain is especially sensitive to hyperthermia because its high cerebral (compared to resting) metabolic rate generates a relative abundance of heat (Caputa, 2004). One way in which human brain temperature is regulated is through arterial blood that is delivered into the cranium, which is cooler than the brain it supplies. As the arterial blood circulates, it removes heat from the brain, so that venous blood exiting the braincase is warmer than the arterial blood supplying it.

4.20.1.1.1 Role of emissary veins Whole-body cooling takes place when arterial blood is cooled through the effects of evaporation of sweat from the body's surface, a process that also contributes to regulation of brain temperature via its arterial supply. Cabanac and Brinnel (1985) proposed an additional mechanism for selectively cooling the

brain under conditions of intense exercise that results in hyperthermia. Because experimental evidence revealed that blood flows out of the cranium through the mastoid, ophthalmic, and parietal emissary veins in hypothermic subjects but into the braincase in hyperthermic subjects, Cabanac and Brinnel reasoned that venous blood that is cooled at the head's surface through the effects of evaporation on dilated veins is selectively delivered into the braincase under, and only under, conditions of hyperthermia (oral temperature of $37.6 \pm 0.18^{\circ}$ C). The authors noted that innumerable, microscopic emissary veins exist in humans, and demonstrated (by massaging a cadaver's skullcap) that blood is capable of flowing through this network from the outside of the skull to the diploic veins within the cranial bones and then to the inside of the braincase.

The three emissary veins that were used to record direction of blood flow are located at dispersed points of the network that supplies the entire skull: at the face (ophthalmic), behind the ear (mastoid), and at the top back part of the skull (parietal) (Figure 1). Cabanac and Brinnel concluded that when blood flows into the braincase in these three emissary veins, it also does so in the innumerable tiny veins that comprise the entire network. According to this hypothesis, venous blood cooled at the head's surface under hyperthermic conditions flows into the braincase over a dispersed network of tiny veins (the cranial radiator). This is a selective brain cooling mechanism that serves to keep brain temperature in check. Cabanac and Brinnel's hypothesis became controversial among physiologists who claimed that existence of an anatomical network of cranial veins capable of delivering cooled blood into the braincase was speculative. This point will be returned to in Section 4.20.3.

4.20.1.1.1.(i) Patterns of cranial blood flow in apes, hominins, and contemporary people All scorable skulls of robust australopithecines (*Paranthropus*) show traces of an unusual enlarged sinus at the lower back ends of their skulls that routed venous blood from their heads, called the occipital/marginal (O/M) sinus. Curiously, only one other hominin shared this high frequency, namely *Australopithecus afarensis* from Ethiopia (the group to which the famous fossil Lucy belongs). Neither living people nor apes manifest enlarged O/M sinuses to any appreciable degree (Figure 2). Instead, apes and humans drain blood from their heads through another route, the transverse/sigmoid sinus system (Figure 2). (*Paranthropus* skulls may or may not

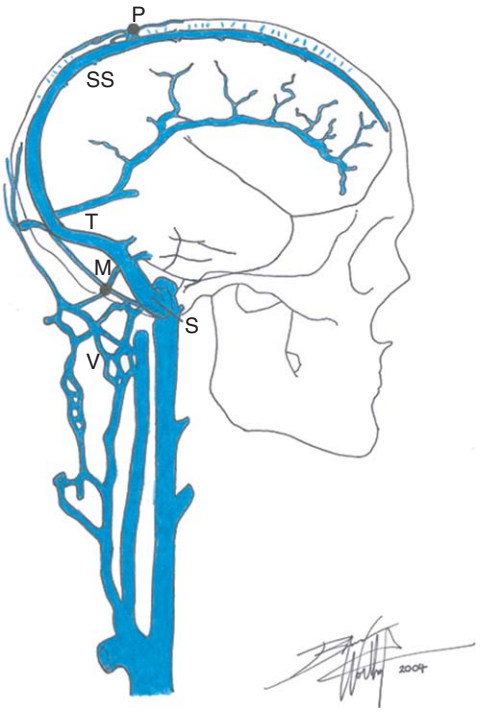

Figure 1 Venous sinuses and parietal (P) and mastoid (M) emissary veins in humans. SS, superior sagittal sinus; T, transverse sinus; S, sigmoid sinus; V, vertebral plexus of veins. Reproduced from Falk, D. Concepts and hypotheses: history of brain evolution, http://www.anthro.fsu.edu/research/falk/braindance.html.

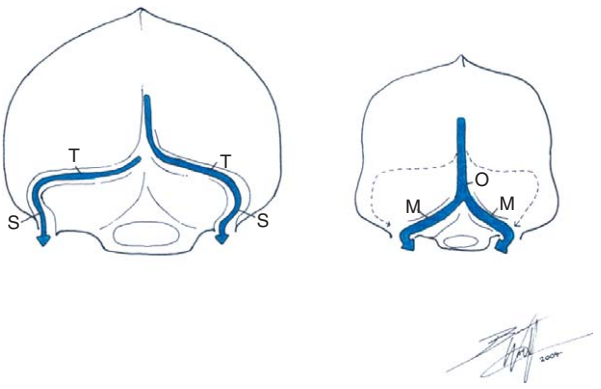

Figure 2 Occipital views of typical cranial venous sinus systems in modern humans (left) and fossil robust australopithecines (*Paranthropus*) and earlier hominins from Hadar, Ethiopia (right). In humans, venous blood exits the skull through the transverse (T)–sigmoid (S) sinuses, which deliver blood to the internal jugular veins. This system may or may not be present in robust and Hadar australopithecines, in which a significant amount of blood is always drained through an enlarged occipital (O)–marginal (M) sinus system that communicates with the vertebral plexus of veins. The O/M system is always present on one or both sides of the latter. Reproduced from Falk, D. Concepts and hypotheses: history of brain evolution, http://www.anthro.fsu.edu/research/falk/braindance.html.

reproduce one or both sides of the transverse/sigmoid route in addition to one or both sides of an enlarged O/M system.) Because the enlarged O/M sinuses that are infrequently found in human cadavers deliver blood to a network of veins around the spinal column (called the vertebral plexus of veins), this is likely to have been an important drainage route for the enlarged O/M sinuses of early hominins.

In order to explore the evolution of cranial blood flow, data were collected on the frequencies of O/M sinuses and mastoid and parietal emissary foramina from skulls of African apes, fossil hominins, and extant humans (Falk, 1986). Apes have low frequencies (appearing in $\leq$25% of specimens) for all three traits (Boyd, 1930). Emissary foramina of *Paranthropus* are equally infrequent but all scorable specimens to date ($n = 11$) have enlarged O/M sinuses (Falk *et al.*, 1995). Humans, on the other hand, have high frequencies of both emissary foramina but very low frequencies for enlarged O/M sinuses. As discussed below, the nonrobust hominins that preceded *Homo* show decreasing frequencies of O/M sinuses and increasing frequencies of parietal and mastoid foramina over time.

4.20.1.1.1.(ii) Relationship of vascular pattern to brain size Figure 3 shows the frequencies of enlarged O/M sinuses and emissary foramina plotted for African apes and various hominins along with their mean cranial capacities (which approximate brain size) expressed as a percentage of the average capacity for extant *Homo sapiens*. The graph reveals that emissary foramina increased in frequency in *Homo* as brain size enlarged over time. These data and experimental evidence that mastoid and parietal emissary veins participate in

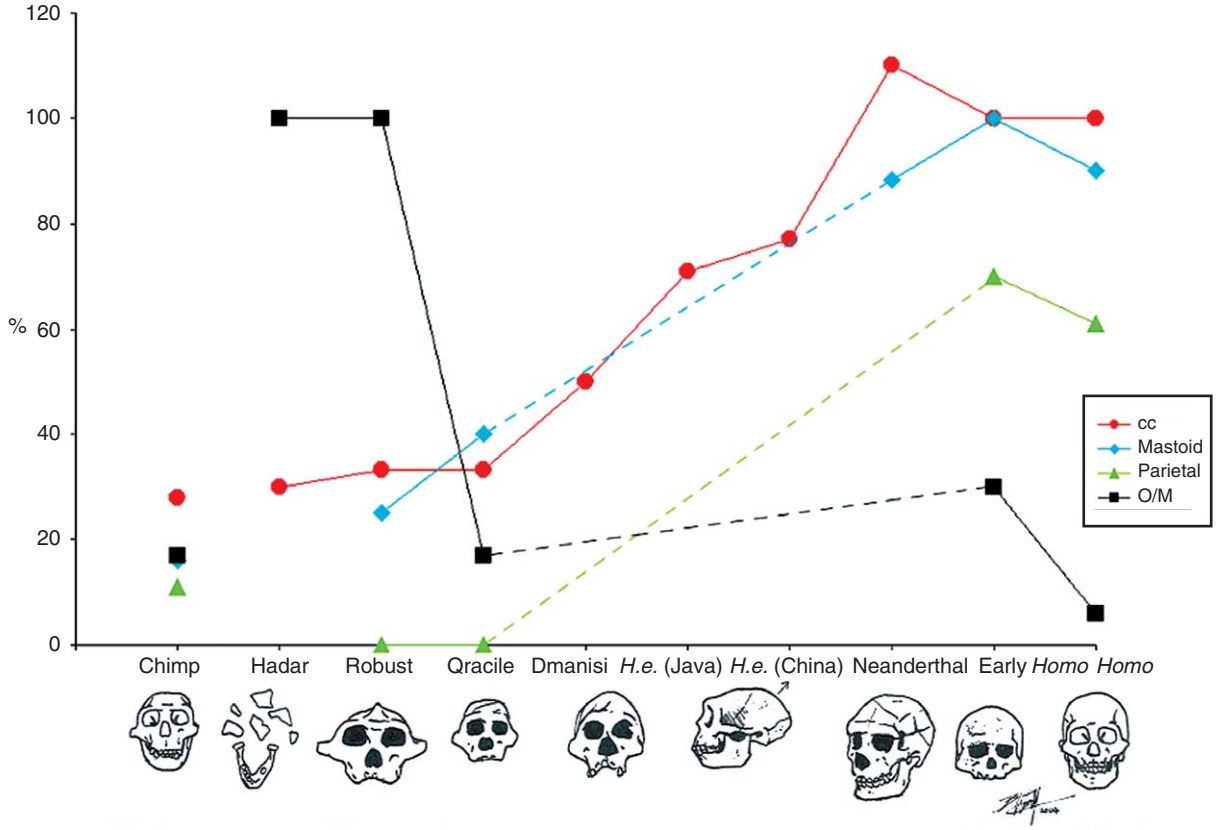

Figure 3 Frequencies of mastoid (blue diamonds) and parietal (green triangles) emissary foramina and enlarged O/M sinuses (black squares) plotted against mean cranial capacities (CCs) expressed as percentages of the 1350 cc mean for modern humans (red circles). Data from Falk (1986), Tobias and Falk (1988), and Falk *et al.* (1995, 2000). Dashed lines indicate fossils that have not been scored; robust = *Paranthropus*, gracile = *Australopithecus africanus*. Chimpanzees are included for comparative purposes; hominins are arranged in approximate chronological order from left to right. In Hadar and robust specimens, O/M is fixed; in the latter emissary foramina occur in low apelike frequencies. The reverse is true for the *Australopithecus* (gracile)–*Homo* lineage in which O/M frequencies fluctuate around those of apes, while those for emissary foramina increase through time in conjunction with brain size. Reproduced from Falk, D. Concepts and hypotheses: History of brain evolution, http://www.anthro.fsu.edu/research/falk/braindance.html.

selective brain cooling in hyperthermic people (Cabanac and Brinnel, 1985) suggest that the wider network of veins that incorporates the two emissary veins increased in size and complexity as cooling requirements became more demanding due to the ongoing enlargement of an evolving brain. Because this network of veins acts to keep brain temperature within check much as an automobile's radiator regulates engine temperature, it has been dubbed a cranial radiator (Falk, 1990).

4.20.1.2 Significance of Bipedalism

Experimental evidence reveals that the vertebral plexus of veins around the spinal column receives significantly more cranial blood when living humans are upright than when they are supine. The enlarged O/M sinuses of an early biped, *A. afarensis*, presumably delivered blood to their vertebral plexuses and have therefore been correlated with the development of upright walking (bipedalism) in that species or its ancestors. Because of their shared enlarged O/M sinuses, it was suggested in 1983 that *A. afarensis* was ancestral to more recent *Paranthropus* (or that the two groups shared a common ancestor) rather than to *Homo* (Falk and Conroy, 1983). The proponents of Lucy as 'the mother of us all' did not take kindly to this suggestion, which nevertheless found support in 1986 with the announcement of the discovery of a remarkably early *Paranthropus* specimen, the Black Skull dated to around 2.5 Mya, which appeared to be a probable descendant of *A. afarensis* (Falk, 1990, 2004a).

Humans do not usually have enlarged O/M sinuses, and appear instead to utilize a network of tiny pathways to deliver blood to their vertebral plexuses when they are standing or walking. The finding that blood drains preferentially to the vertebral plexus (instead of to the jugular veins) in upright but not supine postures (due to hydrostatic and hemodynamic factors) is well documented and has important implications for patients undergoing surgery of the head and neck. The named emissary veins at the back of the head contribute to the routes that drain to the vertebral plexus and, fortunately for paleoanthropology, these veins go through identifiable foramina (Hauser and De Stefano, 1989) that may be observed in fossil skulls (Figure 3). To summarize, both an enlarged O/M sinus system and the cranial radiator channel blood toward the vertebral plexus of veins surrounding the spinal column when individuals are bipedal, but only one of these (the radiator) is involved in selective brain cooling.

4.20.1.2.1 Interaction of bipedalism with climate/solar radiation According to Wheeler (1988), hominins that foraged for food during the heat of the day initially reduced their risk of hyperthermia by behavioral means, namely by maintaining bipedal stances that reduced the amount of body surface exposed to direct sunlight, which allowed them to occupy a noon-day scavenging niche. By the time bipedalism was fully achieved, however, hair had reduced on body surfaces that were no longer exposed to direct radiation from the sun at its zenith, and the newly naked skin and an increased number of cutaneous sweat glands facilitated evaporation and therefore whole-body cooling. Wheeler also suggested that such cooling released a constraint on brain size in *Homo*. Add to this the elaboration of the network of emissary and other small veins that participate in selective brain cooling and one has the radiator hypothesis (Falk, 1990)!

4.20.1.2.1.(i) Two lineages of hominins (Paranthropus, Australopithecus–Homo): *two ways to drain cranial blood* A variety of patchy environments were available to hominins living in Africa during the Plio-Pleistocene, and evidence from dentition and cranial blood flow shows that robust and nonrobust (gracile) australopithecines occupied different niches. *Paranthropus* was a vegetarian who had no need to engage in hunting of game during the heat of the day and therefore no need of a cranial radiator that would kick in during intense exercise. This hominin and its earlier *A. afarensis* relatives from Ethiopia relied on an enlarged O/M sinus system to drain blood from the cranium. Gracile australopithecines and early *Homo*, on the other hand, were more eclectic in their diets and, like living chimpanzees, had a taste for meat. By around 2 Mya, late *Australopithecus* and/or some of its early *Homo* descendants had begun scavenging and hunting on the hot African savanna (Blumenschine, 1987), and cranial radiators began to evolve.

4.20.2 Significance for Understanding Human Encephalization

4.20.2.1 The Cranial Radiator Was a Prime Releaser, Not a Prime Mover of Human Encephalization

During the early development of the network of radiator veins, thermal constraints that had previously kept brain size in check were released by the emergence of selective brain cooling in hominins that were living in niches associated with exposure to intense solar radiation. Consequently, brain size

began to increase in late nonrobust australopithecines and their *Homo* descendants, but remained forever static in *Paranthropus*. The radiator network of veins continued to increase along with brain size during most of *Homo*'s evolution, and today the human brain averages three times the size one would expect for a nonhuman primate of equivalent body size. The radiator network of veins is not hypothesized to have been the 'prime mover' of human brain evolution, i.e., the one trait whose selection was primarily responsible for brain evolution. (Prime mover candidates include hunting, tool production, work, warfare, throwing, language, and Machiavellian intelligence Falk 2004a). Instead, the radiator is viewed as an underlying and dynamic mechanism that helped regulate brain temperature and, as such, released thermal constraints that would otherwise have kept brain size in check in *Homo*, as they had in *Paranthropus*. The radiator is therefore best viewed as a 'prime releaser' (or one of several coordinated physiological releasers), not a prime mover of human brain evolution (see The Evolution of Human Brain and Body Growth Patterns, Evolutionary Specializations for Processing Faces and Objects).

4.20.2.1.1 The prime mover(s) of encephalization and cognitive evolution must be sought elsewhere The radiator hypothesis is purely mechanistic and does not address specific behaviors that may have been favored directly by natural selection as the prime mover(s) of hominin brain evolution. Of all the candidates that have been suggested as prime movers, human-like language may be the most likely because (1) it is not found in other animals and (2) it is germane to many of the advanced cognitive abilities that characterize *Homo sapiens*.

4.20.3 Recent Evidence Bearing on the Hypothesis

4.20.3.1 New Data Are Consistent with the Radiator Hypothesis

4.20.3.1.1 The takeoff in hominin brain size may have begun earlier than previously believed Since the radiator hypothesis was first published in 1990, major changes have occurred in the understanding and interpretation of the hominin fossil record that pertain to brain evolution. Revised cranial capacities for numerous australopithecines and new data for hominins from Dmanisi, Republic of Georgia (Vekua *et al.*, 2002), contradict the traditional view that brain size 'took off' around 2.0 Mya in *Homo* and suggest, instead, that brain size may have begun to increase considerably earlier in the *Australopithecus* ancestors of *Homo* (Falk, 2004b). Such a paradigm shift fits with the radiator hypothesis (Figure 3), and newly discovered skulls offer the opportunity to collect more data pertaining to cranial blood flow.

4.20.3.1.2 New findings regarding early hominin habitats The radiator hypothesis incorporated a controversial assumption that early *Homo* and its direct australopithecine ancestors occupied patchy savanna niches that exposed individuals to risk of hyperthermia from intense solar radiation, and that other hominins, including *A. afarensis* and *Paranthropus*, occupied more wooded, less thermally challenging niches. The assumption that different early hominins occupied different habitats has received recent support from discoveries of 6–7-million-year-old hominins (*Sahelanthropus*, *Orrorin*) that are thought to have lived in forested or wooded habitats rather than savannas. Although it now looks as if bipedalism may not have originated in savanna habitats as previously thought, by around 2 Mya nonrobust hominins were 'standing tall and staying cool' while adding more meat to their diets by scavenging and hunting in thermally stressful savannas. Such a shift to higher quality (and easily digestible) foods at this time is incorporated in the expensive-tissue hypothesis of Leslie Aiello and Pete Wheeler (Aiello and Wheeler, 1995), which postulates a trade-off between metabolically expensive guts and brains, with the former decreasing as the latter increased in mass during hominin evolution. These new data are consistent with the radiator hypothesis' basic assumption that a cranial radiator developed when hominins began to occupy thermally stressful habitats, thus releasing a thermal constraint that had previously kept brain size in check.

4.20.3.1.3 New data for frequency of enlarged occipital/marginal venous sinuses in *Paranthropus* According to the radiator hypothesis, an enlarged O/M sinus occurs in 100% of scorable robust australopithecines. This assertion has met with occasional claims that a particular *Paranthropus* specimen lacks this feature, but in these few cases the specimens have not been complete enough to score (per guidelines published by Tobias and Falk, 1988). When enlarged O/M sinuses were first tallied, seven *Paranthropus* specimens were scorable and all seven had the feature. Today, 11 out of 11 scorable *Paranthropus* specimens have the trait, which lends strong support to the suggestion that enlarged O/M sinuses are, indeed, fixed in *Paranthropus*.

4.20.3.1.4 Anatomical demonstration of the radiator The radiator hypothesis' basic assumption that humans possess an extensive network of tiny veins that participate in selective brain cooling was based on physiological and frequency data collected from a few named emissary veins. Although this assumption was controversial, in 1996 Wolfgang Zenker and Stefan Kubik demonstrated an anatomical basis for a convection process of cooling the brain by evaporation of sweat from the head (Figure 4). Transcranial heat exchange may thus occur between cool venous blood, the CSF (cerebral spinal fluid), and thin-walled subarachnoid and pial arterial branches before they enter the brain. As the authors note, the suggestion that the 'unprecedented vascular bed' shown in Figure 4 may transmit temperature changes to the cerebrospinal compartment, which, in turn, selectively cools the brain is open to physiological verification or falsification.

4.20.4 Summary and Conclusions

4.20.4.1 Physiological Data Such as Those Incorporated in the Radiator Hypothesis Are Beginning to Inform Paleoanthropology

Traditional hypotheses on hominin brain evolution have often included suggestions regarding single behaviors or traits that may have been the prime mover(s) of cognitive evolution targeted by natural selection. Although entertaining, such prime mover hypotheses are extremely speculative and do not lend themselves readily to testing. Physiological hypotheses such as those regarding a cranial radiator and expensive tissue are beginning to emerge in paleoanthropology, and these lend themselves more easily to scientific testing. The radiator and expensive-tissue hypotheses should be viewed as complementary rather than competing. Both incorporate data that suggest the direct ancestors of humans increased their exploitation of resources in open African habitats; and both identify physiological constraints that, when released, permitted brain size to increase.

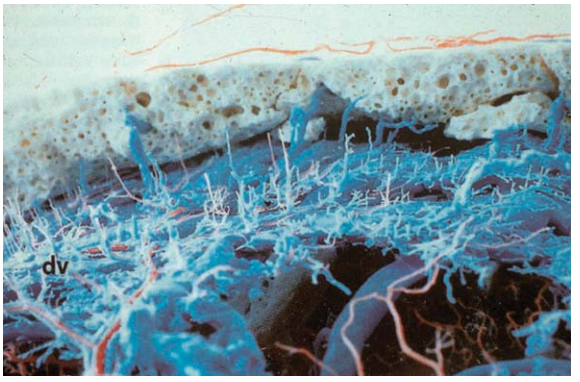

Figure 4 Corrosion cast of cranial blood vessels. Parietal bone preserved. Note vessels arising from the dural vascular rete and entering the bone (dv). Reproduced from *Anat. Embryol.*, Brain cooling in humans – anatomical considerations, W. Zenker, S. Kubik, vol. 193, p. 4, figure 4, 1996, © Springer-Verlag, with permission from Springer-Verlag.

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4.21 The Evolution of Neuron Types and Cortical Histology in Apes and Humans

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Glossary

allometry Many biological traits scale with overall size in a nonlinear fashion. Such allometric scaling relationships can be expressed by the power function: $Y = bX^a$. The logarithmic transformation of the allometric scaling equation yields: $log\ Y = \log\ b + a\ \log\ X$. The exponent of the power function becomes the slope of the log-transformed function. The slope of this line can then be interpreted in terms of a biological scaling relationship between the independent and dependent variable. Positive allometry refers to a scaling relationship with an exponent that is greater than 1, which means that the structure in question grows disproportionately larger or more numerous with increases in the size of the reference variable. Negative allometry refers to a scaling relationship with an exponent that is less than 1, which means that the structure in question becomes proportionally smaller or less numerous with increases in the size of the reference variable.

chemoarchitecture The microanatomical organization of the cerebral cortex revealed by staining for biochemical substances using techniques such as immunohistochemistry and enzyme or lectin histochemistry.

dysgranular cortex A type of cortex that has a weakly defined layer IV because it is variable in thickness. At points, layer IV seems to disappear because neurons from layers IIIc and Va intermingle.

encephalization A relative measure of a species' brain size that represents the degree to which it is larger or smaller than expected for a typical animal of its body size.

granular cortex A type of cortex that has a clearly identifiable layer IV.

gray level index (GLI) The proportion of an area of reference that is occupied by the projected profiles of all

Nissl-stained elements. This value provides an estimate of the fraction of tissue that contains neuronal somata, glial cell nuclei, and endothelial nuclei versus neuropil. GLI values are highly correlated with the volume density occupied by neurons since glial and endothelial cell nuclei contribute only a very small proportion of the total volume.

hominoid A phylogenetic clade that includes lesser apes (gibbons and siamangs), great apes (orangutans, gorillas, chimpanzees, and bonobos), and humans.

infragranular layers Cortical layers that are deep to granular layer IV, i.e., layers V and VI.

minicolumn Morphologically, minicolumns appear as a single vertical row of neurons with strong vertical interconnections among layers, forming a fundamental structural and functional unit. The core region of the column contains the majority of the neurons, their apical dendrites, and both myelinated and unmyelinated fibers. A cell-poor region, containing dendritic arbors, unmyelinated axons, and synapses, surrounds each column.

neuropil The unstained portion of Nissl-stained tissue, which is comprised of dendrites, axons, and synapses.

supragranular layers Cortical layers that are superficial to granular layer IV, i.e., layers I, II, and III.

4.21.1 Introduction

4.21.1.1 Evolutionary History of the Hominoids

Apes and humans are members of the primate superfamily Hominoidea (Figure 1). Molecular evidence indicates that the hominoid lineage split from the Old World monkeys about 25 Mya (Wildman *et al.*, 2003). The extant representatives of this phylogenetic group include two families. The Hylobatidae comprises gibbons and siamangs, and the Hominidae includes great apes (i.e., orangutans, gorillas, chimpanzees, and bonobos) and humans (Groves, 2001). Living hominoids are distinguished by a suite of shared derived traits that point to the key adaptations of this clade. These characters include lack of an external tail, modifications of the shoulder girdle and wrist for greater mobility, and stabilization of the lower back (Begun, 2003). These adaptations allow hominoids to exploit resources in small branches of trees by developing suspensory postures to distribute their body weight. This form of locomotion may have been particularly important in allowing certain species to increase body size. In addition, compared to other primates, hominoids have extended periods of growth and development (Schultz, 1969), an increased complexity of social interactions (Potts, 2004), and larger brains than would be expected for a monkey of the same body size (Rilling and Insel, 1999). The increased encephalization and associated life history elongation of these species suggest that cognitive flexibility and learning were important aspects of the hominoid adaptive complex, which allowed them to deal with locating ephemeral resources from fruiting trees and to negotiate more complicated relationships in fission–fusion societies (Potts, 2004).

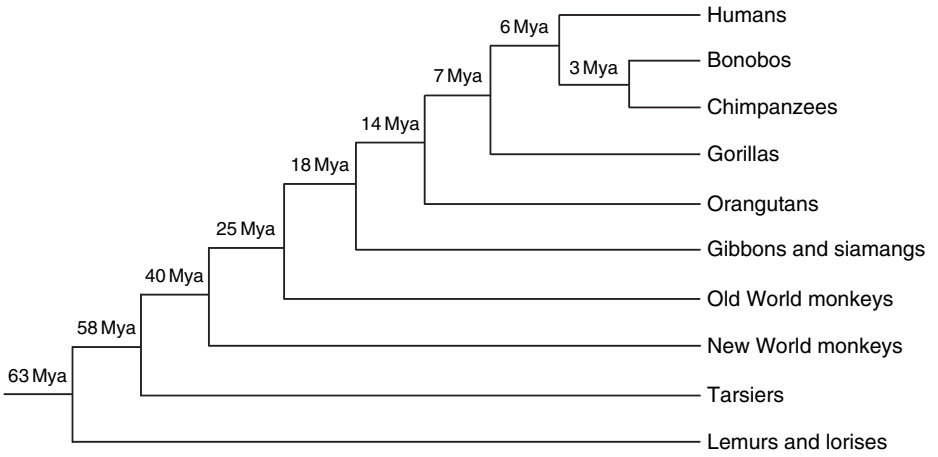

Figure 1 Cladogram showing the phylogenetic relationships of living hominoids and other primates. Estimated divergence dates are taken from Goodman *et al.* (2005).

Although only a small number of hominoid species persist today, the fossil record reveals a diverse array of successive adaptive radiations of hominoids in the past. During the Miocene epoch, global climates were warm and humid, supporting dense forests and lush woodlands extending throughout the tropics and into northern latitudes. These environmental conditions were favorable for the diversification of arboreal specialists, such as the hominoids. In fact, hominoids were the most abundant type of anthropoid primate throughout the Miocene in Africa and Eurasia, occupying a range of different ecological niches (Begun, 2003). The earliest apes in the fossil record are characterized by hominoid-like dental morphology, but monkey-like postcranial anatomy. The best known of these early dental apes is the genus *Proconsul* from the Early Miocene (20–18 Mya) of East Africa. *Proconsul africanus* endocasts show a frontal lobe morphology that is similar to modern hominoids in being gyrified and lacking the simple V-shaped arcuate sulcus that is characteristic of most Old World monkeys (Radinsky, 1974). Furthermore, *P. africanus* had a relatively larger brain than extant monkeys of comparable body size (Walker *et al.*, 1983). Thus, increased encephalization and perhaps a greater degree of frontal lobe gyrification were present early in the evolution of the hominoids.

With the emergence of arid climates in the transition to the Pliocene and the replacement of forests by mosaic habitats, the arboreal specializations of hominoids were less successful. The relatively slow reproductive rates of these taxa, moreover, made it difficult for many to endure habitat loss resulting from climate change and human encroachment in recent times (Jablonski *et al.*, 2000). In the context of these dramatic environmental changes, one lineage adopted a new form of locomotion, upright bipedal walking, which would give rise to modern humans. Other hominoids, however, fared less well and today apes are restricted to a small number of endangered tropical forest species.

4.21.1.2 History of Studies Concerning Hominoid Cortical Histology

At the beginning of the twentieth century, neuroanatomists applied new histological staining techniques to reveal the architecture of the cerebral cortex in numerous species, including apes and humans (Campbell, 1905; Mauss, 1908, 1911; Brodmann, 1909; Beck, 1929; Filimonoff, 1933; Strasburger, 1937a, 1937b). In addition, with the advent of various techniques for tracing neuronal connectivity based on intracellular pathological changes subsequent to ablation, some studies also examined cortical projection systems in apes (Walker, 1938; Lassek and Wheatley, 1945; Kuypers, 1958; Jackson *et al.*, 1969). After the 1950s, however, the amount of research directed toward understanding variation in the hominoid brain declined. There are three main reasons for this. First, the development of molecular biological techniques caused neuroscientists to focus on a small number of model species under the implicit assumption that many aspects of cortical structure are evolutionarily conserved. These ideas were further bolstered by claims of uniformity in the basic columnar architecture of the cerebral cortex (Rockel *et al.*, 1980). Second, findings from the first systematic studies of great ape behavior from the field and laboratory were beginning to be appreciated (e.g., Kortlandt, 1962; Schaller, 1963; Yerkes and Learned, 1925; Yerkes and Yerkes, 1929). These studies contributed to a more sophisticated understanding of cognitive and emotional complexity in great apes and suggested that they deserve special protected status with respect to the ethics of invasive neurobiological experimentation. Third, the book *Evolution of the Brain and Intelligence* (Jerison, 1973) had an enormous influence on the direction of later research in comparative neuroanatomy. This book argued for the predictability of neuroanatomical structure from brain size and encephalization, suggesting that these metrics form the most significant contribution to species diversity in brain organization. Combined with the ready availability of comparative brain region volumetric data in primates and other mammals from the publications of Heinz Stephan, Heiko Frahm, George Baron, and colleagues (e.g., Stephan *et al.*, 1981), a great deal of research effort has been expended in studies of allometric scaling and covariance of large regions of the brain (Finlay and Darlington, 1995; Barton and Harvey, 2000; de Winter and Oxnard, 2001). In contrast, much less attention has been paid to the possibility of phylogenetic variation in cortical histology (Preuss, 2000). Fortunately, advances in quantitative neuroanatomy and immunohistochemical staining techniques have opened new avenues of research to reveal interspecific diversity in the microstructure of hominoid cerebral cortex.

The history of studies of hominoid cortical histology, therefore, has resulted in two eras of research. The early era comprises several qualitative comparative mapping studies of the cerebral cortex based on cyto- and myeloarchitecture, with the occasional comment regarding species differences in the microstructure of homologous cortical areas

(Campbell, 1905; Mauss, 1908, 1911; Brodmann, 1909; Beck, 1929; Bailey *et al.*, 1950). Later studies from this era also contributed quantitative data concerning the surface area of particular cortical areas, as well as cellular sizes and densities in a few nonhuman hominoid species (Mayer, 1912; Tilney and Riley, 1928; von Bonin, 1939; Lassek and Wheatley, 1945; Shariff, 1953; Haug, 1956; Glezer, 1958; Blinkov and Glezer, 1968). These quantitative data, however, were rarely presented in the context of a focused examination of variation among hominoids.

The modern era is characterized by studies that use techniques such as design-based stereology, computerized image analysis, and immunohistochemical and histochemical staining to identify subpopulations of cortical cells. These new approaches are especially appealing for investigations of phylogenetic variation in cortical histology in species that are not common to the laboratory because several enzymatic, cytoskeletal, and other macromolecular constituents that are well preserved in postmortem tissue can be used as reliable markers for subpopulations of distinct neuron types, thereby extending comparative studies of species for which anatomical tracing, electrophysiological mapping, or other experimental procedures would be either unethical or impractical (see The Evolution of Neuron Classes in the Neocortex of Mammals). Subsets of pyramidal neurons, for example, can be distinguished immunohistochemically by staining with an antibody (e.g., SMI-32) to nonphosphorylated epitopes on the medium- and high-molecular-weight subunits of the neurofilament triplet protein. These epitopes are particularly enriched in subpopulations of large neurons of the neocortex that have a specific laminar and regional distribution. Because nonphosphorylated neurofilament protein (NPNFP) is involved in the maintenance and stabilization of the axonal cytoskeleton, its expression is associated with neurons that have thick myelinated axons (Hoffman *et al.*, 1987; Kirkcaldie *et al.*, 2002). In addition, the calcium-binding proteins – calbindin D-28k (CB), calretinin (CR), and parvalbumin (PV) – are useful markers for understanding the cortical interneuron system because each of these molecules is typically co-localized with GABA in morphologically and physiologically distinct nonoverlapping populations (DeFelipe, 1997; Markram *et al.*, 2004).

While the neuroanatomical structure of one hominoid species in particular has been studied most extensively, it is well beyond the scope of this article to provide a comprehensive review of human cortical architecture. Here we focus explicitly on comparative studies of the histology of hominoid cerebral cortex, highlighting evidence concerning shared derived traits of hominoids in comparison to other primates, as well as indicating possible species-specific specializations. Hence, the studies reviewed in this chapter provide the most direct evidence currently available to delimit which aspects of cortical histology are uniquely human, which are derived for all hominoids, and which reflect the specializations of each species.

4.21.2 Comparative Anatomy of the Cerebral Cortex

4.21.2.1 Topology of Cortical Maps

Total brain weight in hominoids ranges from approximately 90 g in Kloss's gibbons (*Hylobates klossii*) to 1400 g in humans (*Homo sapiens*) (Table 1). While there is a large range of variation among hominoids in total brain size, mapping studies of the cortex (Figure 2) generally agree that the location of the primary sensory and motor areas are similar across species (Grünbaum and Sherrington, 1903; Campbell, 1905; Mauss, 1908, 1911; Brodmann, 1909; Leyton and Sherrington, 1917; Beck, 1929; Bailey *et al.*, 1950; Preuss *et al.*, 1999; Hackett *et al.*, 2001; Bush and Allman, 2004a, 2004b; Sherwood *et al.*, 2004b). In particular, the primary visual cortex lies within the banks of the calcarine sulcus. Primary auditory cortex is located on the posterior superior plane of the superior temporal gyrus, usually comprising the transverse gyrus of Heschl in great apes and humans. Primary somatosensory cortex is found within the posterior bank of the central sulcus and extends on to the postcentral gyrus. Primary motor cortex is located mostly on the anterior bank of the central sulcus. One notable difference is the fact that primary visual cortex extends to only a very small portion of the lateral convexity of the occipital lobe in humans, whereas a much larger part of the lateral occipital lobe is comprised of striate cortex in apes (Zilles and Rehkämper, 1988; Holloway *et al.*, 2003). This is because the primary visual cortex in humans is 121% smaller than expected for a primate of the same brain size (Holloway, 1996). Other higher-order areas, particularly within the frontal cortex, have also been shown to occupy similar locations across these species (Strasburger, 1937a, 1937b; Semendeferi *et al.*, 1998, 2001; Sherwood *et al.*, 2003a).

4.21.2.2 Architecture of the Cortex

The general histological architecture of the neocortex in hominoids shares many features in common

Table 1 Endocranial volumes of hominoids (in cc)

Common name	*Species*	*Sex*	*Sample size*	*Mean*	*SD*	*Range*
White-handed gibbon	*Hylobates lar*	M	44	106.3	7.2	92–125
		F	37	104.2	7.0	90–116
Siamang	*Symphalangus syndactylus*	M	8	127.7	8.2	99–140
		F	12	125.9	12.7	102–143
Orangutan	*Pongo pygmaeus*	M	66	415.6	33.6	334–502
		F	63	343.1	33.6	276–431
Gorilla	*Gorilla gorilla*	M	283	535.5	55.3	410–715
		F	199	452.2	41.6	345–553
Chimpanzee	*Pan troglodytes*	M	159	397.2	39.4	322–503
		F	204	365.7	31.9	270–450
Bonobo	*Pan paniscus*	M	28	351.8	30.6	295–440
		F	30	349.0	37.7	265–420
Human	*Homo sapiens*	M	502	1457.2	119.8	1160–1850
		F	165	1317.9	109.8	1040–1615

Endocranial volumes are shown, rather than brain volumes, because larger sample sizes are available for these species. Reproduced from Holloway, R. L. 1996. Evolution of the human brain. In: Handbook of Human Symbolic Evolution (*eds.* A. Lock and C. R. Peters), pp. 74–114. Oxford University Press.

with other primates and mammals in general, such as a fundamental six-layered and columnar organization (Mountcastle, 1998). Compared to other mammals of similar brain size, however, the cerebral cortex of hominoids is reported to have a relatively low density of glial cells and a greater variety of neuron soma sizes (Haug, 1987). Astroglia of the cerebral cortex in great apes, as revealed by immunohistochemistry for glial fibrillary acidic protein (GFAP), resemble other primates in forming long, radially oriented interlaminar processes spanning supragranular cortical layers (Colombo *et al.*, 2004). This configuration may be unique to primates, as GFAP staining in the cortex of other mammals appears distinctly different, comprising a network of stellate astroglial somata with short, branching processes (Colombo, 1996; Colombo *et al.*, 2000; Colombo and Reisin, 2004).

Comparison of mammalian brains indicates that the surface area of the cortical sheet can vary by more than five orders of magnitude, while the thickness of the cortex varies by less than one order of magnitude (Allman, 1990). Accordingly, evolutionary changes in the size of the cerebral cortex have occurred primarily in the tangential dimension, while the vertical dimension of the cortex may be more constrained by the development of columnar units (Rakic, 1988, 1995). Nonetheless, the cortical sheet does tend to display increased thickness in mammals with larger brains (Hofman, 1988). Due to these scaling trends, hominoids have thicker cortices than other smaller-brained primates and homologous cortical areas in humans tend to be thicker than in apes (Figure 3).

With the relative ease of establishing homology among the primary sensory and motor cortical areas on the basis of cytoarchitecture and topology, several studies have compared the microstructure of these areas among different hominoid species (reviewed below). On the whole, the cytoarchitecture of homologous cortical areas shows only subtle differences across hominoid species. Indeed, an early quantitative comparative analysis of the cytoarchitecture of primary cortical areas (areas 3, 4, 17, and 41/42), found marked similarities among species (orangutans, gorillas, chimpanzees, and humans) in terms of the relative thickness of different layers and the proportion of neuropil in each layer (Zilles and Rehkämper, 1988). Only minor differences were noted, such as greater relative thickness of layer III in primary somatosensory cortex (area 3) in humans and gorillas, an increase in the proportion of neuropil in layers V and VI of area 3 in orangutans, and a relatively thicker layer IV of primary auditory cortex (area 41/42) in orangutans. These results were interpreted to corroborate the qualitative observations of Campbell (1905) and Brodmann (1909), indicating that there are not any substantial differences between humans and apes in the cytoarchitecture of these primary cortical areas. The study by Zilles and Rehkämper (1988), however, was based on small samples, which did not permit statistical evaluation of species differences. More recent studies using larger samples, different staining techniques, and more refined quantitative methods have revealed interesting phylogenetic differences among hominoids in cortical histological structure.

4.21.2.3 Primary Visual Cortex

Important modifications of primary visual cortex (Brodmann's area 17) histology, particularly of the

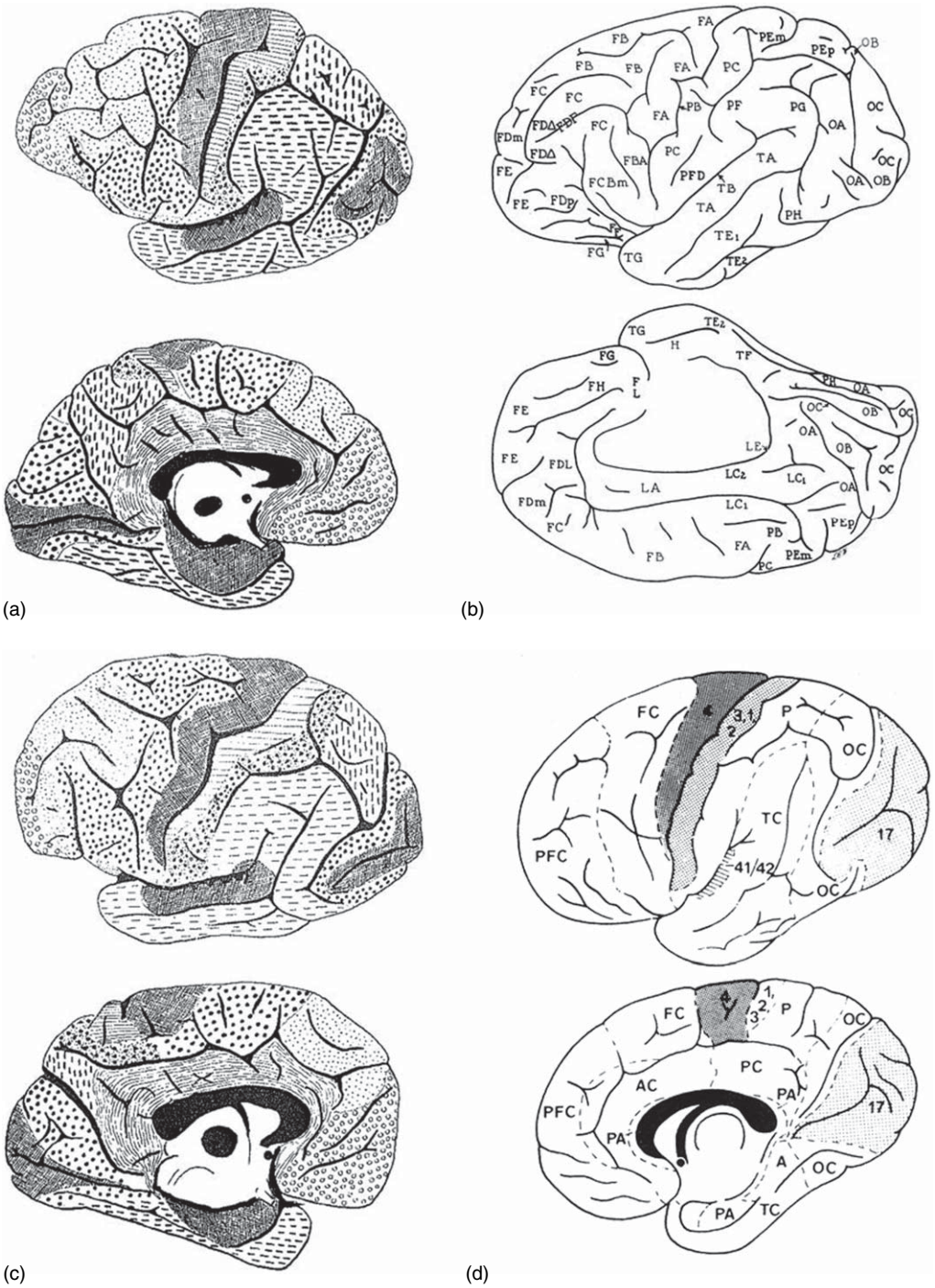

Figure 2 Parcellation maps of the cerebral cortex of apes. Chimpanzee (*Pan troglodytes*) cortical maps reproduced from (a) Campbell, A. W. 1905. Histological Studies on the Localisation of Cerebral Function. Cambridge University Press. and (b) Bailey, P., von Bonin, G., and McCulloch, W. S. 1950. The Isocortex of the Chimpanzee. University of Illinois Press. Orangutan (*Pongo pygmaeus*) cortical maps reproduced from (c) Campbell (1905) and (d) Mauss (1908, 1911) compiled in Zilles and Rekämper (1988). c and d, Reproduced from "figure 12-2", *Orang-utan Biology*, edited by Jeffrey H. Schwartz, copyright © 1988 by Oxford University Press, Inc., used by permission of Oxford University Press, Inc.

thalamic recipient layer IV, have taken place at several points in the evolution of hominoids. Distinct parallel ascending fiber systems arising from retinal ganglion cells project to the lateral geniculate nucleus (LGN) of the thalamus. The M-type retinal ganglion cells give rise to the magnocellular channel, which is involved in the analysis of motion and gross spatial properties of stimuli. The P-type ganglion cells process visual

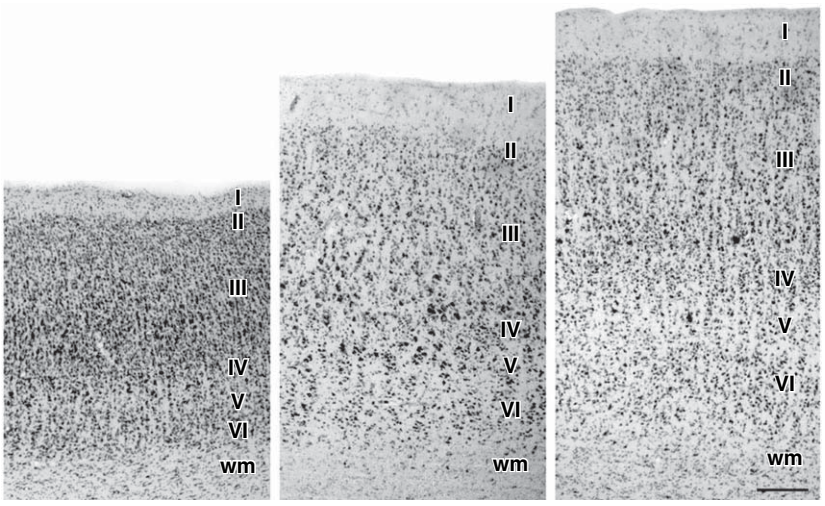

Figure 3 Cytoarchitecture of primary somatosensory cortex (area 3b) in long-tailed macaque (*Macaca fascicularis*), chimpanzee (*Pan troglodytes*), and human (*Homo sapiens*), showing interspecific variation in the thickness of the cortex. Scale bar: 250 μm.

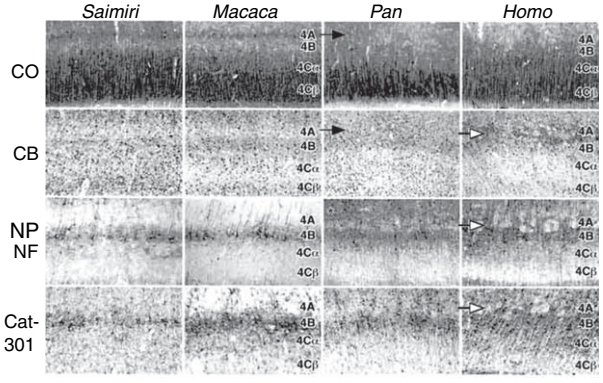

Figure 4 Comparative chemoarchitecture of layer IV in primary visual cortex of squirrel monkeys (*Saimiri*), macaques (*Macaca*), chimpanzees (*Pan*), and humans (*Homo*). Reproduced from Preuss, T. M. and Coleman, G., Human-specific organization of primary visual cortex: Alternating compartments of dense Cat-301 and calbindin immunoreactivity in layer 4A, *Cereb. Cortex*, 2002, 12, 671–691, Oxford University Press.

information with high acuity and color sensitivity, and project to parvocellular layers of the LGN. In the geniculostriate component of these parallel pathways, different systems derive from distinct portions of the LGN and synapse within separate sublayers of layer IV in primary visual cortex. The complexity of segregated geniculostriate projects is reflected in the development of at least three subdivisions of layer IV in primates as demarcated by Brodmann (1909). Two cell-rich layers, IVA and IVC, are separated by a cell-poor layer, IVB, which contains a dense plexus of myelinated axons known as the stria of Gennari. Neurons in the magnocellular layers of the LGN project to the upper half of layer IVC. Neurons in the parvocellular layers of the LGN project to layer IVA.

The chemoarchitecture of layer IVA is markedly different in hominoids compared to other primates (Figure 4). In most monkeys, except the nocturnal owl monkey, there is a dense band of cytochrome oxidase (CO)-rich staining in layer IVA (reviewed in Preuss *et al.*, 1999), reflecting high levels of metabolic activity within this sublayer. However, in the hominoid species examined to date (orangutans, chimpanzees, and humans), intense CO staining in layer IVA is absent, suggesting that the direct parvocellular-geniculate projection to layer IVA was either reduced or more dispersed to include both layers IVA and IVB in the last common ancestor of this phylogenetic group (Preuss *et al.*, 1999; Preuss and Coleman, 2002). Primary visual cortex of great apes and humans is further distinguished from monkeys in having increased staining of CB-immunoreactive (-ir) interneurons and neuropil in layer IVA (Preuss and Coleman, 2002).

Layer IVA of primary visual cortex in humans exhibits additional modifications to the basic hominoid plan described above. In humans, a meshwork pattern is observed in which compartments of neuropil that stain intensively for Cat-301 and nonpyramidal NPNFP-ir cells, and neurites alternate with bands of high densities of CB-ir interneurons (Preuss and Coleman, 2002). These changes in human primary visual cortex have been interpreted to reflect a closer association of this layer to M-pathway inputs than observed in any other primates. The functional implications of

these histological changes are unclear; however, it has been suggested that these alterations are related to specializations in humans for the visual perception of rapid orofacial gestures in speech (Preuss and Coleman, 2002).

Another distinctive feature of primary visual cortex organization in many primates is ocular dominance columns, which correspond to the horizontal segregation of inputs from the two retinas to different compartments in layer IV of primary visual cortex. Ocular dominance columns have been anatomically demonstrated in Old World monkeys, humans, and some other primates, such as the New World spider monkey and the strepsirrhine galago (reviewed in Allman and McGuinness, 1988). A similar pattern of alternating patches of ocular representation within primary visual cortex has been described in a chimpanzee after monocular injection of a transneuronal tritiated tracer substance (Tigges and Tigges, 1979). It is worth noting, however, that this study revealed geniculate projections to layer IVC, but not to layer IVA, as is observed in monkeys.

Large pyramidal neurons, which are found at the boundary between layers V and VI, called Meynert cells, are prominent in the primary visual cortex of primates, and their soma size displays interspecific variation (Figure 5) (Sherwood *et al.*, 2003c). These cells can be distinguished as a unique subtype on the basis of their morphology and connectivity. Their thick axon collaterals project to both area MT/V5 and the superior colliculus, suggesting that these cells are involved in processing visual motion (Fries *et al.*, 1985; Movshon and Newsome, 1996; Livingstone, 1998). In a comparative study, Meynert cell somata were found to be on average 2.8 times lager than other layer V pyramidal neurons across a range of primates (Sherwood *et al.*, 2003c). Because the basal dendrites and axon collaterals of Meynert cells extend horizontally in layers V and VI to integrate information and facilitate responses across widespread areas of visual space, interspecific variation in Meynert cell size appears to be largely constrained by their function in the repetitive stereotyped local circuits that represent the retinal sheet in primary visual cortex. In the context of the general scaling patterns of Meynert cells, it is interesting that soma volumes of these neurons fit closely with predictions among humans (2.84 times larger than neighboring pyramidal cells) and gorillas (2.98 times), whereas they are relatively large in chimpanzees (3.78 times) and relatively small in orangutans (1.94 times). These differences in neural organization for the processing of visual motion may relate to socioecological differences among these apes. Because wild chimpanzees engage in aggressive incursions into the territory of neighboring groups (Watts and Mitani, 2001) and hunt highly mobile prey such as red colobus monkeys (Watts and Mitani, 2002), we speculate that relatively large Meynert cells evolved in this species to enhance detection of visual motion during boundary patrolling and hunting. In contrast, relatively small Meynert cells in orangutans may relate to the fact that these apes are solitary, large-bodied, committed frugivores (van Schaik and van Hooff, 1996), which allows them to maintain low levels of vigilance for motions of predators, competitors, and food items.

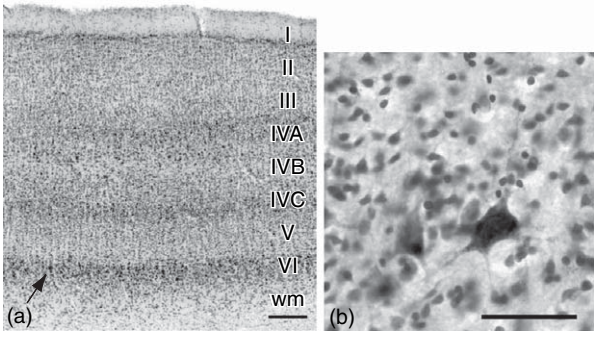

Figure 5 The location and morphology of Meynert cells in an orangutan (*Pongo pygmaeus*). Meynert cells are located at the boundary between layers V and VI, as indicated by the arrow in the Nissl-stained section (a). The morphology of Meynert cells as revealed by immunostaining for NPNFP with Nissl counterstain is shown (b). Scale bar: a, 250 μm; b, 50 μm.

4.21.2.4 Auditory Cortex

There are two parallel thalamocortical projections from the medial geniculate nucleus (MGN) to the superior temporal cortex of primates. Neurons in the ventral division of the MGN supply a tonotopic projection to the core region of auditory cortex (Brodmann's areas 41 and 42). Neurons in the dorsal and medial divisions of the MGN project to areas surrounding the core. The core region of auditory cortex has been identified in macaques, chimpanzees, and humans as a discrete architectural zone as compared to the surrounding belt cortex (Hackett *et al.*, 2001). The core can be recognized by a broad layer IV that receives a dense thalamic projection, heavy myelination, and intense expression of acetylcholinesterase (AChE), CO, and PV in the neuropil of layer IV. In macaques, the relatively high density of cells and fibers makes the auditory cortex core appear structurally homogeneous as compared to the hominoids. In contrast, the medial and lateral domains of the core region of auditory

cortex are more clearly differentiated in humans and chimpanzees because of the lower packing density of structural elements. Additionally, the network of small horizontal and tangential myelinated fibers in layer III appears most complex in humans, intermediate in chimpanzees, and least elaborate in macaques.

The auditory core region is enveloped by several higher-order belt and parabelt fields (Figure 6). The belt areas of auditory cortex, which are less precise in their tonotopic organization, receive major inputs from the core and diffuse input from the dorsal division of the MGN. The belt region is bordered laterally on the superior temporal gyrus by a parabelt region of two or more divisions that are activated by afferents from the belt areas and the dorsal MGN, but not the ventral MGN or the core. Interestingly, AChE-stained pyramidal cells in layer III and V of the belt region are more numerous in chimpanzees and humans compared to macaques (Hackett *et al.*, 2001). Neurons in the belt and parabelt project to auditory-related fields in the temporal, parietal, and frontal cortex. Other types of processing that occur in the other divisions of auditory cortex are not well understood, but they are likely to be important for higher-order processing of natural sounds, including those used in communication. Neurons in the lateral belt cortex areas of rhesus macaques, for example, respond better to species-specific vocalizations than to energy-matched pure tone stimuli (Rauschecker *et al.*, 1995).

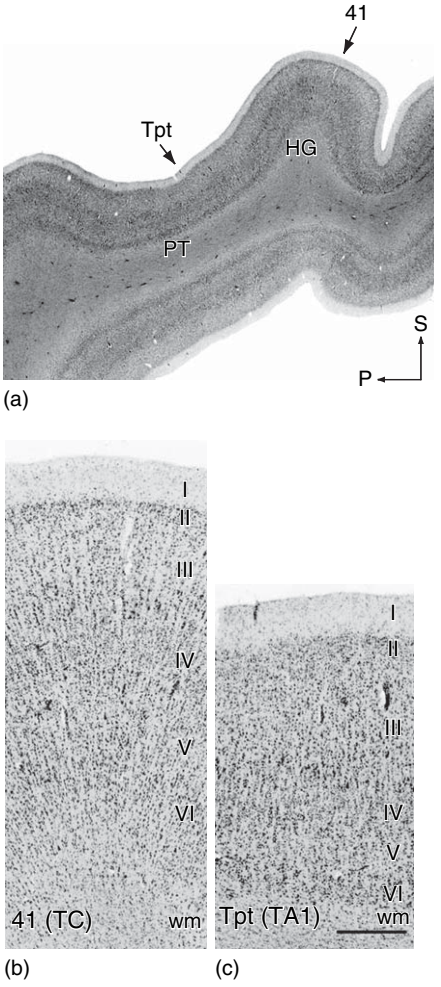

Figure 6 Cytoarchitecture of auditory cortex of an orangutan (*Pongo pygmaeus*). A parasagittal section of the superior temporal gyrus shows the location of regions detailed below (a). Higher-power micrographs show cytoarchitecture in (b) the core of primary auditory cortex (Brodmann's area 41 or von Economo and Koskinas' area TC) and (c) area Tpt (posterior Brodmann's area 22 or von Economo and Koskinas' area TA_1). HG, Heschl's gyrus; PT, planum temporale; S, superior; P, posterior. Scale bar: 500 μm.

The comparative anatomy of one particular region of auditory association cortex has been studied most extensively. In humans, Wernicke's area, a region important for the comprehension of language and speech, is located in the posterior superior temporal cortex. Gross anatomic observations indicate that asymmetries similar to humans are present in the superior temporal lobe of nonhuman primates, such as leftward dominance of the planum temporale in great apes (Gannon *et al.*, 1998; Hopkins *et al.*, 1998) and a longer left sylvian fissure in many anthropoid species (LeMay and Geschwind, 1975; Yeni-Komshian and Benson, 1976; Heilbroner and Holloway, 1988; Hopkins *et al.*, 2000).

Several investigations have examined the microstructure of the cortical area most closely associated with Wernicke's area. Area Tpt (Galaburda *et al.*, 1978) or area TA_1 (von Economo and Koskinas, 1925) comprises a portion of posterior Brodmann's area 22 located on the upper bank of the superior temporal gyrus and sometimes extending to part of the parietal operculum and the convexities of the temporal and parietal lobes (Galaburda *et al.*, 1978). This area represents a transition between auditory association cortex and cortex of the inferior parietal lobule (Shapleske *et al.*, 1999). Cortex with the cytoarchitectural characteristics of area Tpt has been described in galagos, macaques, chimpanzees, and humans (Galaburda and Pandya, 1982; Preuss and Goldman-Rakic, 1991; Buxhoeveden *et al.*, 2001a, 2001b). The microstructure of area Tpt in these primates is distinguished by a eulaminate appearance, with a poorly defined border of layer IV due to the encroachment of pyramidal cells in adjacent layers IIIc and Va, and an indistinct border between layers IV and V due to curvilinear columns of neurons that bridge the two layers (Galaburda and Sanides, 1980).

There are differences among rhesus monkeys, chimpanzees, and humans in the details of minicolumn structure in area Tpt. In the left hemisphere, area Tpt of humans has wider minicolumns as compared to macaques or chimpanzees, whereas the width of minicolumns is similar in the nonhuman species (Buxhoeveden *et al.*, 2001b). These findings suggest that wider minicolumns in human area Tpt may be a species-specific specialization that allows for more extensive neuropil space containing interconnections among neurons.

The microstructure of area Tpt has also been shown to be asymmetric in humans, possibly as a neural substrate of hemispheric dominance in the cerebral representation of language. Long-range intrinsic connections within area Tpt labeled in postmortem brains with lipophilic dyes have revealed greater spacing between interconnected patches in the left hemisphere compared to the right (Galuske *et al.*, 2000). Furthermore, left area Tpt has a greater number of the largest pyramidal cells in layer III, known as magnopyramidal cells, that give rise to long corticocortical association projections (Hutsler, 2003). In addition, AChE-rich pyramidal cells display greater cell soma volumes in the left hemisphere despite lacking asymmetry in number (Hutsler and Gazzaniga, 1996). In humans, left area Tpt has also been shown to contain a greater amount of neuropil and axons with thicker myelin sheaths (Anderson *et al.*, 1999). Of particular significance, a comparative analysis of area Tpt found that only humans, but not rhesus macaques or chimpanzees, exhibit left dominant asymmetry in area Tpt, with wider minicolumns and a greater proportion of neuropil (Buxhoeveden *et al.*, 2001a).

4.21.2.5 Primary Motor Cortex

The primary motor cortex (Brodmann's area 4) has a distinctive cytoarchitectural appearance in primates (Geyer *et al.*, 2000; Sherwood *et al.*, 2004b), containing giant Betz cells in the lower portion of layer V, low cell density, large cellular sizes, an indistinct layer IV, and a diffuse border between layer VI and the subjacent white matter (Figure 7a). In humans, the region of primary motor cortex that corresponds to the representation of the hand exhibits interhemispheric asymmetry in its cytoarchitectural organization. Concomitant with strong population-wide right handedness in humans, most postmortem brains display a greater proportion of neuropil volume in the left hemisphere of this part of primary motor cortex (Amunts *et al.*, 1996). Interestingly, brains of

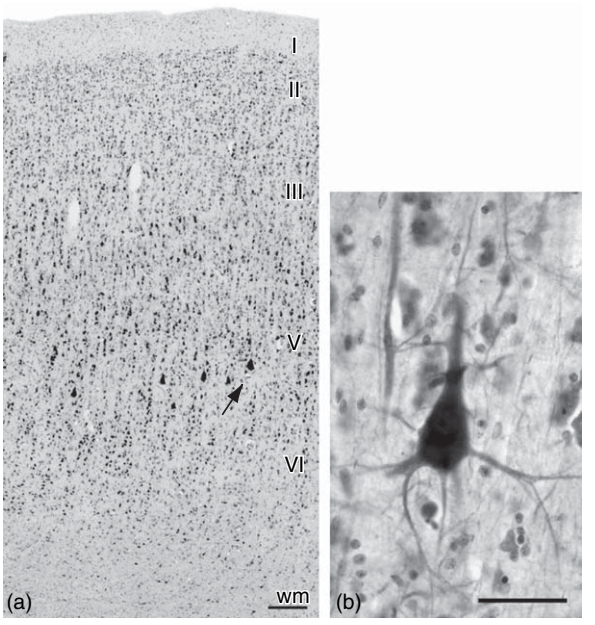

Figure 7 Cytoarchitecture of primary motor cortex and morphology of Betz cells in an orangutan (*Pongo pygmaeus*). Betz cells can be seen in the bottom of layer V, as indicated by the arrow in the Nissl-stained section (a). The morphology of Betz cells as revealed by immunostaining for NPNFP with Nissl counterstain is shown (b). Scale bar: a, 250 μm; b, 50 μm.

captive chimpanzees (Hopkins and Cantalupo, 2004) and capuchin monkeys (Phillips and Sherwood, 2005) show humanlike asymmetries of the hand region of the central sulcus that are correlated with the direction of individual hand preference. However, the histology of primary motor cortex in nonhuman primates has not yet been examined for asymmetry (see The Evolution of Hemispheric Specializations of the Human Brain).

While the cytoarchitectural organization of primary motor cortex is generally similar across species, interspecific differences have been described. The cytoarchitecture of the region corresponding to orofacial representation of primary motor cortex in several catarrhine species (long-tailed macaques, anubis baboon, orangutans, gorillas, chimpanzees, and humans) was analyzed using the gray level index (GLI) method (Sherwood *et al.*, 2004b). Compared to Old World monkeys, great apes and humans displayed an increased relative thickness of layer III and a greater proportion of neuropil space. A stereologic investigation of NPNFP and calcium-binding protein-ir neurons was also conducted in this same comparative sample (Sherwood *et al.*, 2004a). Primary motor cortex in great apes and humans was characterized by a greater percentage of neurons enriched in NPNFP and PV compared to the Old World monkeys

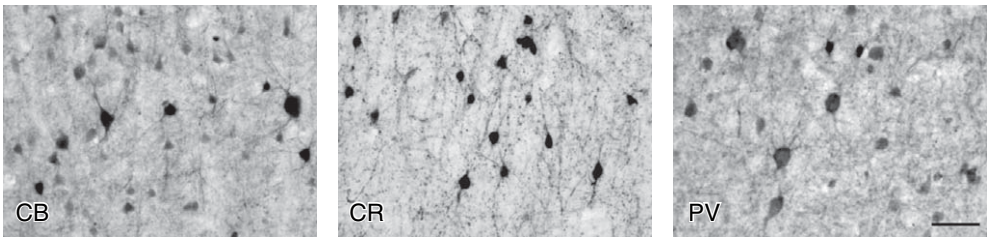

Figure 8 Calcium-binding protein-immunoreactive neurons in layer III of primary motor cortex of a chimpanzee (*Pan troglodytes*). Neurons stained for CB (a), CR (b), and PV (c) are shown. Morphologically, CR-ir interneurons correspond mostly to double-bouquet cells. They are predominantly found in layers II and III, and have narrow vertically oriented axonal arbors that span several layers. CB-ir neurons are morphologically more varied, including double-bouquet and bipolar types, with many showing a predominantly vertical orientation of axons. In contrast, the morphology of PV-ir interneurons includes large multipolar types, such as large basket cells and chandelier cells, which have horizontally spread axonal arbors spanning across cortical columns within the same layer as the parent soma. Scale bar: 50 μm.

(Figure 8). Conversely, the percentage of CB- and CR-ir neuron subtypes did not significantly differ among these species. These modifications of particular subsets of neuron types might contribute to the voluntary dexterous control of orofacial muscles exhibited in the vocal and gestural communication of great apes and humans. Enhancement of PV-ir interneuron-mediated lateral inhibition of cell columns may enhance specificity in the recruitment of different muscle groups for dynamic modulation of fine orofacial movements. Increased proportions of NPNFP-ir pyramidal cells, on the other hand, may be a correlate of greater descending cortical innervation of brainstem cranial motor nuclei by heavily myelinated axons to allow for more voluntary control (Kuypers, 1958).

The giant Betz cells are found in the lower half of layer V of primary motor cortex and possess a large number of primary dendritic shafts that leave the soma at several locations around its surface (Figure 7b) (Braak and Braak, 1976; Scheibel and Scheibel, 1978; Meyer, 1987). They are largest and most numerous in the cortical representation of the leg, where axons project farther along the corticospinal tract to reach large masses of muscles (Lassek, 1948; Rivara *et al.*, 2003). Betz cells are strongly immunoreactive for NPNFP among humans, great apes, and Old World monkeys (Sherwood *et al.*, 2004a). An analysis of scaling of Betz cell somata volumes in the region of hand representation of primates revealed that these cell subtypes become relatively enlarged with increases in brain and body size (Sherwood *et al.*, 2003c). At larger sizes, there is an increase in the distance to the spinal representation of target muscles and a greater number of less densely distributed corticospinal neurons (Nudo *et al.*, 1995). In larger brains and bodies, Betz cell axons need to become thicker to maintain conduction speed to reach more distant targets in the spinal cord. Accordingly, Betz cells are scaled to global connectivity constraints and therefore increase in somatic volume in a manner that is correlated with brain size. Due to these scaling trends, among hominoids Betz cells are relatively largest in humans (10.96 times larger than neighboring pyramidal cells), then gorillas (8.37 times), chimpanzees (7.02 times), and orangutans (6.51 times).

Specializations of biochemical phenotypes are known for certain regionally restricted subsets of pyramidal neurons. Although calcium-binding proteins are expressed transiently during prenatal and early postnatal development (Moon *et al.*, 2002; Ulfig, 2002), their expression in pyramidal neurons of adult mammals is more limited. Neurons expressing the calcium-binding proteins – CB, CR, and PV – are thought to have relatively high metabolic rates associated with fast repolarization for multiple action potentials (Baimbridge *et al.*, 1992). While such calcium buffering mechanisms are most commonly associated with GABAergic interneurons, the presence of calcium-binding proteins in pyramidal cells might reflect a neurochemical specialization for higher rates of activity. In this context, it is interesting that faint CR immunoreactivity is observed in isolated medium- and large-size layer V pyramidal neurons in primary motor cortex of great apes and humans, but not in macaques or baboons (Hof *et al.*, 1999; Sherwood *et al.*, 2004a). PV-ir pyramidal neurons are also very rarely observed in the neocortex of mammals (see The Evolution of Neuron Classes in the Neocortex of Mammals). However, large layer V pyramidal neurons, including Betz cells, have been reported to express PV immunoreactivity in primary motor cortex of humans (Nimchinsky *et al.*, 1997; Sherwood *et al.*, 2004a). Evidence concerning the existence of PV-ir pyramidal neurons in other nonhuman primates is somewhat contradictory. In

one study, PV-ir pyramidal neurons were observed in primary motor and somatosensory cortices of galagos and macaques (Preuss and Kaas, 1996). Another study, however, failed to label PV-ir pyramidal cells in macaques (DeFelipe *et al.*, 1989), probably due to methodological discrepancies among experiments. A comparative study of primary motor cortex using the same immunohistochemical procedures across species found that PV-ir pyramidal neurons were either not present or sparse in Old World monkeys, whereas they were considerably more numerous in great apes and humans (Sherwood *et al.*, 2004a).

4.21.2.6 Inferior Frontal Cortex

The microstructure of the inferior frontal cortex, the region that contains Broca's area in humans, has been described in hominoids on the basis on cyto-, myelo-, and NPNFP-architecture (von Economo, 1929; Bailey and von Bonin, 1951; Braak, 1980; Amunts *et al.*, 1999; Hayes and Lewis, 1995; Sherwood *et al.*, 2003a). Cytoarchitectural studies of chimpanzee and orangutan frontal cortex describe a dysgranular region anterior to the inferior precentral sulcus comprising a part of pars opercularis and designated Brodmann's area 44 (von Bonin, 1949; Sherwood *et al.*, 2003a), FCBm (Bailey *et al.*, 1950), or areas 56 and 57 (Kreht, 1936). In chimpanzees, this region has been shown to receive projections from the mediodorsal nucleus of the thalamus (Walker, 1938). In macaques, a field with similar cytoarchitectural characteristics in the caudal bank of the inferior limb of the arcuate sulcus has been denoted area 44, with area 45 located rostrally (Galaburda and Pandya, 1982; Petrides and Pandya, 1994). The cytoarchitecture of area 44 is characterized by a columnar organization similar to ventral premotor area 6, but it is distinguished by the development of a thin layer IV and clustered magnopyramidal neurons in the deep part of layer III. Layer IV in area 44 has an undulating appearance due to the invasion of pyramidal cells from layer III and layer V. NPNFP staining in area 44 of chimpanzees and humans displays clusters of large pyramidal neurons at the bottom of layer III and a lower band of immunoreactive layer V cells and neuropil (Figure 9) (Hayes and Lewis, 1995; Sherwood *et al.*, 2003a).

The cytoarchitecture of area 45 is distinguished from area 44 by the presence of a more prominent layer IV, a more homogeneous distribution of pyramidal cells in the deep portion of layer III, and the absence of conspicuous cell columns. NPNFP staining in area 45 is characterized by a clearer separation of immunoreactive neurons into upper (layer III) and lower (layer V) populations and by the absence of the intensely stained magnopyramidal clusters, as seen in area 44.

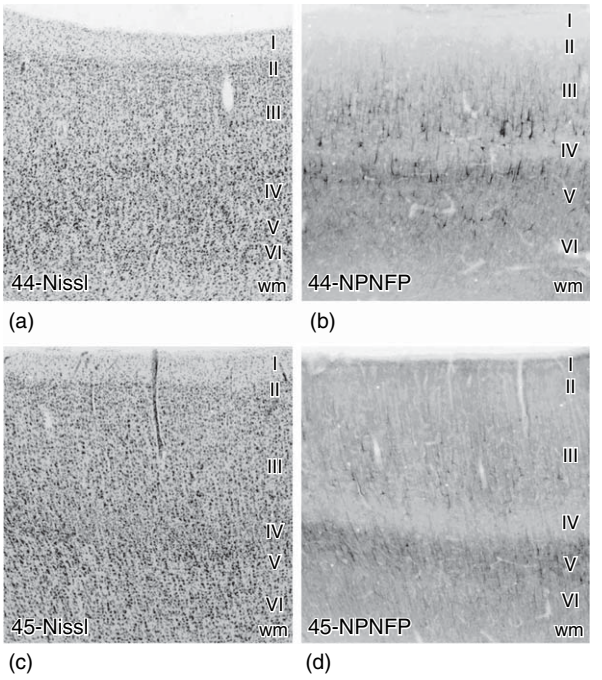

Figure 9 The architecture of areas 44 and 45 in a chimpanzee (*Pan troglodytes*). Area 44 is shown stained for Nissl substance (a) and NPNFP (b). Area 45 is shown stained for Nissl substance (c) and NPNFP (d). Scale bar: 500 μm.

Considering the preponderance of left hemisphere dominant control of language in humans, several studies have examined the cortex of the inferior frontal gyrus in humans for microstructural asymmetries. Using GLI profile analysis methods to quantify regional variation in cytoarchitecture, area 44 has been shown to display left dominance in terms of volume and an increased proportion of neuropil space, whereas area 45 does not show a consistent direction of asymmetry (Amunts *et al.*, 1999). In addition, the total length of pyramidal cell dendrites is longer in the left opercular region of the inferior frontal gyrus due to a selective increase in the length of higher-order segments (Scheibel *et al.*, 1985). Using different methods, another study examined asymmetries in only magnopyramidal cells in layer III of area 45 and found total dendritic length, dendritic complexity (numbers of branches and maximal branch order), and spine densities to be greater in the right (Hayes and Lewis, 1996). In area 45, AChE-positive layer III magnopyramidal cells have larger somata in the left hemisphere, despite lacking asymmetry in their density (Hayes and Lewis, 1995; Garcia *et al.*, 2004).

While asymmetries of the inferior frontal cortex are well established in humans, the condition of nonhuman primates is less clear. Although population-level

leftward asymmetry of the fronto-orbital sulcus, a portion of the inferior frontal gyrus, has been reported in great apes (Cantalupo and Hopkins, 2001), it remains to be known whether humanlike microstructural asymmetries are present in the inferior frontal cortex of these species. In humans and chimpanzees, the borders of areas 44 and 45 have been shown to correspond poorly with external sulcal landmarks (Amunts *et al.*, 1999; Sherwood *et al.*, 2003a). Thus, determination of whether asymmetries are evident in regional volumes and intrinsic circuitry of areas 44 and 45 of great apes will require histological studies.

4.21.2.7 Prefrontal Cortex

While it has been a popular notion that human cognitive abilities are associated with disproportionate enlargement of the frontal or prefrontal cortex, recent data show that the human frontal cortex is no larger than expected for a hominoid of the same brain size (Semendeferi *et al.*, 1997, 2002). Furthermore, progressive increase in the relative size of the frontal cortex accompanies enlarging brain size for primates in general, with hominoids simply continuing this scaling trend (Bush and Allman, 2004a; see Scaling the Brain and Its Connections, Encephalization: Comparative Studies of Brain Size and Structure Volume in Mammals, Do All Mammals Have a Prefrontal Cortex?). At present, the comparative quantitative data available concerning the volume of specific prefrontal cortical areas in hominoids are scanty, representing only areas 10 and 13 in one individual per species (Semendeferi *et al.*, 1998, 2001). Taken together, however, it does not seem that these prefrontal areas are disproportionately enlarged in humans beyond what is expected for a hominoid of the same brain size (Holloway, 2002). Nonetheless, quantitative cytoarchitectural analyses have shown that some prefrontal cortical areas differ in their histological organization among hominoid species.

Area 13 is a dysgranular field located in the posterior orbitofrontal cortex (Figure 10). This cortical area is remarkably integrative, receiving inputs from olfactory, gustatory, and visceral centers, as well as premotor, somatosensory, auditory, visual, and parahippocampal cortices (Carmichael and Price, 1995; Cavada *et al.*, 2000). Damage to this region disrupts performance on tasks that require behavioral inhibition and causes impairments in emotional control (Fuster, 1998; Roberts and Wallis, 2000). In a study of the cytoarchitecture of area 13 across macaques and hominoids, several similarities were observed that suggest homology among these species (Semendeferi *et al.*, 1998). This cortical area is distinguished by a poorly defined layer IV, horizontal striations of cells in layers V and VI, large pyramidal cells in layer V, relatively thick infragranular layers as compared with supragranular layers, and greater neuropil space in supragranular layers as compared with deeper layers. Among hominoids, area 13 is located in the posterior portion of the medial orbital and posterior orbital gyri. This concords with the earlier description of an area labeled FF in the posterior orbitofrontal cortex of chimpanzees that seems to correspond to these cytoarchitectural features (Bailey *et al.*, 1950).

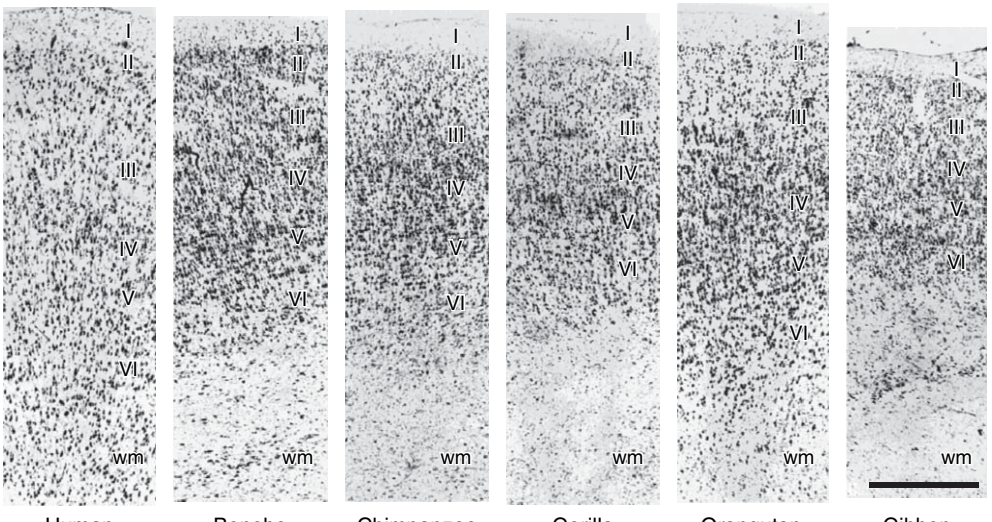

Figure 10 The cytoarchitecture of area 13 in hominoids. Scale bar: 500 μm. Modified from Limbic frontal cortex in hominoids: A comparative study of area 13, *Am. J. Phys. Anthropol.*; Semendeferi, K., Armstrong, E., Schleicher, A., Zilles, K., and Van Hoesen, G. W.; Copyright © 1998, Wiley-Liss. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

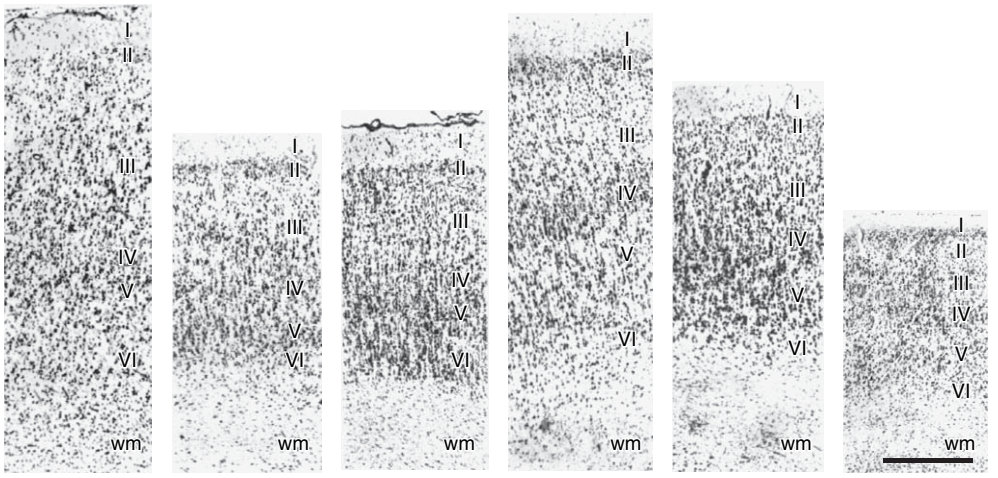

Figure 11 The cytoarchitecture of area 10 in hominoids. Scale bar: 500 μm. Modified from Prefrontal cortex in humans and apes: A comparative study of area 10, *Am. J. Phys. Anthropol.*; Semendeferi, K., Armstrong, E., Schleicher, A., Zilles, K., and Van Hoesen, G. W.; Copyright © 2001, Wiley-Liss. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

While general similarities are found in the cytoarchitecture of area 13 of hominoids, quantitative analyses have identified some interspecific differences. For example, layer IV in orangutans is relatively wide, making this cortex appear more similar to granular prefrontal cortex. Compared to other hominoids, area 13 in humans and bonobos occupies a small proportion of total brain volume and more cytoarchitectonic subdivisions occupy the orbitofrontal cortex adjacent to area 13. In contrast, area 13 of orangutans is relatively large and thus occupies the majority of the orbitofrontal region.

Area 10 is a granular cortex that forms a part of the frontal pole in most hominoid species, including humans, chimpanzees, bonobos, orangutans, and gibbons (Figure 11) (Semendeferi *et al.*, 2001). This cortical area is involved in planning and decision making (Fuster, 1998). Area 10 receives highly processed sensory afferents from corticocortical connections in addition to inputs from the mediodorsal nucleus of the thalamus, striatum, and many limbic structures (Öngür and Price, 2000). In hominoids, the cytoarchitecture of area 10 is characterized by a distinct layer II, a wide layer III with large pyramidal cells in its deep portion, a clearly differentiated granular layer IV, large pyramidal cells in layer Va, and a sharp boundary between layer VI and the white matter. Quantitative analyses reveal a similar pattern of relative laminar widths among humans, chimpanzees, and bonobos, such that the supragranular layers are relatively thick compared to the infragranular layers (Semendeferi *et al.*, 2001). In contrast, the infragranular layers comprise a greater proportion of cortical thickness in the other hominoids. When GLI profile curves describing laminar variation in neuron volume densities are compared among taxa, apes and macaques follow a similar pattern with roughly equal GLI throughout the cortical depth. The proportion of neuropil space in layers II and III relative to infragranular layers, however, is greater in humans. Notably, Semendeferi *et al.* (2001) raise uncertainty regarding whether a homologue of area 10 is present in gorillas. In particular, the cortex of the frontal pole in gorillas has a prominent layer II and Va, features that are not found in macaques or other hominoids.

4.21.2.8 Anterior Cingulate Cortex

In layer Vb of anterior cingulate cortex (subareas 24a, 24b, and 24c), large spindle-shaped cells are found only in great apes and humans, to the exclusion of hylobatids and other primates (Nimchinsky *et al.*, 1999). These neurons have a very elongate, gradually tapering, large soma that is symmetrical about its vertical and horizontal axes (Figure 12). This distinctive somatic morphology arises from the presence of a large apical dendrite that extends toward the pial surface, as well as a single large basal dendrite that extends toward the underlying white matter, without any other dendrites branching from the basal aspect of the cell. These unique neurons are also substantially larger in size than other neighboring pyramidal cells. Interestingly, spindle neurons increase in soma size, density, and clustering from orangutans to gorillas, chimpanzees, bonobos, and humans. Furthermore, spindle-shaped neurons have been observed in layer Vb of area 24b in a fetal chimpanzee (E 224), indicating that this specialized projection cell type differentiates early in

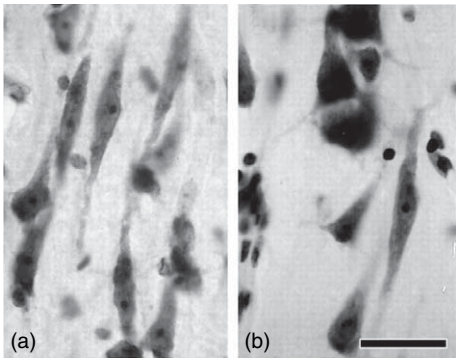

Figure 12 Morphology of spindle-shaped neurons in layer Vb of anterior cingulate cortex in a bonobo (*Pan paniscus*) (a) and gorilla (*Gorilla gorilla*) (b). Scale bar: 80 μm. Modified from Nimchinsky, E. A., Gilissen, E., Allman, J. M., Perl, D. P., Erwin, J. M., and Hof, P. R. 1999. A neuronal morphologic type unique to humans and great apes. *Proc. Natl. Acad. Sci. USA* 96, 5268–5273.

development (Hayashi *et al.*, 2001). Notably, neurons with a spindle phenotype also have a phylogenetically restricted distribution within another region, the frontoinsular cortex. Spindle cells are found in layer V in the frontoinsular cortex only in humans and African great apes (i.e., gorillas, chimpanzees, and bonobos), being far more numerous in humans compared to the apes (see Role of Spindle Cells in the Social Cognition of Apes and Humans; Hakeem *et al.*, 2004).

In addition to spindle-shaped neurons, the anterior cingulate cortex of great apes and humans contains another unique pyramidal cell phenotype. A survey of the anterior cingulate cortex of several primate species revealed a small subpopulation of CR-containing layer V pyramidal neurons that are only found in great apes and humans (Hof *et al.*, 2001). These neurons are located in the superficial part of layer V in areas 24 and 25. In orangutans, they comprise 0.5% of all layer V pyramidal cells in the anterior cingulate, 2% in gorillas, 4.1% in chimpanzees, and 5.3% in humans. The occurrence of these cells decreases sharply at the boundary between anterior and posterior cingulate cortex, suggesting that they are not directly involved in the somatic motor functions associated with the posterior cingulate motor areas.

The restricted phylogenetic distribution of spindle-shaped cells and CR-ir pyramidal neurons in layer V of anterior cingulate cortex may reflect specializations of projection neurons in this region for a role in the control of vocalization, facial expression, attention, the expression and interpretation of emotions, and autonomic functions (Nimchinsky *et al.*, 1999; Allman *et al.*, 2001; Hof *et al.*, 2001). Of particular interest, in humans, CR-immunoreactive layer V pyramidal neurons are also present in the anterior paracingulate cortex (area 32) (Hof *et al.*, 2001). The presence of this distinctive projection cell type in area 32 of humans is intriguing, considering that this cortical area has been found to be recruited in tasks that require theory of mind (Gallagher *et al.*, 2000), which is the capacity to attribute mental states such as attention, intention, and beliefs to others and may be a cognitive capacity that is exclusive to humans (Tomasello *et al.*, 2003).

4.21.3 Patterns of Cortical Organization in Hominoids

4.21.3.1 The Emergence of Cell Types and Their Distribution

Particular cellular subtypes appear to have phylogenetically restricted distributions. It is interesting that among hominoids, the presence of these unique neuron phenotypes accords with the hierarchical nested structure of monophyletic taxa, suggesting that they are indicators of phylogenetic relationships (Table 2). For example, in all great apes and humans, spindle-shaped neurons are found in layer V of anterior cingulate cortex. Also in these taxa, CR-ir pyramidal cells are found in layer V of anterior cingulate cortex and primary motor cortex. In just African great apes and humans, layer V spindle-shaped neurons are found in frontoinsular cortex. And only in humans, CR-ir pyramidal neurons in layer V are found in anterior paracingulate cortex. Thus, novel neuron phenotypes have appeared at several different times in hominoid evolution.

It is tempting to speculate that the evolution of each unique neuron type marks specializations of the cortical areas involved. In particular, the morphomolecular characteristics of these novel neuron types suggest that there have been modifications of specific efferent projections to facilitate high levels of activity or higher conduction velocity for outputs. The possibility that the great ape and human clade is distinguished by such specializations of projection cells is especially intriguing in light of recent hypotheses that intelligence among mammals is correlated with the rate of information processing capacity as represented by axonal conduction speed (Roth and Dicke, 2005). The presence of unique neuron classes in great apes and humans extends this hypothesis to suggest that specific cortical efferents located within behaviorally relevant circuits may be selectively modified. It is also significant that a common feature of these novel projection cells is their localization in layer V. This laminar distribution indicates that evolutionary modifications have

Table 2 Phylogenetic distribution of some cortical histological traits

Cortical area	*Layer*	*Trait*	*Homo sapiens*	*Pan paniscus*	*Pan troglodytes*	*Gorilla gorilla*	*Pongo pygmaeus*	*Hylobates sp.*	*Macaca sp.*
Primary motor cortex[a]	Layer V	CR-ir pyramidal neurons	+	?	+	+	+	?	−
Primary motor cortex[a]	Layer V	PV-ir pyramidal neurons	++	?	++	?	++	?	+
Primary motor cortex[a]	Layers III and V	NPNFP-ir neurons	++	?	++	++	++	?	+
Primary visual cortex[b]	Layer IVA	Loss of CO-dense band	+	?	+	?	+	?	−
Primary visual cortex[b]	Layer IVA	Dense CB-ir neurons and neuropil	+	?	+	?	+	?	−
Primary visual cortex[b]	Layer IVA	Meshwork with dense NPNFP and Cat-301 staining in mesh bands alternating with dense CB in interstitial zones	+	?	−	?	−	?	−
Auditory belt cortex[c]	Layers III and V	AChE-stained pyramidal cells	++	?	++	?	?	?	+
Anterior cingulate cortex[d]	Layer Vb	Spindle-shaped neurons	++	++	++	+	+	−	−
Anterior cingulate cortex[e]	Layer Vb	CR-ir pyramidal neurons	++	?	++	+	+	?	−
Anterior paracingulate cortex[e]	Layer Vb	CR-ir pyramidal neurons	+	?	−	−	−	?	−
Frontoinsular cortex[f]	Layer Vb	Spindle-shaped neurons	++	+	+	+	−	−	−

[a]Sherwood *et al.* (2004a).
[b]Preuss and Coleman (2002).
[c]Hackett *et al.* (2001).
[d]Nimchinsky *et al.* (1999).
[e]Hof *et al.* (2001).
[f]Hakeem *et al.* (2004).
+, present; ++, present and abundant in comparison to other species; −, absent.

been focused upon descending cortical control over targets in the brainstem and spinal cord.

4.21.3.2 The Evolution of Cortical Asymmetries

A substantial body of evidence shows that the human cerebral cortex expresses lateralization in the control of language and fine motor actions of the hand (Toga and Thompson, 2003). Asymmetries in histological structure have been demonstrated across cortical areas implicated in these processes in humans, including Broca's area (areas 44 and 45), Wernicke's area (area Tpt), and the hand representation of primary motor cortex (area 4). Some authors have hypothesized that these anatomical asymmetries are exclusive adaptations of the human brain that are encoded genetically and comprise the chief evolutionary novelty in the speciation of modern humans (Crow, 2000; Annett, 2002).

An alternative view is that functional and anatomical lateralization may be a byproduct of increases in overall brain size (Ringo *et al.*, 1994; Hopkins and Rilling, 2000). One cost of increasing brain size is that axons must propagate action potentials over a greater distance to communicate between the hemispheres (Harrison *et al.*, 2002). While these delays in conduction can be overcome to some extent by increasing axon cross-sectional area and myelination (Changizi, 2001), the design problems associated with large brains ultimately

may necessitate increased modularity of processing and more of an emphasis on local network connectivity (Kaas, 2000). In particular, as brains grow in size, the efficiency of interhemispheric transfer of information by long connections diminishes because costs, in terms of wiring space, dictate that axons cannot increase cross-sectional area sufficiently to keep pace with demands for processing speed (Aboitiz and Montiel, 2003). Hence, it is expected that cortical processes in large brains, especially those that depend on rapid computations, will come to rely on specialized processing that is dominant in one hemisphere. Indeed, it has been shown that increasing brain size is accompanied by reduced hemispheric interconnectivity via the corpus callosum (Ringo *et al.*, 1994; Olivares *et al.*, 2001) and the development of more pronounced gross cerebral asymmetries among anthropoid primates (Hopkins and Rilling, 2000).

One consequence of lateralized hemispheric specialization of function may be divergence in the histological organization of homotopic cortical areas. Unfortunately, there is a surprising absence of data from nonhumans concerning microstructural asymmetries in the homologues of Broca's area, Wernicke's area, and primary motor cortex. At present, the sole study of such histological asymmetry indicates that lateralization is not present in area Tpt of chimpanzees or macaques, while asymmetry of neuropil space and minicolumn widths are observed in humans (Buxhoeveden *et al.*, 2001a). Although this is an important finding, it should be kept in mind that there are many other aspects of microstructural organization that have been demonstrated to be asymmetric in the human cortex, such as distributions of cell volumes and dendritic geometry, which have yet to be investigated in other species. Thus, at the present time, there are still insufficient data to adequately resolve whether many of the observed microstructural asymmetries of the human cerebral cortex are unique species-specific adaptations that are related to language and handedness.

4.21.3.3 How Much Variation in Cortical Architecture Can be Attributed to Scaling versus Specialization?

Interpretation of interspecific differences in the histological structure of the cortex in hominoids requires parsing the source of this variation. Certainly a portion of it can be attributed to specific alterations of circuitry that generate behavioral differences among species. Another cause, however, may be the effects of allometric scaling. That is, as overall brain size changes, predictable changes occur in cell sizes, cell packing density, dendritic geometries, and other aspects of microstructure (Jerison, 1973; Striedter, 2005). Thus, with variation in brain size among hominoids, some of the observed interspecific differences may simply be the result of scaling to maintain functional equivalence and may not indicate any significant differences in computational capacities. For example, how can we know whether greater densities of AChE-stained neurons in the auditory belt of hominoids (Hackett *et al.*, 2001) is of functional importance until we have developed a clearer understanding of the scaling principles that govern the distribution of AChE-enriched neurons in general? Hence, whenever possible it is best to evaluate phylogenetic variation in cortical histology from the perspective of allometric scaling. Accordingly, the case for declaring that a trait is a phylogenetic specialization is strengthened when it can be demonstrated that individual species depart from allometric expectations or that an entire clade scales along a different trajectory (i.e., grade shift).

It is well established that cortical neuron density varies among mammalian species. Across a large sample of mammals ranging from mouse to elephant, there is a negative correlation between cortical neuron density and brain size that follows a $-1/3$ power law (Tower, 1954; Cragg, 1967; Haug, 1987; Prothero, 1997). Despite this broad trend, however, some evidence suggests that neuron densities may be higher in hominoids (gorilla, chimpanzee, and human) than expected for their brain size (Haug, 1987). It has also been shown that the fraction of the cortex that is comprised by neuropil space versus cell somata increases in a negative allometric fashion with greater brain size (Shariff, 1953; Tower, 1954; Bok, 1959; Tower and Young, 1973; Zilles *et al.*, 1982; Armstrong *et al.*, 1986; Haug, 1987). These empirical findings fit with a model predicting that a constant average percent interconnectedness among neurons cannot feasibly be maintained in the face of increasing gray matter volume, so the reach of processing networks cannot keep pace with brain size variation (Changizi, 2001).

Many of these theories concerning the scaling of network connectedness across brain size, however, were developed to explain variation in mouse-to-elephant comparisons. Are these predicted allometric relationships between neuron density, neuropil space, and brain size maintained when comparisons are restricted to the hominoids? Table 3 shows the results of stereologic estimates of neuron density and GLI from recent comparative studies of areas 4, 10, and 13 in hominoids.

Table 3 Neuron densities (in neurons per mm^3) and gray level index (GLI) values for different cortical areas in hominoids and Old World monkeys

	Area 4[a]		Area 10[b]		Area 13[c]	
Species	*GLI*	*Neuron density*	*GLI*	*Neuron density*	*GLI*	*Neuron density*
Homo sapiens	11.65	18 048	15.17	34 014	14.18	30 351
Pan troglodytes	13.19	22 177	17.52	60 468	18.63	50 686
Pan paniscus			18.17	55 690	16.98	44 111
Gorilla gorilla	8.76	24 733	15.87	47 300	14.62	54 783
Pongo pygmaeus	10.61	18 825	20.10	78 182	18.55	42 400
Hylobates lar			19.80	86 190	13.33	53 830
Macaca sp.	15.58	50 798	20.34		18.36	
Papio anubis	14.85	33 661				

[a]Sherwood *et al.* (2003b); Sherwood *et al.* (2004b).
[b]Semendeferi *et al.* (2001).
[c]Semendeferi *et al.* (1998).
In all studies, neuron densities were estimated by the optical disector method.

In most of these cortical areas, there is not a significant correlation between neuron densities or GLI and brain size. The one exception is prefrontal area 10 neuron density, which scales against brain weight with a reduced major axis slope of −0.42 based on log-transformed data ($p = 0.03$, $n = 6$). Therefore, the mammal-wide relationship between these parameters and brain size may not explain interspecific variance in interconnectedness within all cortical areas of hominoids. This raises the interesting possibility that differences among hominoid species in these variables might instead correspond to functionally significant modifications in the organization of cortical interconnections.

Other aspects of network scaling in the cerebral cortex are less well understood. For example, there does not appear to be a correlation between brain size and the density of glial cells (Tower and Young, 1973; Haug, 1987). However, phylogenetic differences in glial cell densities have not yet been systematically examined using modern immunohistochemical markers to identify astrocytes and oligodendrocytes separately. Furthermore, questions regarding the scaling of subpopulations of interneurons and pyramidal cells have only begun to be addressed. Evidence suggests that the proportion of pyramidal neurons that are enriched in NPNFP may increase with brain size. In the orofacial representation of primary motor cortex, there is a striking increase in the percentage of neurons stained for NPNFP in larger-brained great apes and humans in comparison to smaller-brained Old World monkeys (Sherwood *et al.*, 2004a). Tsang *et al.* (2000) also found increasing NPNFP labeling in primary motor cortex across a sample including rats, marmosets, rhesus macaques, and humans. In addition, Campbell and Morrison (1989) found a larger proportion of NPNFP-ir pyramidal neurons, particularly in supragranular layers, in humans compared to macaque monkeys across several different cortical areas.

Interneuron subtypes, as revealed by labeling for calcium-binding proteins, appear to adhere to different scaling trends in anthropoid primates depending on the cortical area. For example, when regressed on total neuron density, the density of PV-ir neurons scales with negative allometry in the primary motor cortex and thus a greater proportion of PV-ir neurons is observed in hominoids compared to Old World monkeys (Sherwood *et al.*, 2004a). In contrast, CB-ir neurons scale against total neuron density with positive allometry in areas V1 and V2, resulting in a smaller percentage of CB-ir interneurons in apes compared to monkeys in these areas (Sherwood *et al.*, 2005). Further studies using allometric approaches to examine the scaling of different neuron subtypes will be necessary to elucidate phylogenetic specializations of cortical circuitry.

4.21.3.4 Genomic Data Provide Insights into Cortical Specializations

Recent studies of phylogenetic variation in gene sequences and expression provide additional insights into cortical specializations among hominoids. While most of these studies have been directed at determining the genetic basis for human neural uniqueness (Enard *et al.*, 2002a, 2002b; Caceres *et al.*, 2003; Dorus *et al.*, 2004; Uddin *et al.*, 2004), some molecular data point to changes that occurred at earlier times in the hominoid

radiation. For instance, all hominoids have evolved a novel biochemical mechanism to support high levels of glutamate flux in neurotransmission through the retroposition of the gene *GLUD1* (Burki and Kaessmann, 2004). This duplicated gene, *GLUD2*, which is unique to hominoids, encodes an isotype of the enzyme glutamate dehydrogenase that is expressed in astrocytes. All hominoid *GLUD2* sequences contain two key amino acid substitutions that allow the GLUD2 enzyme to be activated in astrocytes during conditions of high glutamatergic neurotransmitter flux. Concordant with this evidence for alterations in the molecular machinery necessary for enhanced neuronal activity in apes, it has been shown that the gene encoding the cytochrome *c* oxidase subunit 4-1 underwent rapid nonsynonymous evolution in the hominoid stem, followed by purifying selection in descendent lineages (Wildman *et al.*, 2002). Because these nucleotide substitutions have functional consequences for the manner and rate at which electrons are transferred from cytochrome *c* to oxygen, it is likely that these modifications were selected to serve the needs of cells with high aerobic energy demands, such as neurons.

Also of significance, an alternative splice variant of neuropsin (type II) has originated in recent hominoid evolution (Li *et al.*, 2004). Neuropsin is expressed in hippocampal pyramidal neurons and is involved in neuronal plasticity. The high incidence of polymorphisms in the coding region of this protein in gibbons and orangutans, however, suggests that it may not be functional in these species. In contrast, the coding region of the type II splice form of neuropsin shows relatively little variation in gorillas, chimpanzees, and humans, signifying that it is maintained by functional constraint and that it might be involved in a molecular pathway important for learning and memory in these hominoids.

With respect to brain size, several genes that are involved in controlling the development of cerebral cortex size have undergone accelerated rates of sequence evolution in the hominoid lineage. The *microcephalin* gene shows an upsurge of nonsynonymous amino acid substitutions in a protein-coding domain of the last common ancestor of great apes and humans (Wang and Su, 2004). Additionally, the *ASPM* gene shows evidence of adaptive sequence evolution in all African hominoids (i.e., gorillas, chimpanzees, bonobos, and humans) (Kouprina *et al.*, 2004).

These data put into phylogenetic context evidence that, in the lineage leading to humans, several genes important in the development, physiology, and function of the cerebral cortex show positive selection (Enard *et al.*, 2002b; Dorus *et al.*, 2004; Evans *et al.*, 2004). Furthermore, findings from studies that have compared human and chimpanzee transcriptomes indicate that the human cerebral cortex is distinguished by elevated expression levels of many genes associated with energy metabolism (Caceres *et al.*, 2003; Uddin *et al.*, 2004), suggesting that levels of neuronal activity might be higher in humans compared to chimpanzees (Preuss *et al.*, 2004). While the phenotypic correlates of many of these genetic changes await characterization by *in situ* hybridization and immunohistochemical studies, it is clear that intensified efforts at analyzing variation in the histological organization of the hominoid cerebral cortex will be necessary if there is any hope of understanding how such molecular differences translate into modifications of the computational capacities of cortical circuits.

4.21.3.5 On the Horizon

There remains an extraordinary amount to learn regarding the microstructure of the cerebral cortex of hominoids. Even the basic cytoarchitecture of many cortical areas, such as the posterior parietal cortex, inferior temporal cortex, posterior cingulate cortex, and premotor cortex, has not yet been explored using the methods of modern quantitative neuroanatomy. Moreover, there is not a single recent study of parcellation for any part of the cerebral cortex using chemoarchitectural staining techniques in apes. It will also be important to examine the scaling patterns that govern the distribution of neurochemically identified subsets of pyramidal neurons, interneurons, and glia across different cortical areas from a broad phylogenetic perspective in order to clearly distinguish network allometric scaling from phylogenetic specialization. Finally, determination of whether humanlike histological asymmetries of cortical areas important in language and control of the hand are present in other apes still requires systematic study. By taking seriously the task of understanding such species-specific neural adaptations, we stand to learn an extraordinary amount about the underlying substrates of the cognitive abilities of humans and our closest relatives.

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4.22 The Evolution of Hemispheric Specializations of the Human Brain

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Glossary

allele One of several possible forms of a gene.

antisymmetry Form of bilateral asymmetry in which there are equal numbers of sinistral and dextral forms (e.g., equal numbers of left- and right-handers) in the population.

bilateral asymmetry Condition in which one side of the body is not the mirror image of the other.

bilateral symmetry Condition in which one side of the body is the mirror image of the other.

Bilateria The phylum of ancient origin that includes all organisms (including humans) whose body plan is for the most part bilaterally symmetrical.

bipedalism Standing or walking on two legs rather than four.

Broca's area Region in the left frontal convolution of the brain, usually on the left side, that plays a major role in the organization of speech.

cerebral asymmetry Systematic difference in structure or function between the two sides of the brain.

commissurotomy Section of the commissures connecting the two cerebral hemispheres of the brain, sometimes known as splitting the brain.

directional asymmetry Form of bilateral asymmetry in which the majority of the population exhibit the same direction of asymmetry (e.g., right-handedness in humans).

functional magnetic resonance imaging (fMRI) Technique for measuring blood flow in the intact brain, indicating which parts of the brain are active.

handedness Systematic difference between the two hands, usually favoring the right hand in humans. Can be defined either in terms of preference for one hand over the other or in terms of greater skill or strength on one hand relative to the other.

hemispheric dominance Dominance of one side of the brain over the other.

hemispheric specialization Function performed exclusively or preferentially by one side of the brain rather than the other.

heterozygosity Condition in which an individual carries different alleles on a gene.

homozygosity	Condition in which an individual carries identical alleles at one or more loci in chromosome segments.
human revolution	Emergence of so-called modern behavior in humans, including cave art, more sophisticated tools, and bodily ornamentation, and thought to date from around 40 000 years ago.
right shift	Theory that, in humans, the distribution of differences in function between the two hands is shifted to the right, explaining why most people are right-handed. The right shift is thought to be due to a genetic effect.
temporal planum	Region in the posterior temporal lobe of the brain. This region on the left is part of Wernicke's area, and plays a major role in the comprehension of language.

4.22.1 Introduction

The most obvious mark of hemispheric specialization in the human brain is the near-universal preference for the right hand, implying a left-hemispheric dominance for manual action. This remarkable asymmetry has long been a source of fascination. It seems to apply to all human cultures and has served as a potent source of symbolism (Hertz, 1960). Because the right hand is generally the more skilled, the right is associated with positive values and the left with negative ones, as is evident in the contrast between terms such as 'dexterous', 'adroit', and even 'right' itself, with terms such as 'gauche' and 'sinister'. We speak of a 'right-hand man', but a 'left-handed compliment'. The Bible is said to contain over 100 favorable references to the right hand, and 25 unfavorable references to the left (Barsley, 1970). The negative values associated with the left are often manifest as discrimination against left-handers, even though left-handers often triumph in sports such as tennis or baseball, or even in intellectual ability. The quintessential Renaissance Man, Leonard da Vinci, was a left-hander.

The left cerebral dominance for speech and language, since it was first discovered in the nineteenth century, has been an equally potent source of myth, as is evident in the frequent references in popular culture to left-brain and right-brain values. In this case, though, the differences are not seen as quite so value laden, and if anything there may be more positive associations with the right hemisphere than with the left, even though it is the left hemisphere that controls the characteristically human functions of language and manual manipulation. The right is often portrayed as more creative, artistic, emotionally sensitive, and holistic than the more linear, rule-bound left (Corballis, 1980).

The potency of left and right may be due in part to the fact that both handedness and cerebral asymmetries are functional asymmetries that appear to emerge from symmetrical structures. Our hands look alike, yet function very differently. Similarly, the two sides of the brain are anatomically more or less left–right mirror images, yet one side can produce articulate speech and the other cannot. This discrepancy between function and structure seems to imply some nonmaterial, Cartesian influence, perhaps reinforcing our sense that we humans are unique and superior to other animals, closer to angels than to apes. We are, it has been argued, the lopsided ape (Corballis, 1991). Genetic theories of handedness and cerebral asymmetry are typically based on the premise that a genetic mutation at some point in the hominid lineage gave rise to both handedness and cerebral asymmetry in the majority of humans (e.g., Annett, 1995, 2002; Corballis, 1997; McManus, 1999). It has even been proposed that cerebral asymmetry is the result of a genetic mutation that not only resulted in cerebral asymmetry, but also gave birth to language, theory of mind, a vulnerability to psychosis, and the speciation of *Homo sapiens* (Crow, 2002).

In this article, I propose to challenge this view by summarizing the evidence on cerebral asymmetries in other species and showing that human cerebral asymmetry may in fact have ancient origins. It is almost certainly true that language itself is a uniquely human accomplishment, but its lateralized representation probably derives from asymmetries that go well back in evolution (see Cortical Commissural Connections in Primates). A second aim of this article is to show that bilateral symmetry is as much a property of organisms, including humans, as is lateralization of function. The obsession with handedness and hemispheric specialization has tended to obscure the more obvious fact that our brains and bodies are built on a plan that duplicates the left and right sides in mirror fashion. The main theme of this article, then, is that there is a trade-off between bilateral symmetry and lateral specialization, and this trade-off informs differences both between and within species.

4.22.2 Bilateral Symmetry

We belong to the phylum known as the Bilateria, an ancient lineage that includes over 1.5 million

present-day animal species, including such diverse creatures as soil nematodes, fruit flies, and mammals. The emergence of this lineage is said to mark the transition from stationary or drifting planktonic animals to active swimmers and burrowers. bilaterian fossils have been dated to some 600 Mya, well before the Cambrian (Chen *et al.*, 2004). Bilateral symmetry may even precede the Bilateria, since it is also present in some species of the phylum Cnidaria, which is outside the Bilateria. In the sea anemone *Nematostella vectensis*, for example, bilateral symmetry is dependent on the expression of homologous *Hox* genes much as it is in the Bilateria, suggesting that bilateral symmetry arose even before the evolutionary split between the Cnidaria and Bilateria (Finnerty *et al.*, 2004).

Bilateral symmetry probably evolved in the first instance as an adaptation to directional movement, which effectively defines an anterior–posterior axis in addition to the dorsal–ventral one. These two axes are asymmetrical: the tops of organisms differ from their bottoms, and their fronts differ from their backs. The third axis, orthogonal to these two, is the left–right axis, and organs of movement are then arranged symmetrically to either side of this axis. It seems reasonable to suppose, then, that bilateral symmetry was selected to ensure linear movement, the most efficient way to travel between two points. With very few exceptions, legs, wings, swimming muscles (in fish), and flippers are bilaterally symmetrical. *Nematostella* is a directional swimmer, and the common ancestor we share with this interesting creature presumably lay close in time to the emergence of bilateral symmetry, perhaps as much as 900 Mya (Finnerty *et al.*, 2003).

Given linear movement, there is pressure to ensure bilateral symmetry of the sense organs. To a freely moving organism, it is as well to have the eyes, ears, and skin senses equally placed on either side, since any asymmetry could leave the organism exposed to attack or impervious to prey on the less receptive flank. It follows from the bilateral symmetry of both motor and sensory organs that the neural machinery for both action and perception is also likely to be symmetrical. For most animals, behavior is dominated by sensorimotor activity, involving reactions to an environment that is without overall left–right bias. Symmetry is much less apparent in parts of the body not involved in perception, locomotion, or sensorimotor behavior. The internal organs, including the heart, lungs, stomach, and liver, are arranged asymmetrically, presumably in the interests of more efficient packaging. Even the molecules of living tissue are asymmetrical, a property often taken to be a fundamental property of living matter (e.g., Monod, 1969). In a review of the evolution of bilateral asymmetry, Palmer (2004, p. 828) remarks that "bilateral symmetry is a default state once the anteroposterior and dorsoventral axes are defined." An extra step is therefore required to create bilateral asymmetry. Even the brain, with its origins in the control of sensorimotor activity, remains for the most part built on a bilaterally symmetrical plan.

Besides being largely symmetrical, the brain is also 'double'. In the treatment of intractable epilepsy in humans, surgeons have sometimes resorted to commissurotomy, in which the brain is split through the midsagittal plane, disconnecting the left and right halves. Roger W. Sperry, who pioneered the psychological investigation of the split brain in both humans and animals, wrote that split-brained patients behave as if they had "two separate conscious entities or minds running in parallel in the same cranium, each with its own sensations, perceptions, cognitive processes, learning experiences, memories and so on" (Sperry, 1966–1967, p. 318). Studies have also shown that people can function remarkably well following the removal of an entire cerebral cortex (e.g., Vargha-Khadem *et al.*, 1997; Hertz-Pannier *et al.*, 2002). Although differences between the two halves of the brain have been well documented, and are discussed further below, it is clear that there is considerable overlap.

4.22.2.1 The Trade-Off between Symmetry and Specialization

With respect to basic sensory and motor functions, then, there are clear benefits to bilateral symmetry, given that we live in a world that is for the most part without systematic left–right biases. We humans have nevertheless created left–right asymmetries in the manufactured world, such as the direction of script, traffic conventions, and so forth, and some of these work to the disadvantage of left handers: scissors, golf clubs, corkscrews, the placement of door handles, the sequence of pages in books and magazines. As a general rule, the advantages of symmetry apply more strongly to behaviors that are reactions to the environment than to behaviors that are operations on the environment. There may be advantages to having one hand or its controlling cerebral hemisphere specialized for intricate activities involving tools, as in writing, for example. One advantage of unilateral control is that it is not constrained by the relatively slow conduction time between hemispheres, so that computations can be carried out with greater speed (Ringo *et al.*, 1994), although an alternative solution, of course, would

have been to evolve faster interhemispheric transfer! Another advantage of hemispheric specialization is that it avoids duplication, and this may be especially important in complex functions, like language, that require large amounts of neural circuitry. Duplication may therefore be too wasteful of neural space, and also lead to interhemispheric conflict (Corballis, 1991).

Humans are cognitive specialists, with large brains even by primate standards (Passingham, 1982), and this may well have favored specialization to a greater extent than in other species. That is, the evolution of cognition and the relative independence from stimulus control may well have favored hemispheric specialization at the expense of bilateral symmetry. But it is becoming increasingly clear that cerebral and behavioral asymmetries are widespread in nature, and that at least some of the asymmetries observed in other species seem to operate according to principles similar to those documented in humans. Consequently, any sense that humans are special may derive not from cerebral asymmetry *per se*, but from the nature of the functions that are lateralized.

4.22.3 Hemispheric Specialization in Nonhuman Species

In this section, I review some of the evidence for directional asymmetries in animals, with an emphasis on those that that may provide insight into the nature and origins of our own lopsidedness. First, though, we need to distinguish two different kinds of asymmetry (see Palmer, 2004). One is antisymmetry, in which there are equal numbers of sinistral and dextral forms, as in the claws of male fiddler crabs, which are sometimes larger on the left and sometimes larger on the right. The other is directional asymmetry, in which most of the members of a species are asymmetrical in the same direction, as in the case of the vertebrate heart. In the case of antisymmetry, the direction of asymmetry is almost never inherited, while in the case of directional asymmetry, it typically is. This article is concerned primarily with directional asymmetry, which might arise directly, through some genetic mutation, from symmetry, or it might arise through genetic assimilation from antisymmetry.

4.22.3.1 Handedness

In humans, at least, the most obvious manifestation of cerebral asymmetry is handedness, since the majority of us are right-handed. It seems likely that handedness is one example of a directional asymmetry that arose through genetic assimilation of one form of asymmetry from a character that was previously antisymmetric. Mice are equally divided into left- and right-handers, and selection for left-handedness fails to influence the relative proportions in succeeding generations (Collins, 1969). There does appear to be a genetic component underlying the direction of the handedness component in chimpanzees, however, where the evidence suggests a 65:35 split in favor of right-handedness (Hopkins *et al.*, 2001). In humans, the split is about 90:10 in favor of right-handedness, and there is strong evidence for a genetic component to human handedness (McManus, 1999; Annett, 2002). This progression suggests canalization and genetic assimilation, and may apply more generally to cerebral asymmetry.

Aside from the evidence for weak right-handedness in chimpanzees, and perhaps in other great apes (Hopkins *et al.*, 2001), there is relatively little evidence for comparable asymmetries in other species. One reason for this is that the limbs are generally involved in locomotion, which, as noted earlier, creates a pressure toward bilateral symmetry. The hominids are characterized by bipedalism, which freed the hands from locomotion, allowing for manual specialization to emerge, or at least to become apparent in everyday activities. Nevertheless, there are other isolated examples of consistent handedness in nonhuman species.

Oddly enough, the clearest case of limb asymmetry comes not from primates, but from parrots. Most species of parrot show a strong preference for the left foot in picking up objects, and the proportion of left footers is close to 90%, comparable to the proportion of right-handed humans (Rogers, 1980). Second place may go to the walrus, since there is evidence that 77% of walruses display a preference for the right flipper when feeding, and there is evidence that several bones (scapula, humerus, and ulna) are longer in the right than in the left flipper (Levermann *et al.*, 2003). There have been claims that monkeys show a slight population-level preference for the left hand (MacNeilage *et al.*, 1987). Subsequent evidence has been mixed (see commentaries to the article by MacNeilage *et al.*, 1987), but if true the asymmetry may reflect a right-hemispheric bias for spatial perception. At least one study has shown a slight right-hand advantage for rhesus monkeys, but no bias in capuchins (Westergaard and Suomi, 1996).

McGrew and Marchant (1997) sound a cautionary note. In a comprehensive review, they conclude that among nonhuman primates, "only chimpanzees show signs of a population bias ... to the

right, but only in captivity and only incompletely" (McGrew and Marchant, 1997, p. 201); see also McGrew and Marchant (2001). The evidence for a population-level right-hand preference in chimpanzees comes from Hopkins and his colleagues, but appears to be restricted to certain activities, such as extracting peanut butter from a glass tube (Hopkins, 1996), gestural communication (Hopkins and Leavens, 1998), and throwing (Hopkins *et al.*, 2005), and the ratio of right-to-left-handers is only about 2:1, whereas in humans the ratio is about 8:1. McGrew and Marchant (2001) suggest that the bias in captive chimpanzees is a consequence of contact with right-handed humans, although Hopkins *et al.* (2004) have disputed this, claiming that right-handedness occurs in three distinct populations of captive chimpanzees and is unrelated to the proportion of animals raised by humans, and more recently Lonsdorf and Hopkins (2005) have documented population-level right-handedness for tool use in wild chimpanzees.

4.22.3.2 Vocalization

The left-hemispheric specialization for speech and language in humans may well derive from left-hemispheric control of vocalization. This has been demonstrated even in the frog, suggesting an ancestry that may go back to the very origins of the vocal cords some 170 Mya (Bauer, 1993). In passerine birds, too, vocalization seems to be controlled by the left hemisphere (Nottebohm, 1977), although it has been argued that the mechanisms underlying this asymmetry are not comparable to those in humans (Goller and Suthers, 1995).

Asymmetries for vocalization apply to perception as well as to production. A left-hemispheric advantage for the perception of species-specific vocalizations has been demonstrated in mice (Ehert, 1987), rhesus monkeys (Hauser and Anderson, 1994), and Japanese macaques (Heffner and Heffner, 1984). In chimpanzees, the left temporal planum is larger on the left than on the right (Gannon *et al.*, 1998; Hopkins *et al.*, 1998), an asymmetry that seems not to be present in rhesus monkeys or baboons (Wada *et al.*, 1975) but is well documented in humans (Geschwind and Levitsky, 1968; Jäncke and Steinmetz, 1993; Foundas *et al.*, 1996). This too may reflect an asymmetry in the perception, and perhaps comprehension, of species-specific vocal communication.

4.22.3.3 Facial Asymmetries

The asymmetry for species-specific vocalization may also be manifested in facial movements, but may be reversed for vocalizations and facial movements that are more emotionally based. Hook-Costigan and Rogers (1998) found that marmosets opened the right side of the mouth wider when making social contact calls, implying left cerebral dominance, but the right side of the mouth wider when expressing fear, implying right cerebral dominance for emotion. Curiously, however, Hauser and Akre (2001) found only a bias toward the left side of the mouth in rhesus monkeys, regardless of the nature of the calls, implying uniform right cerebral dominance. Hook-Costigan and Rogers' finding mimics that found in humans, with the right side of the mouth dominant for speech (e.g., Graves and Potter, 1988) and the left for emotional expression. These asymmetries are also evident in 5- to 12-month-old human babies, who open the right side of the mouth wider when babbling, and the left side when smiling (Holowka and Petitto, 2002). In adults, the asymmetry of the mouth when speaking also influences the McGurk effect, in which the perception of spoken syllables depends on movements of the mouth as much as on the actual sounds emitted (McGurk and MacDonald, 1976). This effect depends on movements of the right side of the mouth, not the left (Nicholls *et al.*, 2004).

More generally, there are facial asymmetries associated with emotional expression. For example, human observers see the left side of chimpanzee faces as more emotional than the right side (Fernández-Carriba *et al.*, 2004), implying right-hemispheric dominance. In humans, though, there is controversy as to whether there is a general bias to the left (and thus the right hemisphere) for emotional expression, or whether there is a leftward bias for negative emotions and a rightward bias for positive ones (see Davidson, 1995, for a review of human evidence). The bulk of evidence now seems to support this second view (e.g., Brockmeier and Ulrich, 1993; Jansari *et al.*, 2000).

4.22.3.4 Visual Asymmetries

Lateral asymmetries in the visual system have been widely documented in birds. One striking example comes from the New Caledonian crow, which appears to favor the right eye, and therefore the left hemisphere, when constructing digging tools from Pandanus leaves (Hunt *et al.*, 2001). Not only does this finding parallel the human preference for the right hand (and therefore the left hemisphere) in tool-making and tool use, but it also suggests that manufacture itself, as well as cultural transmission of tool-making techniques, may not be unique to humans (Hunt and Gray, 2003).

Other birds also show visual asymmetries. For example, chicks show a right-eye advantage in fine visual discrimination, suggesting a left-hemispheric advantage. This advantage arises because the right eye is exposed to light in the egg, prior to hatching, whereas the left eye is not (Rogers, 1990). Nevertheless it appears to have adaptive significance. Chicks raised without this prehatching asymmetry do not show the discrimination bias and are at a disadvantage relative to lateralized birds in a situation where they monitor a hovering predator while at the same time discriminating grain from nonedible grit (Rogers, 2002a). This suggests that the lateralized brain is better able to carry out two tasks at once, with the right eye (left hemisphere) picking out the grain and the left eye (right hemisphere) monitoring the hawk. Other evidence shows that the avian right hemisphere is better able to make use of the large-scale geometry of the environment to deal with problems in spatial reorientation (Vallortigara *et al.*, 2004). A left-hemispheric advantage for fine-grained visual analysis and a right-hemisphere advantage for more global vision have also been well documented in humans (Ivry and Robertson, 1998).

The advantage of asymmetry over symmetry is further illustrated in a study with pigeons (Güntürkun *et al.*, 2000). Like chickens, pigeons show a right-eye advantage in discriminating grain from grit. There was a positive correlation between the degree of asymmetry under monocular conditions and the discrimination performance under binocular conditions, suggesting that visual foraging is accomplished more effectively if mediated by a single hemisphere, perhaps because there is less risk of interhemispheric conflict (cf. Corballis, 1991).

4.22.3.5 Behavioral Asymmetries

Many species also show biases in overt behavior, such as turning to escape predators or to attack prey. Faced with a barrier through which a learned predator was visible, some species of fish showed population-level biases to turn left or right, while others did not (Bisazza *et al.*, 2000). This bias was related to the gregariousness of the species, suggesting a social influence: presumably, species that swim together must turn together to avoid collisions. Tadpoles have been shown to have a bias to turn left when escaping a predator, but a bias to turn right when turning to take in air at the surface (Rogers, 2002b), suggesting hemispheric differences. A right-hemisphere bias has also been documented for social responses in a number of species of fish (Sovrano *et al.*, 2001), chicks (Vallortigara and Andrew, 1994), sheep (Peirce *et al.*, 2000), and monkeys (Vermeire, *et al.*, 1998), and may relate to the right-hemispheric involvement in social understanding in humans (e.g., Sperry *et al.*, 1979). There may be a dark side to this, as there is also evidence that the right hemisphere is the more specialized for aggressive behavior in a number of species, including toads (e.g., Rogers, 2002b), lizards (Deckel, 1995), chicks (Howard, *et al.*, 1980), baboons (Casperd and Dunbar, 1996), and humans (Devinsky *et al.*, 1994). Right-handed boxers typically hold a stance in which their opponents are in their left visual fields, perhaps to ratchet up the aggression in their right hemispheres, but also, of course, to give greater momentum to the stronger right hand.

Complementary to the right-hemispheric dominance for attack, there is a left-hemisphere dominance for feeding. Chicks (Deng and Rogers, 1997), pigeons (Güntürkun, 1985), zebra finches (Alonso, 1998), and toads (Vallortigara *et al.*, 1998) respond to prey or to feeding matter preferentially with the right eye. Andrew *et al.* (2000) have suggested that this asymmetry may be related to left-hemispheric control of the mouth structures, an asymmetry that may be widespread in vertebrates and may relate to the left-hemispheric control of vocalization.

4.22.3.6 Summary

The above review is by no means an exhaustive coverage of the now voluminous literature on behavioral and cerebral asymmetries in nonhuman species. It should serve, however, to illustrate that humans are not unique in displaying such asymmetries. Moreover, some of the principles underlying these asymmetries seem to apply to both human and nonhuman species. There appears to be right-hemispheric specialization for emotion (or perhaps for negative emotions), aggression, social behavior, and for the more holistic aspects of perception. The left hemisphere seems to be the more specialized for detailed visual analysis, feeding behavior, and species-specific communication. It is likely that these asymmetries vary between species, perhaps depending on ecological or social factors.

Rarely, if ever, are the asymmetries absolute: each hemisphere, for example, appears to have some capacity to undertake the speciality of the other. Further, not all members of the species show the same directional asymmetry, despite the overall population bias. The percentage of individuals who reverse the asymmetry shown by the majority ranges from 10% to 35% (Ghirlanda and

Vallortigara, 2004). This again suggests that there is a trade-off between symmetry and asymmetry, and one or the other may dominate depending on survival contingencies.

4.22.4 Cerebral and Manual Asymmetries in Humans

As the foregoing review illustrates, the idea that cerebral asymmetry is unique to humans is wrong and may reflect an age-old desire to place humans on a pedestal above other species, closer to angels than to apes. Nevertheless, part of the reason our own lopsidedness seems so salient is that it applies to activities that are themselves characteristically human, if not uniquely so. Handedness is most obvious in tool use, as in writing, hammering, throwing, or, in the present age, texting. Such activities are indeed essentially and in most cases uniquely human, although whether they place us close to angels may be debated. Moreover, it is generally agreed that true language is uniquely human (e.g., Chomsky, 1966; Pinker, 1994). Hence any sense of human uniqueness applies to the activities that are lateralized, rather than to the lateralization itself.

4.22.4.1 Cerebral Asymmetry

The left-cerebral dominance for language nevertheless remains a distinctive feature of the human brain, and may well be more pronounced than asymmetries associated with communication in other species, although this has yet to be established. Patients with damage to the language-mediating areas of the left hemisphere effectively lose the power of speech or of comprehension if the damage occurs in adolescence or adulthood. Since the pioneering discoveries of Broca (1861), speech production has been typically identified with an area in the third frontal convolution of the left hemisphere, known as Broca's area, but more exacting analysis now suggests that the left precentral area of the insula, a cortical structure underling the frontal and temporal lobes, may be more critical (Dronkers, 1996). The important characteristic of language that distinguishes it from other forms of communication is grammar, and although grammar has also been associated with Broca's area, it probably also involves widespread and diffuse regions of the left hemisphere (Dick *et al.*, 2001).

Cerebral asymmetry for language was corroborated by Sperry's work on the split brain, which again revealed that only the left side of the brain was capable of producing articulate speech (Sperry, 1966–1967, 1974, 1982). This work also rather surprisingly showed that the right hemispheres of at least some of these patients were capable of comprehension, albeit at a less sophisticated level than that displayed by the left hemisphere (Zaidel, 1976). Gazzaniga (1983) has maintained, however, that right-hemisphere comprehension is the exception rather than the rule among commissurotomized patients, but Zaidel (1983) has in turn disputed this. This issue remains unresolved.

Brain imaging has further confirmed the dominant role of the left hemisphere in language. Broca's area is typically larger on the left than on the right in most people (Foundas *et al.*, 1995, 1996), as is the temporal planum, as we have seen. We have also seen that the asymmetry of the temporal planum appears to be present in chimpanzees, suggesting that the asymmetry may not be related to language *per se*, but may originate in a left-hemisphere advantage in the processing of species-specific vocalizations. Nevertheless any such asymmetry was no doubt carried over into the processing of language in humans. Functional imaging also shows that that areas of the left hemisphere are activated during both the production (e.g., Huang *et al.*, 2002; Heim and Friederici, 2003) and comprehension (e.g., Springer *et al.*, 1999) of spoken language. Left-hemisphere dominance for speech perception is evident from functional magnetic resonance imaging (fMRI) recordings even in 3-month-old infants (Dehaene-Lambertz *et al.*, 2002).

Although the right hemisphere has some involvement in language processing (e.g., Gernsbacher and Kaschak, 2003), it is clear that in most people language is largely a left-hemispheric enterprise, and indeed occupies widespread circuits in that hemisphere. It is probably the sheer complexity of language, therefore, that makes the asymmetry stand out. It may also explain a striking right-hemisphere dominance for spatial attention. Patients with lesions to the right hemisphere often show a neglect of the left side of space, whereas those with comparable lesions of the left side do not show the reverse asymmetry, or show it only transiently. This follows especially from lesions in the posterior half of the brain, and although the parietal lobe is usually implicated, it has been claimed that the critical area involves the temporal lobe rather than the parietal lobe. According to Karnath *et al.* (2001), this area is homologous to Wernicke's area in the left hemisphere, suggesting that the asymmetry may have been a secondary consequence of language representation in the left hemisphere (cf. Corballis, 1991). There is no evidence for any asymmetry in spatial attention in animals comparable to that

demonstrated by left hemineglect in humans (Driver and Vuilleumier, 2001), although it could be argued that it is related to the right-hemispheric advantage for more global aspects of perception, which has also been documented in birds, as outlined earlier.

4.22.4.2 Handedness

Right-handedness in humans may also be a consequence of cerebral asymmetry for speech. Along with others, I have argued that language itself may derive from manual gestures, rather than from primate calls (Hewes, 1973; Armstrong *et al.*, 1995; Givón, 1995; Rizzolatti and Arbib, 1998; Corballis, 2001a). If this is so, the shift from gestural to vocal language was presumably gradual, so that language for much of our recent evolutionary history was a combination of the two. As we saw earlier, left-hemispheric control of vocalization may go back far in evolution, perhaps to the origins of the vocalization, so the gradual assimilation of vocalization into the language system may have lateralized the neural circuits involved (Corballis, 2003). Indeed, people habitually gesture with their hands while they speak, and in right-handers the right hand predominates (Kimura, 1973). This may have had a spin-off. As vocalization gradually took over, so the hands were released for other activities, such as toolmaking, resulting in the manual specialization that we see in present-day human activities. The release of the hands, and along with it the release also of right-handedness, may also explain what has come to be termed the human revolution that took place some 40 000 years ago in Europe and probably earlier in Africa (Corballis, 2004).

This account explains why the extreme right-handedness observed in the human population is not evident in nonhuman primates. It is likely, though, that right-handedness itself was a species-wide characteristic well before 40 000 years ago. Cornford (1986) analyzed the asymmetries of flakes recovered from La Cotte de St. Brelade in Jersey, dating from 150 000 to 200 000 years ago, and estimated that the incidence of right-handedness among the toolmakers was between 80% and 90%, which is close to present-day estimates. Toth (1985) provides even earlier estimates from asymmetrical flakes found in Lower Pleistocene sites at Koobi Fora in Kenya, dated at 1.4–1.9 Mya. Flakes favoring right-handed action outnumbered those favoring left-handed action in a ratio of about 57:43. Although this bias may seem relatively slight, Toth found that the same ratio was obtained by present-day right-handers given a similar task, which suggested to McManus (1999, 2002) that right-handedness was universal among the early flakers, and that left-handedness was the result of a later mutation. Yet the ratio observed by Toth is not dissimilar to the ratio of right- to left-handers among present-day captive chimpanzees, as reported by Hopkins (1996), and I have suggested that a later mutation may have raised the ratio from 2:1 to about 8:1 (Corballis, 1997). A more conservative conclusion, more aligned with my current thinking, is that right-handedness emerged gradually as vocalization increasingly accompanied manual gesture in the evolution of language. However, the data do not yet permit a clear distinction between big bang and continuity theories of the emergence of handedness.

4.22.5 Genetic Models

Regardless of which of these theories is correct, there seems good reason to suppose that handedness is at least partially under genetic control. Despite the strong human bias toward right-handedness, a minority of the population remains stubbornly left-handed, and a few ambidextrous individuals display no overall preference. Similarly, the right hemisphere controls language in a minority of people, and in some language appears to be represented bilaterally. The asymmetry in language representation, moreover, is loosely correlated with handedness (Knecht *et al.*, 2000). There is at least a weak parental influence on handedness, as revealed in data summarized by McManus and Bryden (1992) and shown in Table 1. Although it is clear that handedness does not 'breed true', single-gene models can accommodate the data reasonably well.

Over 30 years ago, Annett (1972) proposed that the true distinction was not between left- and right-handers, but between those carrying a right shift (RS) factor and those not carrying this factor; in more recent terminology, there is a right-shift allele, RS+, and an allele without directional specification, RS–. To put it simply, right-handedness is inherited but left-handedness is not (Annett, 2002). It should

Table 1 Percentage of left-handed offspring by parental combination, and prediction from McManus' model

	Parental handedness		
	R–R	*R–L*	*L–L*
% Left-handed offspring	9.5	19.5	26.1
Predicted by McManus' model with $p(D) = 0.76$	9.45	20.24	28.87

be emphasized, though, that in Annett's model most of the variation in handedness is random, and the RS+ allele shifts a normal distribution of intermanual differences to the right. For individuals homozygous for the RS+ allele, designated RS++, the shift is about two standard deviations to the right of neutrality; for heterozygotes, designated RS+–, the shift is about one standard deviation to the right; and for those homozygous for the RS– allele, designated RS– –, the distribution is centered on the point of neutrality, that is, the direction of handedness is essentially assigned at random. Annett also makes it clear that the RS gene influences cerebral dominance rather than handedness *per se*. (Since the left hemisphere controls the right hand, it should really be termed a left-shift gene.)

The idea that genes can influence the presence versus the absence of an asymmetry, rather than the direction of the asymmetry, may be a general principle in the genetics of asymmetry (Morgan and Corballis, 1978) and applies for example to the asymmetry of the heart and other visceral organs (Layton, 1976). The same principle is embodied in McManus' (1999) genetic model of handedness. Like Annett, McManus proposes a two-allele gene, with a dextral (D) allele specifying right-handedness and a chance (C) allele, which does not specify the direction of handedness, but leaves it to chance. Unlike Annett, though, McManus argues that handedness is fundamentally dichotomous, so that all DD individuals are right-handed, 75% of CD individuals are right-handed, and CC individuals are equally divided between left- and right-handers. This model makes predictions about the inheritance of handedness that are essentially indistinguishable from those of Annett's model. Table 1 shows how McManus' version fits the data, with the proportion p(D) of D alleles in the population estimated at 0.76.

This estimate might seem high, but perhaps reflects a society in which dextrality, or left cerebral dominance, has greater adaptive fitness than the lack of consistent handedness or cerebral dominance. Variations in this parameter might explain cultural differences in handedness, although the heterozygotic advantage ensures that both alleles are maintained in the population. Figure 1 shows how the asymptotic value of p(D) varies depending on the relative fitness of CC and DD genotypes.

The models can also account for the relations between handedness and cerebral dominance for language. Studies based on the Wada test (Rasmussen and Milner, 1977), electroconvulsive therapy (ECT) (Warrington and Pratt, 1973), and brain imaging (Pujol *et al.*, 1999; Knecht *et al.*, 2000) are reasonably consistent in showing that over 90% of right-handers are left-cerebrally dominant for language, as are some 70% of left-handers. McManus' model, on the assumption that $p(D) = 0.76$ and that handedness and cerebral asymmetry are assigned independently in CD and CC genotypes, predicts that 90.6% of right-handers and 69% of left-handers will be left-cerebrally dominant for language, figures reasonably close to empirically determined values. Annett's model makes similar predictions.

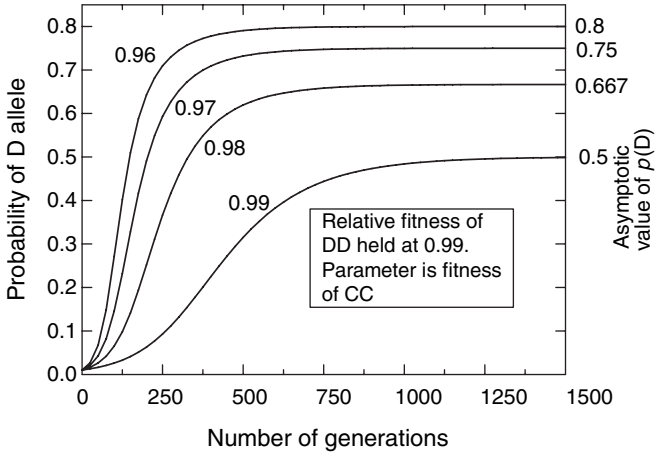

Figure 1 Suppose a mutation occurs creating a dextral allele D in 1% of a population. The figure shows the increase in the probability of the D allele, p(D), over successive generations, given a fitness advantage to the CD genotype. In this example, the relative fitness of the CD genotype is held at 1.0 and that of the DD genotype at 0.99. The rise of the D allele is shown for four different fitnesses: 0.99, 0.98, 0.97, and 0.96, yielding different asymptotic values of p(D). The asymptote would be less than 0.5 if the fitness of the CC genotype were to exceed that of the DD genotype. At asymptote, the ratio of p(D):p(C) is given by $(1–f_{CC}):(1–f_{DD})$, where f_{DD} and f_{CC} are the fitnesses of DD and CC genotypes relative to that of the CD genotype. Reprinted from Corballis, M. C. 1997. The genetics and evolution of handedness. *Psychol. Rev.* 104, 714–727, with permission from APA.

Both Annett and McManus assume that the mutation that gave rise to the RS+ or D allele occurred in hominid evolution. McManus (1999) has speculated that it may have occurred in a copy of one of the genes creating the leftward asymmetry of the heart, perhaps allowing the asymmetry to affect neural tissue as well as the heart rudiment. It seems entirely possible, though, that similar genetic influences may explain the cerebral asymmetries evident in other species. We have seen, in fact, that the proportion of individuals that reverse the population-level bias ranges from approximately 10% to approximately 35% (Ghirlanda and Vallortigara, 2004), and in fact both extremes are evident in humans. Previc (1991) has summarized evidence on what he terms natural forms of auditory and motor asymmetries in humans, and these favor one side over the other in a ratio of 2:1. They include the right-ear advantage in dichotic listening, right-eye dominance, a host of postural asymmetries, and a tendency, especially among newborns, to turn the head to the right. These asymmetries may relate to the fact that about two-thirds of human fetuses are confined to an asymmetrical fetal position, with the right side facing toward the mother's front, during the final trimester. Consequently, approximately 33% of people reverse this asymmetry. The variations between these different asymmetry ratios may reflect the relative fitnesses of homozygotes, under the assumption of an overall heterozygotic advantage, as illustrated in Figure 1.

The gene or genes that create these biases remain hypothetical, although there have been some leads. For example, Crow (2002) has suggested that the laterality gene is located in the *Xq21.3/Yp11.2* region of homology on the X and Y chromosomes, and suggested protocadherin XY as a likely candidate. I have myself argued against this on the grounds that polymorphisms are unstable on the Y chromosome, but suggested, following McKeever (2000), that the gene may be on the X chromosome (Corballis, 2001b). However, a genome-wide search for the handedness gene has since offered little support for X-linkage, and suggests that the region *2p11.2-12* on chromosome 2 may be a better bet (Francks *et al.*, 2002). Although the authors report that this failed to replicate in an independent sample, a further analysis has revealed significant paternal linkage within this site (Francks *et al.*, 2003), suggesting that imprinting may play a role. It is perhaps unlikely that the single-gene models proposed by Annett and McManus will provide the whole answer, but they provide a useful starting point.

4.22.5.1 The Trade-Off between Symmetry and Specialization: A Genetic Perspective

The models proposed by Annett and McManus capture something of the trade-off between symmetry and asymmetry, at least insofar as the D allele stands for asymmetry and the C allele for symmetry. (For simplicity, I use McManus' terminology rather than Annett's, but I do not mean to imply that one model is to be preferred to the other.) Thus DD genotypes are strongly lateralized, with right-handedness and left-cerebral dominance for language, whereas CC genotypes are subject only to random lateralizing influences. The two genes are held in balance by the superior fitness of the CD genotype.

Part of the trade-off may have to do with the relative advantages of language and spatial ability. There is some evidence that left-cerebral dominance for language is achieved through the pruning of the right hemisphere, with some loss of spatial function (Annett, 2002). Thus DD individuals may benefit from having language mechanisms contained within a hemisphere, but while displaying superior skills of oratory may tend to get lost on the way to the Forum. CC individuals may be at risk of divided hemispheric control over speech, with increased risk of stuttering (Foundas *et al.*, 2003) and reading disability (Annett, 2002), but may benefit from greater spatial awareness and more balanced motor skill. Heterozygotic DC individuals may be less prone to either deficiency, and so have the better of both worlds.

A study of 12 770 11-year-olds in the United Kingdom suggests, however, that the academic deficits shown by CC individuals may extend beyond language abilities. Handedness in these children was assessed in a test of skill (checking squares) and ranged from extreme left- to extreme right-handedness. Their scores on tests of verbal ability, nonverbal ability, reading comprehension, and mathematical ability showed a pronounced dip at the point of equality between the hands (Crow *et al.*, 1998). That is, both left- and right-handers scored above those who were ambidextrous. Since CC individuals were more likely to be represented at the point of equality than either DC or DD individuals, this result suggests that they are at greater risk of poor academic performance. This result was not replicated in a German study by Mayringer and Wimmer (2002), who tested a smaller (but still large) sample of 530 boys. Crow *et al.* (1998) refer to ambidexterity as "the point of hemispheric indecision"; the symmetrical brain, so to speak, is unable to make up its mind. The risk of impediment in CC individuals is still relatively small, since many with

this genotype will display asymmetry in one or the other direction by chance.

What, then, are compensatory advantages of a bilaterally symmetrical brain? Many prominent athletes and sportspeople have mixed dominance, and may derive their benefit from an extra degree of perceptual and motor balance. Annett (2002) also claims that surgeons include a disproportionately large number of non-right-handers. It is possible that the benefits of altered handedness derive precisely from being in a minority. Suppose, for example, that members of a group tend to stick together to avoid predation and run off to the left when a predator threatens. By being one of many, each individual is less likely to be singled out by the predator. The predator may nevertheless choose to attack the mob rather than the strays, since the chances of catching at least one victim are maximized. Some individuals may therefore benefit from joining a minority that veers off to the right, a strategy that works only if this group remains a minority. This may have resulted in a subtle selection dynamic that held left- and right-turning in balance (Ghirlanda and Vallortigara, 2004), but with left-turning implying a right-hemisphere dominance for this behavior, maintained for the majority. One might argue similarly that left-handers hold an advantage in fighting, but only so long as they are in the minority (Raymond *et al.*, 1996).

There is also some reason to suppose that the lack of consistent cerebral asymmetry may lead to different styles of thought. In a study of magical ideation and handedness, Barnett and Corballis (2002) reported a relationship that was exactly the reverse of that reported for intellectual achievements by Crow *et al.* (1998). People with mixed-handedness were the most prone to magical ideation, characterized by mild paranoia and superstition, and scores on magical ideation decreased systematically as handedness became more extreme in either direction.

There is also evidence that mixed-handedness – or perhaps hemispheric indecision (Crow *et al.*, 1998) – is associated with a greater sensitivity to sensory illusions (Niebauer *et al.*, 2002) – which was not replicated, however, in a study by Barnett-Cowan and Peters (2004) – and a higher risk of schizophrenia (Claridge *et al.*, 1998; Upadhyay *et al.*, 2004) and strong belief in the paranormal seems to be associated with symmetrical brain activity (Pizzagalli *et al.*, 2000). Another study has shown that people who score relatively low on magical ideation show a left visual field advantage on a lexical-decision task, whereas those who score relatively high show no difference between visual fields, implying a lack of cerebral dominance (Pizzagalli *et al.*, 2001).

Jaynes (1976) speculated that cerebral asymmetry emerged in the second millennium BCE, in response to assorted catastrophes, such as floods, invasions, and the like. Prior to this, people were governed by hallucinations, invoking the gods, but cerebral asymmetry allowed the left hemisphere to create a sense of self, so that people took responsibility for their own actions. Jaynes' theory makes little evolutionary sense, since handedness and cerebral asymmetry almost certainly go back at least 2 My, and perhaps even earlier, in hominid evolution (Corballis, 1997). Nevertheless, there may well be some truth to the idea that cerebral asymmetry underlies rational thought, and that a lack of asymmetry may well lead to more delusional and perhaps hallucinatory thought processes.

At least some of the characteristics associated with the lack of cerebral asymmetry, including paranormal experience, hallucinations, and so on, may be linked to religion. Although religious activities may seem irrational and sometimes counterproductive, it has been argued that religious behaviors has been favored by selection because they promote intergroup alliances (Hayden, 1987). Others have argued that religion is a system used by elites to maintain social control (e.g., Cronk, 1994). While this implies a social rather than an evolutionary origin, there may well have been selection of those predisposed to accept arbitrary leadership. Obedience is a common religious virtue. Religion is undoubtedly a complex phenomenon, but still a universal one, and is in many respects at odds with scientific rationalism. This raises the possibility that the trade-off between symmetry and asymmetry, or the C and D alleles, may have a bearing on the age-old struggle between religion and science, as exemplified in religious opposition to such scientific luminaries as Charles Darwin and Galileo.

Nevertheless, magical thinking may also be related to creativity, with positive implications for science and mathematics. Leonhard and Brugger (1988) note a link between paranormal thought, delusional thought, and creativity, and suggest that these characteristics relate to heightened right-hemispheric activation and relatively coarse semantic activation in that hemisphere. This in turn results in a loosening of associations and enhanced creativity. Although Leonhard and Brugger's account focuses on the right hemisphere, it is possible that the profile has to do with lack of cerebral dominance rather that any specialization of the right hemisphere itself. Despite the evidence of Crow *et al.* (1998) that mixed-handers are deficient in

arithmetic ability, Singh and O'Boyle (2004) report that mathematically gifted adolescents show no hemispheric asymmetry on tasks involving global–local judgments and matching letters, whereas average-ability adolescents and college students show a left-hemispheric advantage, suggesting that the mathematically gifted may lack consistent cerebral asymmetry. Although Singh and O'Boyle selected right-handers for this study, they also characterize the mathematically gifted as "typically male, left-handed, and myopic" (Singh and O'Boyle, 2004, p. 371).

Two individuals who may serve as CC icons are Leonardo da Vinci and Albert Einstein. Leonardo – the prototypical Renaissance Man, artist, scientist, and inventor – is generally regarded as being several centuries before his time. He was left-handed, and habitually wrote backwards, in mirror writing, but was also capable of writing normally. Einstein seems to have been right-handed, but was said to be slow to develop speech and a slow learner, and postmortem analysis of his brain revealed "an unusual symmetry between the hemispheres" (Witelson *et al.*, 1999, p. 2151), especially in the occipital and parietal lobes. He is also reported to have declared "I want to know how God created this world."

4.22.6 Conclusions

The brain and nervous system are built according to a plan that is bilaterally symmetrical. This probably goes back to the first organisms that moved linearly, perhaps even earlier than Bilateria, the lineage that includes nearly all present-day insects and animals, from humans to fruit flies to nematodes. Yet bilateral symmetry is readily broken if there are advantages in lateralization of function. Human handedness and cerebral asymmetry are examples, but there are countless other examples in the animal world, some of which are precursors to our own characteristic lopsidedness. Yet these asymmetries are not absolute. There is considerable overlap of function even in the lateralized human brain, and there is nearly always a minority of individuals within each lateralized species who show the opposite direction of asymmetry, although this may be born of chance fluctuation rather than systematic reversal. This minority appears to range from approximately 10% to some 35%, a range that may also apply to asymmetries that occur within our own species. Again, this suggests phylogenetic continuity rather than human uniqueness.

Genetic theories can explain a number of features of individual differences, at least in humans. The simplest theories are those in which there is a single laterality gene, with two alleles, one specifying a directional asymmetry and the other leaving the direction of asymmetry to chance. Such models remain speculative, since there is no sure evidence as to where the gene might be located in the genome, or even whether such a gene exists. If there is a genetic component to cerebral asymmetry, it may well provide an important basis for individual differences. Despite the widespread belief that right-handedness and left-cerebral dominance for language arose from a genetic mutation that occurred in hominid evolution, the more parsimonious view is that the same mechanisms that underlie these asymmetries also underlie the asymmetries observed in other species. A balance between directional and chance influences is then maintained by a heterozygotic advantage, and the relative costs of the two homozygotic genotypes then determines the relative proportions of lateralized and nonlateralized individuals. This, then, could be the universal mechanism underlying the trade-off between symmetry and specialization.

In any event, the widespread notion that cerebral asymmetry is uniquely human is wrong. Nevertheless, some of the functions that are lateralized in the human brain may well be unique to our species. This applies especially to language, which requires widespread neural circuitry, and if lateralized occupies a good proportion of the hemisphere in which it is housed. This in turn may have created a complementary asymmetry in the other side of the brain for spatial attention. Hand skill is another characteristic that is exceptionally highly developed in humans, and is an asymmetry that is strikingly obvious in everyday human behavior. Yet not all humans show this high degree of asymmetry, and there may be some advantages to an unlateralized brain that offset the advantages of lateralization. Some of these advantages may come about precisely because those who possess them may be in a minority. If nearly all tennis players were left-handed, the advantage of surprise and unorthodoxy would be transferred to the right-handed. If a lack of cerebral dominance were also a characteristic of creative visionaries, the impact of such people would be lessened if we were all like that. The world needs accountants.

Is there a common source for the evolution of lateralization? Perhaps the best candidate is the mouth. MacNeilage (1998) has proposed that speech is based on masticatory movements of the mouth, and Andrew *et al.* (2000) note that left cerebral control of the mouth and its internal structures is widespread in vertebrates. The left-hemispheric control of speech may therefore have

ancient origins. I have proposed that handedness may then have come about because of the association of speech and manual gesture (Corballis, 2003): if language went from hand to mouth, so lateralization went from mouth to hand. But there are perhaps earlier associations between hand and mouth that could have favored the right-hand preference. In some animals, the forelimbs are involved in bringing food to the mouth. We have seen that walruses show a general preference for the right flipper in feeding, and there is controversial evidence that the great apes also show a right-hand preference for a number of activities, including feeding (Hopkins, 1996). Any such preference, however, might be countered by the advantages of bilaterality, allowing an animal to reach with equal facility to either side. The mouth is also a more general manipulative organ, and manipulation also involves the hands, so that right-handedness may have emerged in the context of manipulation, again driven by lateralized control of the mouth. These hand–mouth associations are likely to have been especially decisive in the most manipulative of creatures, *H. sapiens*.

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4.23 Neurological Specializations for Manual Gesture and Tool Use in Humans

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Glossary

apraxia	A deficit affecting manual skills that cannot be attributed to elementary sensory or motor disturbances. Generally pronounced when pantomiming or imitating transitive actions.
prehension	Manual reaching, grasping, and object manipulation.
transitive actions	Behaviors that involve the use of objects other than one's own body (e.g., using tools or utensils).

4.23.1 Neural Bases of Manual Prehension in Primates

Establishing homologies between brain structures of species whose most recent common ancestor lived 30 Mya is a nontrivial challenge (Kaas and Reiner, 1999). This is made even more difficult when comparisons involve contrasting the response properties of single neurons in macaques with indirect measures of the activity of several million neurons in the human brain recorded with functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) (Orban *et al.*, 2004). With growing evidence for differences between human and macaque cortical architecture (Preuss and Coleman, 2002) including areas of the dorsal visual processing stream that are implicated in manual prehension (Vanduffel *et al.*, 2002; Orban *et al.*, 2004), caution is warranted when evaluating claims of homologies between species. Nevertheless, human research in this area is guided largely by results in macaques, and, as reviewed below, there appear to be marked similarities between species in the gross organization of systems involved in manual actions (see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates, Hand Use and the Evolution of Posterior Parietal Cortex in Primates, Nuclear Schizophrenic Symptoms as the Key to the Evolution of the Human Brain).

4.23.1.1 Ventral and Dorsal Pathways

The extrastriate visual areas of nonhuman primates are grossly organized into two primary pathways with significant reciprocal interconnections (Morel and Bullier, 1990): (1) an occipital temporal (i.e., ventral) stream; and (2) an occipital parietal (i.e., dorsal) stream (Ungerleider and Mishkin, 1982; Mishkin *et al.*, 1983; Felleman and Van Essen, 1991). Areas within these streams are also reciprocally interconnected with regions of prefrontal cortex involved in working memory and planning, and their sensory responses may be modulated via feedback from these higher cognitive centers (Wilson *et al.*, 1993; Goldman-Rakic, 1996). This organization is found in both Old and New World monkeys (Preuss and Goldman-Rakic, 1991), and it is believed to be relatively preserved in human beings (Ungerleider and Haxby, 1994; Ungerleider *et al.*, 1998; Braddick *et al.*, 2000). It is generally agreed that areas within the dorsal stream constitute the major source of input to premotor regions of frontal cortex involved in the organization and control of action (Jeannerod *et al.*, 1995; Johnson *et al.*, 1996; Andersen *et al.*, 1997; Luppino and

Rizzolatti, 2000; Marconi *et al.*, 2001; Rizzolatti and Luppino, 2001).

4.23.1.2 Subdivisions Within the Dorsal Pathway

As illustrated in Figure 1, on the basis of anatomical connectivity with frontal cortex, the macaque's dorsal visual pathway can be subdivided further into dorsal–dorsal (d–d) and ventral–dorsal (v–d) streams (Rizzolatti and Matelli, 2003). Inputs to dorsal (PMd) or ventral premotor cortex (PMv) arise from segregated regions of parietal cortex (Tanne-Gariepy *et al.*, 2002), and these frontal areas are not highly interconnected. In macaques, the primary visual input to the d–d stream is from area V6 along with V6A, parietooccipital (PO), and medial intraparietal (MIP) area in the superior parietal lobule (SPL).

The primary visual input to the v–d pathway is from areas MT/MST (middle temporal/medial superior temporal) along with visual areas of the inferior parietal lobule (IPL: anterior intraparietal area (AIP), PG, PFG, and PF). The v–d pathway projects to PMv cortex and can be further subdivided: neural tracing studies demonstrate that parietal area AIP projects to F5, while the adjacent ventral intraparietal area (VIP) projects selectively to F4 (Luppino *et al.*, 1999). As discussed below, these subdivisions may be involved in constructing representations for grasping objects versus delineating peripersonal space, respectively.

Consensus on the functional significance of the v–d and d–d subdivisions of the dorsal stream is currently

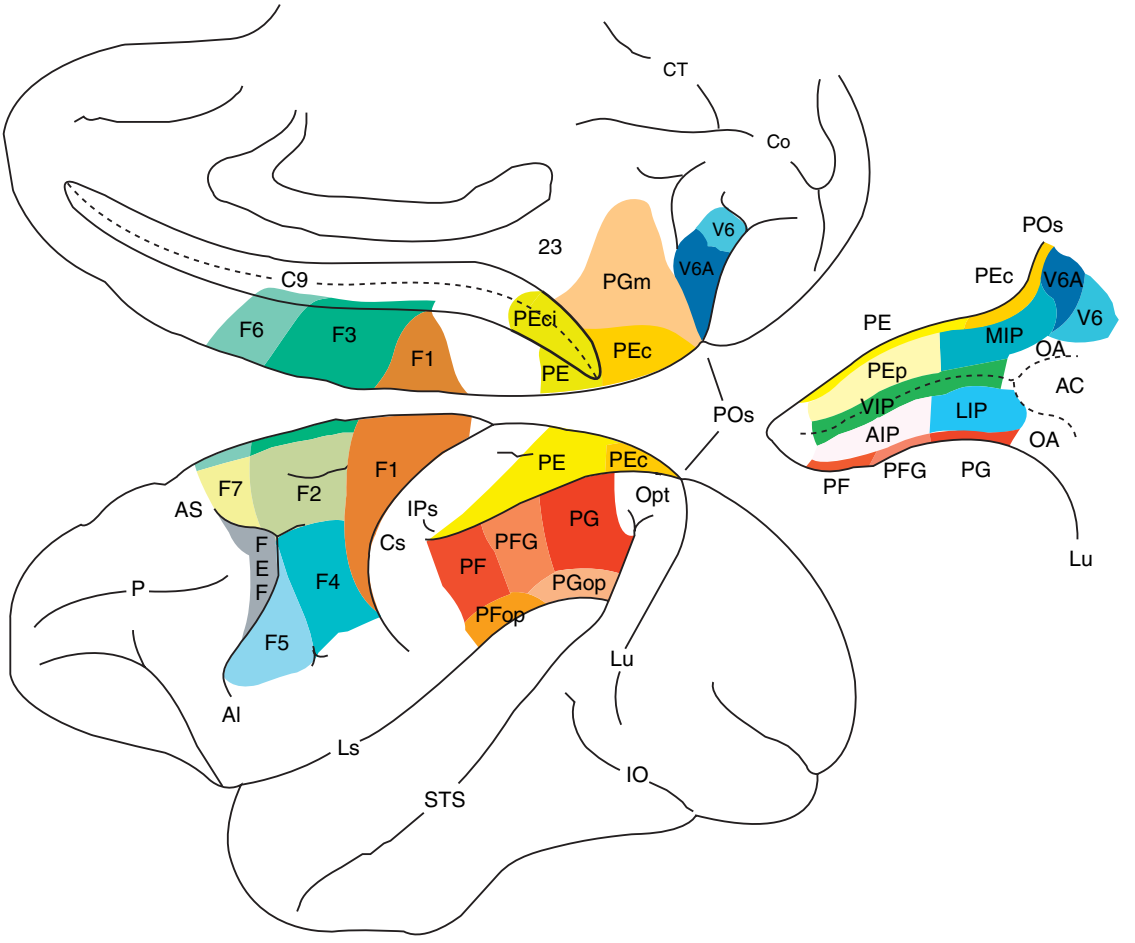

Figure 1 A schematic representation of the parietal and frontal areas of the macaque brain involved in manual prehension. Frontal areas are labeled according to the systems of Matelli and colleagues (Matelli *et al.*, 1985, 1991), and parietal areas use the system of Pandya and Seltzer (Pandya and Seltzer, 1982). Lower: View of the lateral surface of the left cerebral hemisphere. Upper: The medial surface of the left cerebral hemisphere. Inset: The intraparietal sulcus unfolded to reveal areas located within. These include medial (MIP), ventral (VIP), and anterior (AIP) intraparietal areas (from Rizzolatti and Matelli, 2003). AG, annectant gyrus; AI, inferior arcuate sulcus; AS, superior arcuate sulcus; Ca, calcarine sulcus; Cg, cingulated sulcus; Cs, central sulcus; Ls, lateral sulcus; Lu, lunate sulcus; OT, occipitotemporal sulcus; P, principal sulcus; POs, parietooccipital sulcus; STS, superior temporal sulcus. Reproduced from *Exp. Brain Res.*, vol. 153, 2003, pp. 146–157, Two different streams form the dorsal visual system; Anatomy and function, Rizzolatti, G. and Matelli, M. With kind permission of Springer Science and Business Media.

lacking. For the past decade the predominant view has been that reaching and grasping are controlled by parallel dorsal versus ventral visuomotor channels, respectively (Jeannerod *et al.*, 1995). Yet, as articulated by Tanne-Gariepy *et al.* (2002), accruing data suggest that the truth is likely more complex. PMd and PMv both contain representations of distal (F5) and proximal (F4) musculature of the upper limbs involved in grasping versus reaching, respectively. Likewise, both areas contain cells that code movement direction (Kakei *et al.*, 2001). As an alternative, Rizzolatti and Matelli (2003) hypothesize that the d–d pathway is exclusively involved in online motor control while the v–d stream participates in both the execution of prehensile actions as well as premovement organization (Glover, 2004). Another view is that premotor representations are entirely goal-specific and effector-independent (Rijntjes *et al.*, 1999). Consistent with this hypothesis are findings showing that microstimulation of motor and premotor cortex can evoke complex, multijoint movements that appear to be organized on the basis of the final position or action goal (e.g., bringing the grasping hand to the opening mouth regardless of the direction of movement) (Graziano *et al.*, 2002). Regardless of which organizational scheme is ultimately shown best to capture the properties of these systems, the majority of research in this area has been organized around the use of specific functional tasks. In the following sections I will consider evidence regarding those areas contributing to three requisites for dexterous manual prehension in macaques and humans: reaching, grasping, and the representation of peripersonal space.

4.23.1.3 The Dorsal–Dorsal Subdivision

Cells within the medial intraparietal sulcus of the SPL (area MIP), including the so-called parietal reach region (PRR), are involved in the representation of reaching actions (Wise *et al.*, 1997; Snyder *et al.*, 2000; Batista and Andersen, 2001; Andersen and Buneo, 2002). Area PMd also receives visual information via a direct connection with area PO (Caminiti *et al.*, 1996) and proprioceptive input via a circuit interconnecting PEc/PEip-F2 (Lacquaniti *et al.*, 1995; Matelli *et al.*, 1998). Neurons in PMd use this input to compute representations of both the location of visual targets and the direction of forelimb movements needed to acquire them (Johnson and Ferraina, 1996; Johnson *et al.*, 1993, 1996). A subpopulation of PMd neurons responds to specific combinations of sensory cues specifying target location and which limb to use when performing a manual pointing task, suggesting that single PMd units represent plans for specific reaching actions (Hoshi and Tanji, 2000).

Early PET studies identified activation within PMd, intraparietal sulcus (IPS), and SPL during reaching, pointing, and finger-tracking movements in humans (Colebatch *et al.*, 1991; Deiber *et al.*, 1991; Grafton *et al.*, 1992; Kertzman *et al.*, 1997). More recent findings using fMRI are consistent with these earlier results in suggesting the existence of a parietofrontal reach circuit in humans that can be activated by either overt movements (Connolly *et al.*, 2003) or motor imagery (Johnson *et al.*, 2002).

4.23.1.4 The Ventral–Dorsal Subdivision: Grasping

Single-unit electrophysiological recordings indicate that a parietofrontal circuit interconnecting areas AIP and F5 is involved in the transformation of sensory information into motor commands for grasping (Jeannerod *et al.*, 1995; Luppino and Rizzolatti, 2000; Rizzolatti and Luppino, 2001). Reversible inactivation studies with muscimol injections to either AIP (Gallese *et al.*, 1994) or F5 (Fogassi *et al.*, 2001) cause a selective deficit in visually guided grasping without affecting reaching. Electrophysiological recordings in macaques identified cells in the lateral bank of the IPS that are involved in the visual guidance of object-oriented hand movements (Taira *et al.*, 1990). A subpopulation of these cells are highly shape-selective in their responses (Sakata *et al.*, 1995). These object-selective cells can be divided into three categories on the basis of their receptive field (RF) properties. Motor dominant neurons require no visual input and therefore discharge in either the light or dark during manipulation. These cells do not respond to object fixation, and may therefore be coding hand movements necessary to engage objects. Visuomotor neurons respond strongest when objects are manipulated in the light, and less when either the hand or target object is invisible. A subpopulation of the visuomotor neurons also responds when the preferred object is fixated in the absence of manipulation, suggesting that these cells are coding hand movements relative to objects' visual properties. Finally, visual neurons only respond when an object is manipulated in the light, or when it is fixated. These cells are likely coding visual properties of objects that are useful for manipulation (Sakata *et al.*, 1995, 1997).

The object-selective cells within these three classes are distributed in a gradient along the lateral bank of the IPS. Visual neurons are found in higher concentrations in the lateral intraparietal (LIP) areas, known to also be involved in saccades (Colby *et al.*, 1995, 1996; Andersen *et al.*, 1998).

Movement-related motor and visuomotor units are also found in LIP, but are more concentrated immediately posterior to the primary somatosensory area (SI) hand representation in area AIP (Sakata *et al.*, 1995). Injections of a gamma-aminobutyric acid (GABA) agonist (muscimol) into area AIP cause a reversible deficit in preshaping the hand when grasping visual objects while leaving reaching intact (Gallese *et al.*, 1994).

Cells within area F5 of the macaque code the goals of specific prehensile actions rather than the movements of which they are composed. These units can be categorized on the basis of their RF properties into those that represent specific actions such as holding, grasping, or tearing objects. If the same hand movements are made as part of a different action, e.g., grooming instead of feeding, responses are weak or absent (Rizzolatti *et al.*, 1988). This observation has led to the hypothesis that area F5 contains a vocabulary of hand actions (Rizzolatti *et al.*, 1988), in which the goals of hand–object interactions are represented explicitly.

As discussed extensively in Michael Arbib's article (see Premotor Cortex and the Mirror Neuron Hypothesis for the Evolution of the Language), a subclass of neurons in area F5 (Gallese *et al.*, 1996; Rizzolatti *et al.*, 1996) and rostral inferior parietal cortex (PF; Rizzolatti and Craighero, 2004), known as mirror neurons, discharge not only when the monkey produces a specific action but also when it observes the experimenter undertake a comparable behavior (Rizzolatti and Luppino, 2001). This mirror system may be relevant to understanding the acquisition of complex skills through observation (Buccino *et al.*, 2004), as they provide a mechanism for mapping perceived actions on to one's own motor representations. Of potential relevance to the upcoming discussion of tool use, a recent paper reports tool-responding mirror neurons that respond selectively when macaques observe food items being captured with a simple tool and not the hand (Ferrari *et al.*, 2005).

On the basis of cytoarchitectonic similarities, it has been suggested that macaque area F5 is homologous with pars opercularis of the human inferior frontal gyrus (Petrides and Pandya, 1984; Preuss *et al.*, 1996), while F4 may be homologous with human inferior precentral gyrus (Rizzolatti *et al.*, 2002). Several studies report activation of pars opercularis as well as inferior precentral gyrus during visually guided grasping (Ehrsson *et al.*, 2000, 2001), haptic object manipulation (Binkofski *et al.*, 1999), and action observation (Iacoboni *et al.*, 1999; Johnson-Frey *et al.*, 2003). Because the RF characteristics of neurons in AIP and VIP are not strictly segregated, but rather distributed along a gradient (Sakata *et al.*, 1995), it may not be possible to differentiate these regions and adjacent areas of cortex (PF) using current neuroimaging techniques. I will therefore refer to the anterior portion of the human IPS and adjacent cortex as aIPS.

Recent fMRI studies identify activity within aIPS and surrounding cortex in tasks similar to those that evoke responses in cells of macaque AIP and/or VIP. Manipulation of complex versus simple shapes without vision is associated with mean activation in human aIPS (Binkofski *et al.*, 1999). This location is also activated during haptic recognition of shapes (Jancke *et al.*, 2001). Activation within this vicinity is also observed during object discrimination tasks involving both visual-to-tactile and tactile-to-visual transfer (Grefkes *et al.*, 2002). Activity associated with grasping visually presented three-dimensional objects (Binkofski *et al.*, 1998; Johnson-Frey *et al.*, 2005a), or grasping at two-dimensional projected objects (Culham *et al.*, 2003), is centered within a slightly more lateral and anterior site, as is visual discrimination of objects' surface orientations (Shikata *et al.*, 2001). Finally, as reviewed in Johnson-Frey (2004), a number of studies report activation in aIPS and surrounding areas of the IPL when viewing manipulable tools (Chao and Martin, 2000; Kellenbach *et al.*, 2003). Recent work using transcranial magnetic stimulation demonstrates that disruption of aIPS compromises the ability to update plans for visually guided grasping on the basis of rapidly changing visual feedback (Tunik *et al.*, 2005).

4.23.1.5 The Ventral–Dorsal Subdivision: Peripersonal Space

Electrophysiological investigations suggest that visuotactile representations of peripersonal space are constructed in a circuit connecting IPL area VIP with area F4 in PMv (Fogassi *et al.*, 1992, 1996). Area F4 contains a representation of the face, neck, trunk, and limbs and lies caudal to grasp-related area F5 (Figure 1). The majority of units in F4 are bimodal, having tactile RFs that are in register with three-dimensional visual RFs of space immediately adjacent to the animal and are not affected by variations in gaze direction. Similar RF properties can be found in area VIP (Colby *et al.*, 1993; Duhamel *et al.*, 1998), which provide direct afferent input to F4 (Luppino *et al.*, 1999). These observations have prompted the hypothesis that the VIP–F4 circuit constructs representations of peripersonal space in a frame of reference centered on the body part involved in a given visually guided action such as object manipulation (Graziano *et al.*, 1994; Fogassi *et al.*, 1996).

The paucity of neuroimaging studies attempting to identify areas of the human brain involved in the representation of peripersonal space may reflect the technical challenges associated with stimulus delivery and response recording. One successful PET investigation found differential activation in ventral premotor cortex and the IPS in association with bisecting lines located in near versus far space (Weiss *et al.*, 2000). While more work is necessary, these results are consistent with those predicted by the VIP–F4 circuit in macaques.

4.23.2 Primate Tool Use and the Ventral–Dorsal Stream

An interesting possibility to consider is whether tool use in primates might be accounted for by extensions of the sensorimotor mechanisms involved in manual prehension. Similar to using a limb, tool use involves implementing a sensorimotor transformation. However, this transformation must now include parameters that capture the physical and mechanical properties of the tool. Macaques can be trained to use simple tools in the laboratory, and this work suggests that these behaviors may involve acute plasticity within existing neural circuits involved in manual prehension. As noted above, cells in macaque area VIP have visuotactile RF properties similar to those observed in area F4 (Iriki *et al.*, 2001; Obayashi *et al.*, 2000). Interestingly, as illustrated in Figure 2, the RFs of these units appear to increase over time as Japanese macaques learn to use rakes to extend their reach (Iriki *et al.*, 1996). Specifically, visual RFs that are normally in register with tactile RFs of the hand expand to encompass peripersonal space now occupied by the tool. This expansion is only observed when tools are actively used to accomplish a goal-directed action (food retrieval), and not when tools are merely held or when physically similar yet ineffective tools are manipulated. Learning to use tools is associated with increased expression of brain-derived neurotrophic factor within the anterior bank of the IPS, that may reflect neuronal remodeling via axonal sprouting (Ishibashi *et al.*, 2002). Some neurons in PF and AIP also exhibit plasticity with tool use, but the highest density of such cells is

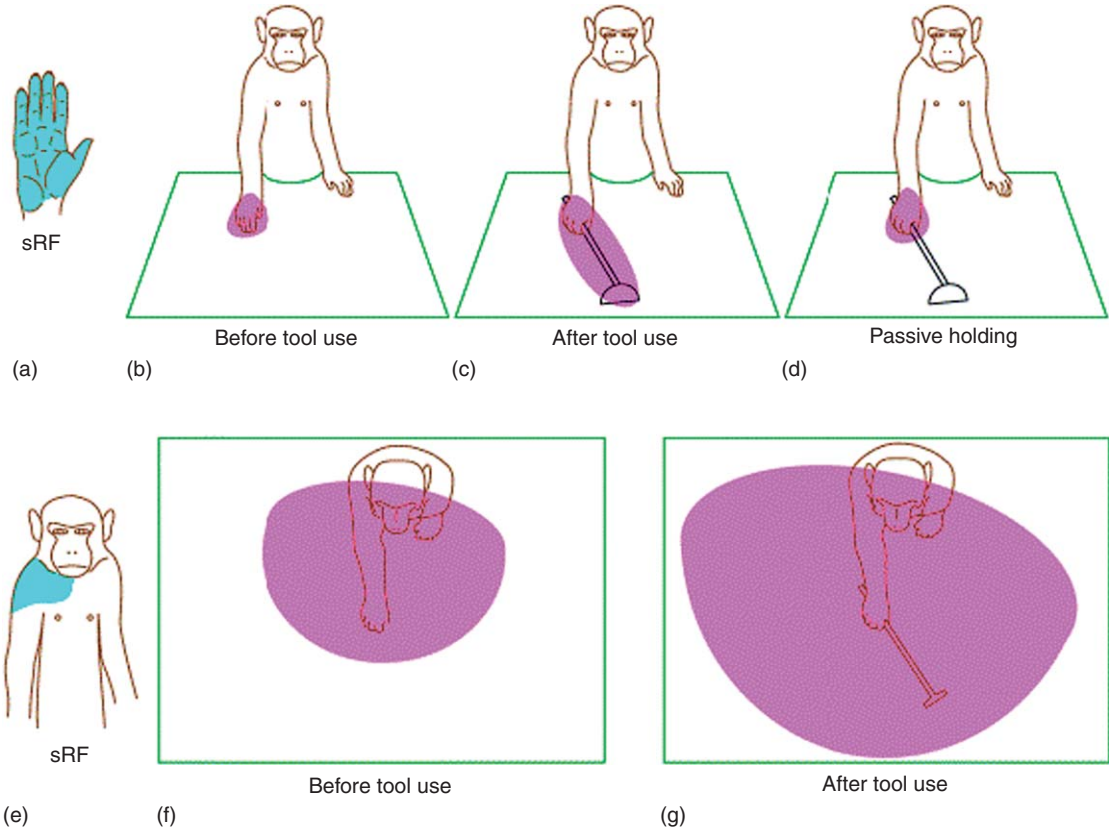

Figure 2 Experience-dependent changes in area VIP neurons with tool use. Distal-type neurons (top): Changes associated with extended tool use in the receptive field of a visuotactile neuron in area VIP that represents the distal forelimb. Proximal-type neurons (bottom): Changes associated with extended tool use in the receptive field of a visuotactile neuron in area VIP that represents the proximal forelimb. Reproduced from Maravita, A. and Iriki, A. 2004. Tools for the body (schema). *Trends Cogn. Sci.* 8, 79–86, Elsevier.

found on the other side of the IPS, i.e., roughly lateral (or anterior) to MIP including VIP and the fundus of the sulcus (Iriki, personal communication). A PET study reveals that performance of these simple tool use behaviors is accompanied by increased activity within a widely distributed network in the macaque brain including regions within v–d pathway (IPS and PMv), as well as basal ganglia, pre-supplementary motor area (pre-SMA), and cerebellum (Obayashi *et al.*, 2001).

Recent studies of humans with parietal lesions reveal behavioral effects that nicely complement the observations of expanded visuotactile hand representations with tool use in macaques (Farne and Ladavas, 2000; Maravita *et al.*, 2001; for a comprehensive review, see Maravita and Iriki, 2004). For instance, some patients suffering right parietal injuries demonstrate left hemineglect when performing a line bisection task positioned within reach. That is, they neglect portions of the line located to the left of fixation, biasing their estimates of center toward the right. However, when bisecting distant lines with a laser pointer they may perform normally. Importantly, when bisecting lines at a distance with a hand-held stick, one such patient again demonstrated neglect (Berti *et al.*, 2001). Similar to changes in the RF properties of IPS neurons in monkeys, use of a stick appears to cause distant space to be remapped as within reach, leading to neglect-related bias in performance. Likewise, there is kinematic evidence in healthy adults suggesting that the same motor representation may be involved in grasping with the fingers and grasping with a tool (Gentilucci *et al.*, 2004).

Results of an fMRI experiment with humans show that employing a set of tongs to extend one's grasp is associated with increased activity centered within the IPS (Inoue *et al.*, 2001). Curiously, these activations are ipsilateral rather than contralateral to the involved limb, as would be expected on the basis of macaque electrophysiology. Reasons for this pattern are uncertain and more work is necessary to determine the source. However, because the control condition involved performing the same grasping actions with the hand without a tool, it is possible that activations within the contralateral sensorimotor regions may have been eliminated during the subtractive comparison.

4.23.3 Human Specializations for Tool Use and Manual Gesture

Whether the explosion of tool use behaviors in hominids reflects the emergence of specialized brain mechanisms is an important and unresolved question. This seems probable for at least two reasons. First, human tool use differs in a variety of ways from that known to occur in nonhumans. Only hominids are known to fashion compound tools by joining together multiple parts and/or materials. Likewise, nonhominids do not appear to make one tool in order to create another (e.g., shaping a rock cutter to manufacture a wooden spear). This latter behavior has been taking place for a relatively long time; the available fossil record indicates that ancestral hominids were using rocks to manufacture stone-cutting implements for at least the past 2.5 million years (Ambrose, 2001). In contrast to other species in which tool use is typically found in specific subpopulations, tool use is a universal characteristic of all human cultures, and highly refined procedures for skillful use have co-evolved with complex tools, and are actively transmitted to successive generations.

Second, it is not possible to understand many complex forms of human tool use exclusively in terms of sensorimotor transformations (Johnson-Frey, 2003a; Johnson-Frey and Grafton, 2003). As is illustrated in Figure 3a, a variety of different postures can be used to achieve a stable grip that will enable an actor to grasp familiar tools stably for

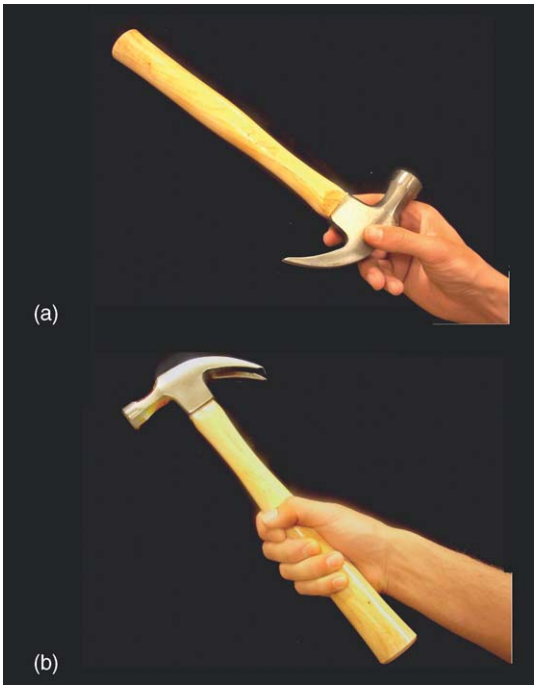

Figure 3 Different ways of grasping a familiar tool. a, The most stable way of gripping a hammer is at its perceived center of mass. b, However, effective use of a hammer requires adopting a less stable grip that is better suited to generating force when pounding. This latter grip depends on both perceived visual properties of the object and access to semantic knowledge.

purposes such as moving them from one location to another, handing them to another individual, or manipulating them. These solutions are clearly not suitable for using familiar tools to perform their ordinary functions, however. As shown in Figure 3b, grips appropriate for tool use frequently differ in nontrivial ways from those chosen solely on the basis of perceived structural information. In addition to mechanisms for online sensorimotor transformations, these actions are influenced by semantic knowledge of the objects' functional properties, the goals it can be used to accomplish, and the user's specific intentions on that occasion. The critical question for understanding these behaviors is how semantic knowledge interacts with and influences sensorimotor representations during the planning and performance of these skills (Johnson-Frey, 2003b). As detailed below, available evidence suggests that the human left cerebral hemisphere may be specialized for constructing representations of these meaningful actions (Johnson-Frey, 2004; Johnson-Frey *et al.*, 2005c; Lewis, 2006; Rumiati *et al.*, 2004).

The majority of what is known about the neural bases of skilled tool use in humans derives primarily from over a century of studies of apraxic patients who have difficulties with praxis skills that cannot be explained in terms of more elementary sensory or motor deficits. A review of this vast literature is well beyond the scope of this article. I will focus instead on findings from recent human neuroimaging studies that have attempted to delineate the neural substrates involved in planning and/or producing familiar tool use actions in the healthy brain.

4.23.3.1 Functional Neuroimaging Investigations of Human Tool Use

As summarized in a review (Johnson-Frey, 2004), the available data from functional neuroimaging studies have two points of convergence with the apraxia literature: First, while systems involved in sensorimotor control are organized in a largely contralateral fashion, the vast majority of evidence shows that mechanisms involved in representing familiar tool use actions are lateralized to the left cerebral hemisphere. Maximal lesion overlap in these patients is found in the left cerebral hemisphere in parietal regions (within and adjacent to the IPs, including angular and supramarginal gyri and ventral SPL) and/or the middle frontal gyrus (GFm) (Haaland *et al.*, 2000).

Second, within this left-lateralized system, mechanisms involved in representing conceptual knowledge about tools and their functions appear to be relatively independent yet interactive with those areas supporting the manual skills involved in their usage.

4.23.3.2 Conceptual-Level Representations

Functional neuroimaging studies show that naming tools selectively activates posterior-left middle temporal gyrus (MTG) (Martin *et al.*, 1996), an area that is also engaged when subjects generate action words (Martin *et al.*, 1995) or answer questions about tools (Chao *et al.*, 1999). Likewise, Damasio and colleagues report activation in this region when subjects identify actions or spatial relations performed with or without a tool (Damasio *et al.*, 2001). On the basis of its proximity to motion-processing centers (putative MT/MST) and its selectivity for manipulable versus nonmanipuable artifacts, it has been suggested that activations in left MTG may be involved in representing nonbiological motions associated with tool use (Heilman *et al.*, 1997; Chao *et al.*, 1999; Beauchamp *et al.*, 2002).

In addition to left posterior temporal cortex, functional neuroimaging studies consistently demonstrate that identification of tools and conceptualization of associated actions activate left frontal and anterior parietal areas. As introduced above, PMv is involved in visuomotor transformations for grasping and manipulating objects in both macaques (Rizzolatti *et al.*, 2002) and humans (Binkofski *et al.*, 1999). In the left hemisphere, this region is also activated when naming (Martin *et al.*, 1996; Chao and Martin, 2000) and viewing (Chao and Martin, 2000) tools, while a larger region including left GFm is activated when identifying the actions with which tools are associated (Grabowski *et al.*, 1998). Activations of left posterior parietal cortex (aIPS/supramarginal gyrus) are also frequently observed in association with these tasks (Martin *et al.*, 1996; Chao and Martin, 2000; Damasio *et al.*, 2001; Kellenbach *et al.*, 2003).

4.23.3.3 Production-Level Representations

In contrast to the sizable literature on conceptual-level action representations, relatively few studies have employed functional neuroimaging techniques to investigate mechanisms involved in organizing and/or producing tool use skills. When activations associated with complex yet meaningless finger and limb movements are removed, pantomiming tool use gestures with either hand activates left posterior parietal cortex in and around the IPS and dorsal lateral premotor cortex (Moll *et al.*, 2000). A similar pattern is also present when tool use pantomimes involving either hand are contrasted with repetitive

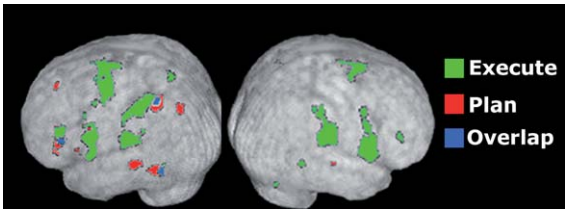

Figure 4 Human cortical regions associated with planning and executing tool use actions. When preparing to pantomime how familiar tools are used, areas of the left frontal, temporal, and parietal cortices are activated, regardless of the hand involved (red). During the execution of tool use pantomimes with either hand, additional regions of frontal and parietal cortex are engaged in both cerebral hemispheres (green). There is modest overlap between regions that are active during both planning and execution (blue). Reproduced from Johnson-Frey, S. H., Newman-Norlund, R., and Grafton, S. T. 2005a. A distributed left hemisphere network active during planning of everyday tool use skills. *Cereb. Cortex* 15, 681–695, by permission of Oxford University Press.

finger movements (Choi *et al.*, 2001), and when gestures are made as though the limb itself is the object (Ohgami *et al.*, 2004). A recent PET study of tool use pantomime found activations of left dorsolateral prefrontal cortex (DLFPC), inferior frontal cortex, and supramarginal gyrus when controlling for effects related to lexical and semantic processing (Rumiati *et al.*, 2004). Interestingly, none of these experiments observed activations in left GFm, as would be expected given the lesion analysis data introduced above. This is also true for results of two recent fMRI studies that used event-related designs to distinguish regions involved in planning (i.e., identifying, retrieving, and preparing actions associated with a familiar tool's usages) versus executing tool use gestures with the dominant right and nondominant left hands (Johnson-Frey *et al.*, 2005b). As illustrated in Figure 4, planning tool use actions for either limb activates a distributed network in the left cerebral hemisphere consisting of areas within the IPL (supramarginal and angular gyri) and frontal cortex (inferior frontal and ventral premotor cortices as well as DLFPC). These studies also detected activation during action planning within the ventral visual pathway (posterior superior temporal sulcus along with proximal regions of the MTG and superior temporal gyri). Involvement of these latter areas likely reflects activation of conceptual-level representations.

An important and unresolved question concerns whether these distributed cortical networks are specific to behaviors involving tools (i.e., transitive skills) or whether they are more generally involved in all manner of familiar skills, including those that do not involve objects (i.e., intransitive skills). In apraxia transitive gestures are often more substantially affected than intransitive gestures (Roy *et al.*, 1991; Rapcsak *et al.*, 1993) and both left and right brain-injured patients may show difficulties pantomiming and/or imitating intransitive actions (Heath *et al.*, 2001). There have been few studies investigating neural bases of familiar intransitive gestures to date, and unfortunately none that has directly contrasted familiar transitive and intransitive skills.

Although the focus has largely been on cortical contributions to skilled actions, both the apraxia (Pramstaller and Marsden, 1996; Hanna-Pladdy *et al.*, 2001) and neuroimaging literatures suggest involvement of subcortical structures. Imamizu and colleagues have demonstrated involvement of the posterior superior cerebellar fissure in learning to control computer mice with novel input–output mappings (Imamizu *et al.*, 2000, 2003).

4.23.4 Conclusions

In summary, results from studies of nonhuman primates indicate the existence of parallel parietofrontal networks involved in the organization and/or execution of prehensile behaviors. Functional neuroimaging studies suggest that aspects of this organization are preserved in the human brain. Findings in macaques demonstrate that use of a tool to extend reach induces an expansion of RFs within parietal visuotactile neurons. Specifically, representations of peripersonal space are remapped to include areas accessible with the tool. Along with complementary results in the human literature, these findings suggest that some forms of tool use may arise from relatively rapid, experience-dependent changes in existing sensorimotor circuits. More complex forms of tool use, that constitute a large potion of the human repertoire, demand input from semantic as well as sensorimotor representations, however. Data from apraxic patients and results of recent functional neuroimaging investigations of healthy adults converge on two points. First, the human left cerebral hemisphere is dominant for the representation of complex tool use actions. Second, although both are necessary for complex tool use behaviors, there appears to be a separation between conceptual and production-level representations.

The story of how complex manual actions are acquired and represented in the primate brain is only now beginning to unfold. Future efforts should be directed at identifying both the similarities in functional organization across primate species, as well as differences that may account for unique, species-specific behaviors.

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4.24 Hand Use and the Evolution of Posterior Parietal Cortex in Primates

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Glossary

3b	Brodmann's area 3b, synonymous with S1.
AIP	Anterior intraparietal area.
area 5	Brodmann's area 5.
area 7	Brodmann's area 7.
haptic shape perception	Tactile sampling of a shape.
LIP	Lateral intraparietal area.
MIP	Medial intraparietal area.
multimodal	Neurons that respond to more than one type of sensory stimulus.
PRR	Parietal reach region.
PV	Parietal ventral area.
retinotopy	An ordered representation of the visual field in areas of the visual cortex.
S1	Primary somatosensory area.
S2	Secondary somatosensory area.
saccade	Rapid eye movements resulting in fixation from one point in the visual field to another.
somatotopy	An ordered representation of the skin surface in areas of the somatosensory cortex.
VIP	Ventral intraparietal area.

4.24.1 Introduction

What distinguishes humans from other primates? The most common answer to this question is language. Humans have a unique ability to communicate, and the organization and connectivity of the human brain reflect this specialty (see Primate Brain Evolution in Phylogenetic Context). We would argue that of equal importance is humans' ability to manipulate their environment with their hands. We are unparalleled in our facility to shape and influence our surroundings. As with the special skills associated with language production and comprehension, the human brain has specific features of organization and connectivity underlying their remarkable manual abilities (see Neurological Specializations for Manual Gesture and Tool Use in Humans). One region involved with these abilities is the posterior parietal cortex.

The posterior parietal cortex consists of discrete areas that are proposed to perform different functions (Mountcastle *et al.*, 1975; Andersen *et al.*, 1997; Kalaska *et al.*, 1997; Snyder *et al.*, 1997; Graziano *et al.*, 2000; Gregoriou and Savaki, 2001). In macaque monkeys, the posterior parietal cortex has recently been subdivided into a number of cortical fields, including areas 5, 7 anterior intraparietal area (AIP), lateral intraparietal area (LIP), medial intraparietal area (MIP), ventral intraparietal area (VIP), and parietal reach region (PRR; Anderson *et al.*, 1997; Andersen and Buneo, 2002). Most studies of posterior parietal cortex in human and nonhuman primates examine its visual functions; however, this region also appears to be involved in visuospatial processing, including monitoring limb location during visually guided reaching (Mountcastle *et al.*, 1975; Lacquaniti *et al.*, 1995; Johnson *et al.*, 1996; Gregoriou and Savaki, 2001; Buneo *et al.*, 2002), and grasping (Taira *et al.*, 1990; Sakata *et al.*, 1995, 1998), converting sensory locations into motor coordinates for intentional movement (Andersen *et al.*, 1985; Battaglia-Mayer *et al.*, 2000), and perceiving the movements of the body in extrapersonal space (Andersen *et al.*, 1997; Snyder *et al.*, 1997). Further, the posterior parietal cortex is involved in saccadic eye movements (Colby *et al.*, 1996; Snyder *et al.*, 2000; see The Role of Vision in the Origin and Evolution of Primates), and the processing of visual and tactile shape and orientation information (Murata *et al.*, 2000; Taira *et al.*, 2000; Tsutsui *et al.*, 2001, 2002; see The Evolution of Sensory and

Motor Systems in Primates). There is also evidence that human posterior parietal cortex plays a role in shape perception (Faillenot *et al.*, 1997; Binkofski *et al.*, 1999a, 1999b; Kourtzi and Kanwisher, 2000; Amedi *et al.*, 2001; Bodegard *et al.*, 2001; Grefkes *et al.*, 2002). Thus, it appears that much of the region has evolved in primates as a consequence of, and for the generation of, specialized hand use.

While great strides have been made in understanding the organization and function of the posterior parietal cortex, there are difficulties associated with the study of this region. First, in the macaque monkey the designation of the location of various cortical fields is not consistent across laboratories (Cavada, 2001). Traditionally cortical fields are defined using several criteria. A cortical field is characterized by: (1) architectonic distinctiveness; (2) unique neural response properties; (3) unique connectivity; (4) a complete representation of the receptor surface; and (5) specific deficits after removal (Kaas, 1983). While criteria for defining a cortical field work well for primary fields such as 3b (S1) or S2 and parietal ventral (PV), they are not as useful for defining fields in the posterior parietal cortex. Traditional staining techniques are not adequate for distinguishing architectural boundaries of fields in posterior parietal cortex, and neural response properties are complex and often multimodal. Without clear anatomically defined borders, patterns of connectivity are difficult to determine, and fields appear to lack obvious visuo- or somatotopic organization, or even a complete representation of the receptor array (e.g., Colby and Duhamel, 1991).

These problems are compounded in humans by the striking anatomical differences between macaque monkey and human posterior parietal cortex. In the macaque, the intraparietal sulcus (IPS) is an easily identified relatively shallow sulcus just caudal to the central sulcus that runs in a mediolateral direction (Figure 1). In the human, the sulcal anatomy is quite different, with the bulk of the IPS running in a rostrocaudal direction, often with additional sulci in this region. While attempts have been made to draw parallels between work in macaques and humans (see Culham and Kanwisher, 2001, for review), comparisons are tentative at best. Further, human hand use diverges dramatically from that of macaque monkey hand use. Thus, the second difficulty in determining the organization and function of this region in humans comes from the lack of an animal model.

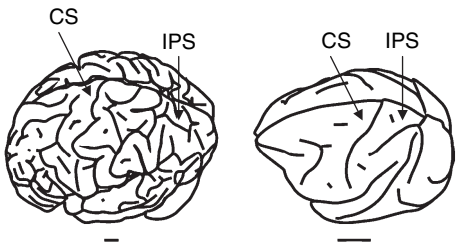

Figure 1 Comparison of human (left) and macaque brains. Note the complexity of the region surrounding the IPS. CS, central sulcus. Scale bars: 1 cm.

4.24.2 Posterior Parietal Area 5

Because of the difficulties associated with identifying particular posterior parietal cortical fields in humans, we began our studies of this region by examining area 5. Several consistent features of area 5 have emerged from the monkey literature regarding its organization and receptive field characteristics. Area 5 is dominated by the representation of the hand and forelimb; neurons in area 5 have contralateral, ipsilateral, and bilateral receptive fields (particularly on the hand and forelimb), and most neurons respond to stimulation of deep receptors of the skin and joints (Mountcastle *et al.*, 1975; Pons *et al.*, 1985; Iwamura *et al.*, 1994, 2002; Taoka *et al.*, 1998, 2000; Iwamura, 2000). Single-unit studies in macaque monkeys indicate that area 5 is involved in intention of movement (Snyder *et al.*, 1997; Debowy *et al.*, 2001), and the generation of body or shoulder rather than eye-centered coordinates for reaching (Ferraina and Bianchi, 1994; Lacquaniti *et al.*, 1995; see Wise *et al.*, 1997, for review). While this field has traditionally been considered a somatosensory area, our work in human (Disbrow *et al.*, 2001) and nonhuman (Padberg *et al.*, 2004, 2005) primates indicates that neurons in area 5 respond to visual stimulation as well.

In our study of titi monkeys we used multiunit electrophysiological recording techniques in an anesthetized preparation to make several interesting observations about area 5 and the surrounding cortex (Padberg *et al.*, 2005). First, the field was dominated by the representation of the hand and forelimb. Second, neurons in area 5 respond to both deep somatic and visual stimulation. Finally, unlike anterior somatosensory fields in which the hand representation is mostly acallosal (Killackey *et al.*, 1983), area 5 receives interhemispheric input in the expected location of the hand representation. Dense label was also observed in area 7, S2/PV, and moderate label was observed in motor, premotor, extrastriate, and cingulate cortex.

Similarly, in preliminary studies of macaque monkeys, we examined responses to somatosensory and visual stimulation in area 5. As in the titi monkey we found that this region was dominated by a somatosensory representation of the hand and forelimb, and

that neurons at several sites within this representation also responded to visual stimulation (Figure 2). Injections placed into the forelimb representation of area 5 revealed connections with anterior parietal and lateral somatosensory areas, additional regions of posterior parietal cortex, as well as M1, supplementary motor area (SMA), and anterior cingulate (Figure 3). These mapping and connectional data indicate that area 5 is involved in integrating visual and somatosensory inputs specifically relating to the hand, and may be generating a motor output for visually guided reaching behaviors.

Based on these findings, we designed a simple functional magnetic resonance imaging (fMRI) experiment to identify area 5 in humans (Disbrow *et al.*, 2001). Stimuli were a moving tactile stimulus applied to the hand, foot, or face, and a visual flow field. Tactile and visual stimuli were presented both individually and simultaneously. When stimuli were presented individually, only primary fields were active; however, simultaneous presentation of visual and tactile stimulation resulted in activation of cortex caudal to anterior parietal somatosensory fields (Figure 4). This field, which we called area 5, was roughly somatotopically organized, and dominated by the hand representation as in the macaque monkey. We used this field as a landmark, and extended our examination of posterior parietal cortex to include more complex stimuli.

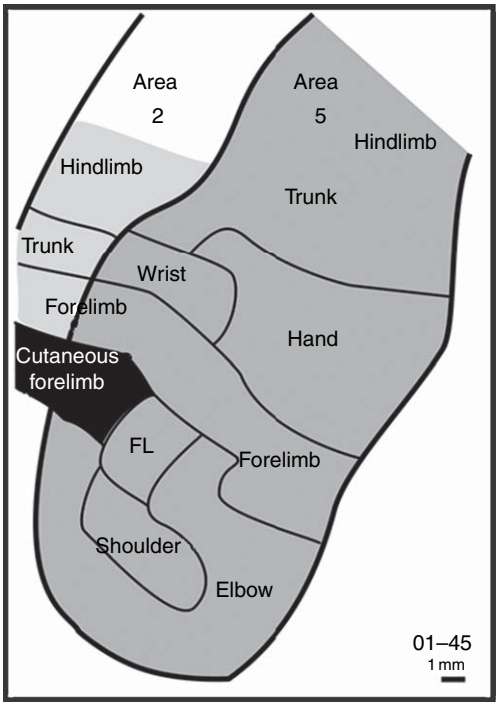

Figure 2 Summary of an electrophysiological map. Area 2 was observed to contain neurons that responded to stimulation of deep receptors, and a zone of cortex containing neurons that responded to cutaneous somatosensory stimulation was observed within both areas 2 and 5. Neurons across a large extent of area 5 were observed to respond to stimulation of deep receptors of the hand and forelimb (FL). Additionally, many of the sites surveyed in area 5 were observed to contain neurons that responded to both deep somatosensory and visual stimulation. Adapted from Disbrow, E. A., Murray, S. O., Roberts, T. P., Litinas, E. D., and Krubitzer, L. A. 2001. Sensory integration in human posterior parietal area 5. *Soc. Neurosci. Abstr.* 511.26.

4.24.3 Effector Specific Network

In posterior parietal cortex cortical field organization based on somatotopy or retinotopy is inadequate. However, there is emerging evidence from studies of the macaque monkey that cortex is organized based on effector – the part of the body performing a movement (for review, see Andersen and Buneo, 2002). In order to identify areas of posterior parietal cortex selective for movements of the eyes, arms, and hands, the same group of subjects also performed a

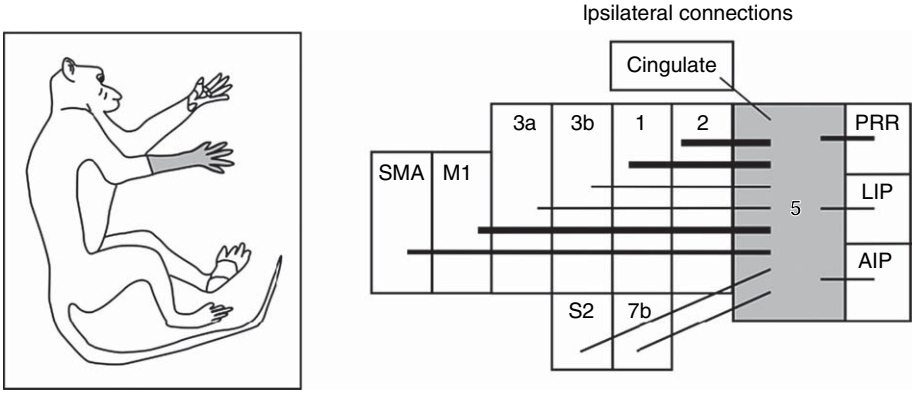

Figure 3 A summary of connections in case 01–45 resulting from injections in the distal forelimb representation in area 5. Labeled cells resulting from injections in area 5 were widespread and were found to be very dense in areas M1, 1, and 2. The PRR and SMA were observed to have dense label. Areas 3a, 3b, anterior cingulate cortex, S2, 7b, and extrastriate cortex, including LIP and AIP, were observed to have moderate label.

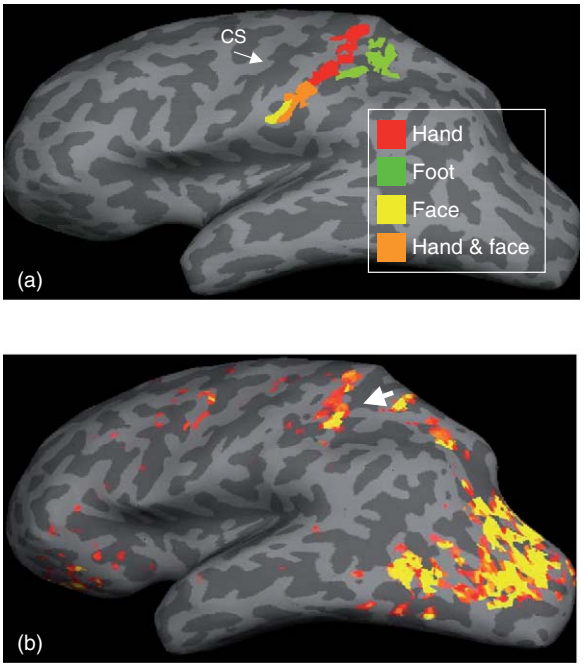

Figure 4 Human area 5. a, Area 5 was only active during a combination of visual and tactile stimulation. Activation from tactile stimulation alone was rostral to area 5 on the central sulcus (CS: arrow) and is not shown in this figure. Area 5 was roughly somatotopically organized, dominated by the representation of the hand (red and orange) as in other primates. b, Activation from a single subject performing a visually guided reaching and grasping task, contrasted with a motor control (eyes closed). Note the late activation in the hand representation of area 5 (arrow), as defined in (a). Visual cortex is active because subjects closed their eyes in the control but not the reaching condition. Adding a visual control reduced the volume of area 5 activation (not shown). Images are displayed on inflated brains (rostral is to the right). Dark gray indicates the location of sulci.

saccade task, a visually guided reaching task, and a haptic shape discrimination task (Disbrow *et al.*, 2001; Hinkley *et al.*, 2004). We hoped to identify a network involved in visually locating an object in space, reaching for and grasping that object, and manually exploring it.

First, we identified a group of cortical fields active during saccadic eye movements. The LIP region is believed to be involved in converting retinotopic visual information to coordinates for oculomotor intention (Mazzoni *et al.*, 1996). Along with LIP, areas of cortex more active during saccades in our subjects also included bilateral regions along the upper bank of the mid-IPS, extending caudally to the parieto-occipital sulcus (Figure 5). We believe that this pattern of activation corresponds to saccade-specific regions identified in monkeys (Duhamel *et al.*, 1992), and its location on a normalized Talairach atlas matches those identified in other human fMRI studies examining the preparation of saccadic eye movements (Connolly *et al.*, 2002; Sereno *et al.*, 2001) in retinotopic space.

Next, we identified fields active during the guidance of a hand toward a visual target. In the macaque monkey the PRR consists of a number of different areas (Snyder *et al.*, 1997), including regions along the MIP portions of area 5 and cortex along the medial wall (7 m, Caminiti *et al.*, 1999). From our human fMRI study, areas more active when subjects are performing a visually-guided reach versus a motor control include cortex within the postcentral sulcus, and cortex along the upper bank of the IPS extending on to the superior parietal lobe at its junction with the parieto-occipital sulcus (Figure 5). Thus we saw unique activation within the PRR region, as well as overlap with activation observed during saccadic eye movements (Table 1).

During conditions where subjects were instructed to manipulate a plastic shape (haptic discrimination task) versus a nonshape (clay), the junction of the postcentral sulcus and lower bank of the IPS were bilaterally active, in a region of cortex other investigators have labeled as human AIP (Binkofski *et al.*, 1998; Culham *et al.*, 2003). In these human fMRI studies, this portion of posterior parietal cortex is more active during object exploration and identification. In macaque monkeys, neurons in the anterior regions of the IPS (AIP) are more active prior to the formation of the hand to interact with the shape of a given object (Sakata *et al.*, 1998) and are found to be selective for the visual and kinesthetic (motor) information from a specific shape (Murata *et al.*, 2000). In addition to AIP, the medial portion of the superior parietal lobe was also active bilaterally. This region of human cortex is also active during the discrimination of the three-dimensional features of an object based on both visual and somatosensory information (Shikata *et al.*, 2003). Similar areas in macaque monkey cortex caudal to AIP (a caudal intraparietal area; Shikata *et al.*, 1996 or posterior intraparietal area; Colby *et al.*, 1988) respond selectively to the orientation of a surface in three dimensions for visual stimuli (Taira *et al.*, 2000). This information is then transferred to anterior intraparietal fields, in order to guide the formation of the hand around an object, a process known as prehension (Gardner, 1998).

4.24.4 The Evolution of Posterior Parietal Cortex

Primates are unique in that they have an expanded posterior parietal cortex compared to other mammals, and our data support the contention that primate brain size and complexity increase in proportion to the ratio of brain to body size, for example, from Old World monkeys to humans (Kaas, 2004). Although the

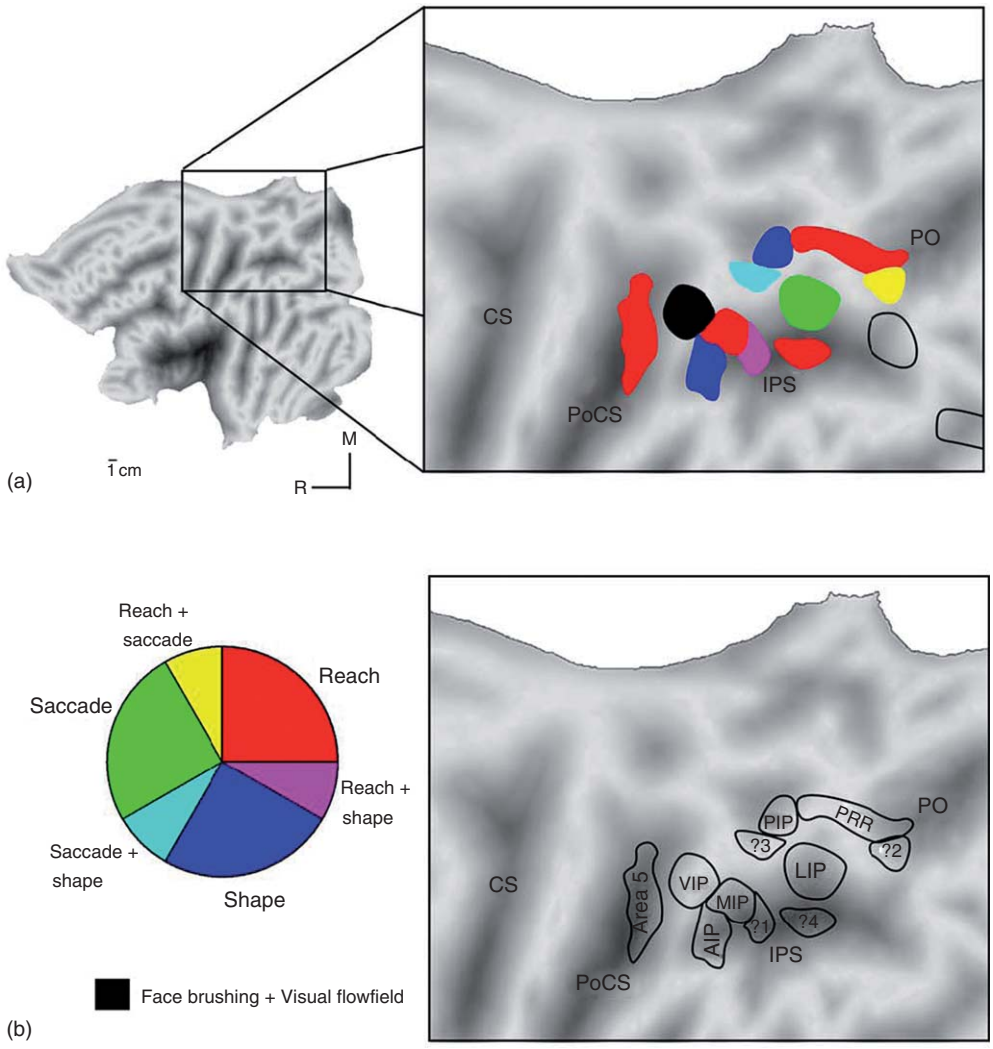

Figure 5 a, Schematic of a group analysis of data from 12 subjects on a flattened brain. Data from three stimulus conditions are overlayed (inset): (1) saccadic eye movements vs. fixation; (2) visually guided reaching and grasping vs. motor control; and (3) haptic exploration of object shape vs. manipulation of clay. b, Location of putative homologues of several cortical fields described in the macaque monkey. Note that there are four areas of activation that do not correspond in location or activation pattern to any field described in the macaque.

Table 1 Pattern of activation for saccade reach and shape tasks in various human cortical fields and their putative homologues

Task	*Caudal ISPL*	*Medial ubIPS*	*AIPS+ PoCS*	*Caudal PoCS*	*Caudal mSPL*	*Anterior ubIPS*	*PO + SPL*	*Lateral SPL*	*Medial SPL*	*Fundal IPS*
Saccade	■	■					■	■		
Reach		■		■	■	■	■			■
Shape		■	■					■	■	
Flowfield + face				■						
Putative homologue	LIP	?1	AIP	VIP	PRR	MIP	?2	?3	PIP	?4

AIPS, anterior intraparietal sulcus; mSPL, medial superior parietal lobule; PO, parietal occipital; PoCS, posterior central sulcus; ubIPS, upper bank, Intraparietal sulcus.

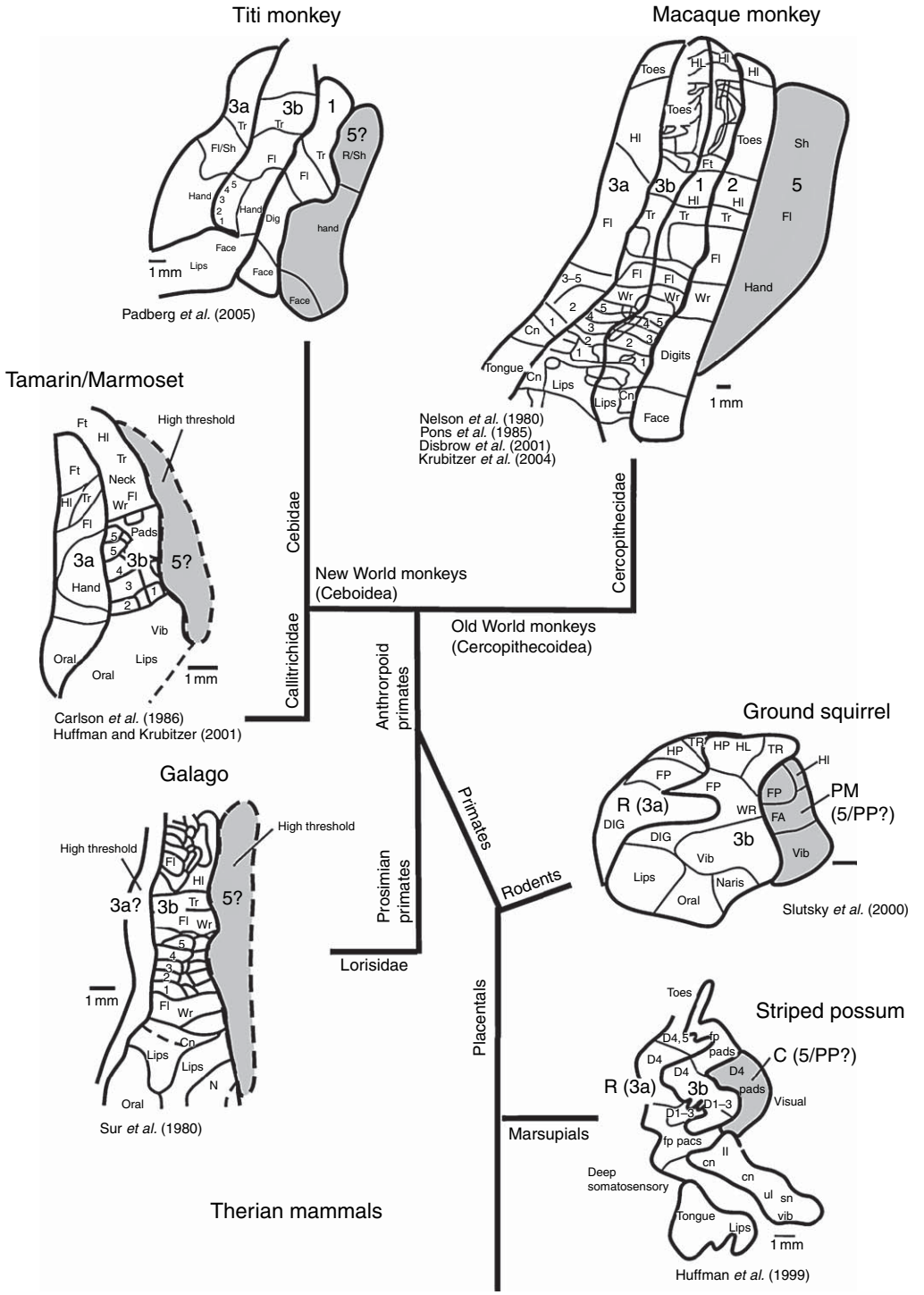

Figure 6 A simplified cladogram depicting the phylogenetic relationship of primates and other mammals, and the organization of anterior and posterior parietal cortex in several species. Comparative data from these and other mammals indicate that early therian mammals possessed a primary somatosensory area (3b or S1), a rostral field (3a or R), and a caudal area (5 or PP, gray). Some species such as simian primates have evolved a low-threshold cutaneous (or deep) representation just caudal to 3b, termed area 1. Since area 1 has not been identified in other mammals, or even in prosimian primates, it is likely that area 1 evolved after the simian and prosimian divergence. Area 2 has only been identified in macaque monkeys. Comparisons across mammals indicate that areas 3a, 3b, and 5 are evolutionarily old fields, and that new, unimodal somatosensory fields such as areas 1 and 2 evolved later in some lineages, and are interspersed between existing fields (i.e., are not added hierarchically). We propose that areas 1 and 2 evolved with the modified morphology of the hand in anthropoid primates, and that older, retained fields, such as area 5, were modified both functionally and connectionally for sophisticated hand use. Phylogenetic relationships come from Murphy *et al.* (2001) and Eisenberg (1981). Cortical organization of different species depicted here is modified from studies listed for each species. Adapted from Padberg, J., Disbrow, E., and Krubitzer, L. 2005. The organization and connections of anterior and posterior parietal cortex in titi monkeys: Do New World monkeys have an area 2? *Cereb. Cortex* 15(12): 1938–1963.

relative locations of the functionally defined areas on the human cortical sheet are similar to the patterns seen in the macaque, additional regions in parietal cortex are active outside our putative homologues and are likely specializations of human neocortex (Figure 5). Unique areas of human posterior parietal cortex have also been described using visual stimuli (Vanduffel *et al.*, 2002).

The relationship to the macaque data can be determined through patterns of overlap of activation as well. For example, at the caudal end of the superior parietal lobe, reach-selective and saccade-selective areas of activation exhibit a degree of overlap similar to the border between PRR and LIP in the macaque monkey (Calton *et al.*, 2002). Conversely, unique patterns of overlap in our data set which do not fit existing macaque literature might represent regions of cortex independently derived in humans. For example, a region of the lateral portion of the superior parietal lobe was active during both saccadic eye movements and haptic shape exploration – a pattern which has not been described in macaques. While we leave open the possibility that such an area selective for both complex movements of the hands and eyes has yet to be identified in nonhuman primates, such novel regions might provide the neural substrate for higher-order functions that would require fast sensorimotor transforms, such as eye–hand coordination.

In contrast, a region resembling area 5, in which neurons respond to deep somatic stimulation and often visual stimulation, has been identified in a variety of mammals (Figure 6), such as squirrels (Slutsky *et al.*, 2000), insectivores (Krubitzer *et al.*, 1997), and marsupials such as the striped possum (Huffman *et al.*, 1999) and the flying fox (Krubitzer and Calford, 1992), as well as New and Old World monkeys and humans. Thus, area 5 appears to be part of a common plan of organization in all primates, and in all mammals, and were interleaved between area 5 and V3a in the human. These new areas may be related to the evolution of the hand and complex cortical processing networks associated with hand use.

While area 5 may be a retained or homologous field in mammals, the addition of new areas and new connections likely promotes new functions of retained cortical fields. Our work in the macaque and titi monkey indicates that area 5 has a unique pattern of connections providing the anatomical substrate for its role in the motor aspects of visually guided reaching, the motivational state of reaching, as well as its role in intermanual transfer of information across hemispheres necessary for bilateral coordination of the hands (Padberg *et al.*, 2004, 2005). Thus, area 5 in squirrels and flying foxes may be homologous to area 5 in primates, but not strictly analogous. Indeed, much like the magnification of behaviorally relevant body parts in area 3b, in area 5 these representations and associated functions appear to magnify to the extreme in particular lineages. In primates, the hand representation dominates area 5 and much of posterior parietal cortex appears to be specialized for hand use.

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4.25 Premotor Cortex and the Mirror Neuron Hypothesis for the Evolution of Language

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Glossary

language-ready brain	A species (only humans, as far as we know) has a language-ready brain if it equips infants of the species to learn languages akin to that of modern humans; it is controversial whether such a brain includes an innate specification of general structures of syntax.
mirror neuron	A neuron active during both the execution of an action and the observation of a similar action.
mirror system	A neural region active during both the execution of a class of actions and the observation of actions from that class. Note that a mirror system need not contain mirror neurons, though it is generally assumed that it will.
parity property of communication	The meaning of a message intended by the sender is approximately the meaning extracted from the message by the receiver.
protolanguage	A hypothetical form of communication employed by hominids before they had language in its modern sense; unlike the vocalizations of nonhuman primates, it supported the open-ended creation of new symbols but did not employ the syntax and compositional semantics of full human languages.

4.25.1 Parity and the Language-Ready Brain

Any normal human child reared in a modern human society will acquire language. In this sense, the human brain and body is language-ready in a way in which the brains of other species are not. Some would argue that the basic structures of language must be innate, forming a universal grammar encoded in the human genome, so that the child need simply hear a few sentences to set the parameter for each key feature of the grammar of his first language. Against this, others have argued that the modern child has both a rich set of language stimuli linked to action and perception and powerful learning mechanisms, so that the child can learn these key features of the grammar from its social interactions. Of course, this is consistent with the view that the different experiences of a modern human and early *Homo sapiens* means that developmental self-organization will yield adult brains of somewhat different structure.

The parity requirement for language in humans – that what counts for the speaker must count approximately the same for the hearer – roots language (and communication more generally) in a social context in which speaker and hearer are motivated to communicate with each other. Since normal face-to-face speech involves manual and facial as well as vocal gestures, and because signed languages are fully developed human languages, the speaker and hearer may use hand and face gestures rather than vocal gestures. This motivates a study of the evolution of the language-ready brain not restricted to speech mechanisms (see The Evolution of Language Systems in the Human Brain).

4.25.2 The Original Mirror System Hypothesis

The system of the macaque brain for visuomotor control of grasping has its premotor outpost in an area called F5 (see Figure 1, left), which contains a

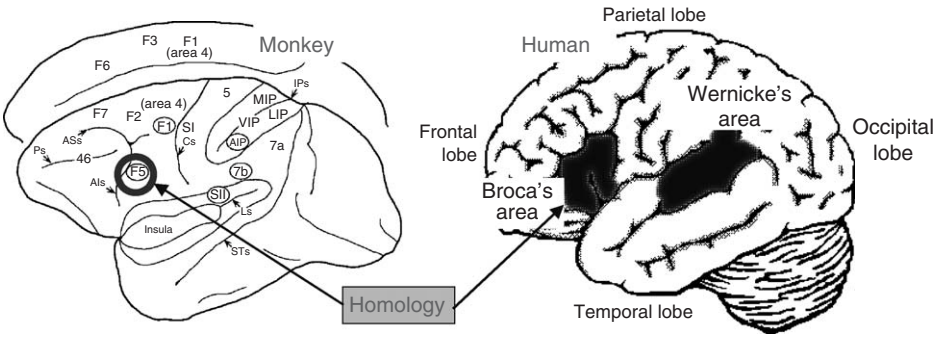

Figure 1 A comparative side view of the monkey brain (left) and human brain (right), not to scale. The left view emphasizes premotor area F5; the right view emphasizes Broca's area and Wernicke's area, considered crucial for language processing. F5 and Broca's area are considered homologous.

set of neurons, called mirror neurons, such that each mirror neuron is active not only when the monkey executes a specific grasp, but also when the monkey observes a human or other monkey execute a more or less similar grasp (Rizzolatti *et al.*, 1996). Thus, macaque F5 contains a mirror system for grasping that employs a common neural code for executed and observed manual actions. The homologous region of the human brain is in or near Broca's area (the posterior part of the inferior frontal gyrus; Figure 1), traditionally thought of as a speech area, but which has been shown by brain imaging studies to be active when humans both execute and observe grasps. It is posited that the mirror system for grasping was also present in the common ancestor of humans and monkeys (perhaps 20 Mya) and that of humans and chimpanzees (perhaps 5 Mya). These findings ground the mirror system hypothesis (MSH; Rizzolatti and Arbib, 1998): the parity requirement for language in humans is met because Broca's area evolved atop the mirror system for grasping which provides the capacity to generate and recognize a set of actions.

Rizzolatti and Arbib posited three stages in the evolutionary progression:

- *R&A1*. Use of the mirror system for recognizing praxic grasping actions of others.
- *R&A2*. Extension of the mirror system to allow the generation and recognition of manual gestures to serve an open system of communication, yielding a proto-Broca's area.
- *R&A3*. Collateralization to allow the system to incorporate vocal as well as manual gestures, yielding a true Broca's area.

Barrett *et al.* (2005) summarize functional and structural evidence supporting differential localization of mechanisms for limb praxis, speech and language, and emotional communication, showing further that in most humans, the left hemisphere may be dominant in the control of vocalization associated with propositional speech, but the right hemisphere often controls vocalization associated with emotional prosody. Such data must be taken into account in refining the MSH, but in no way contradict it. As general principles of cortical evolution, one may state that increasing complexity of behavior is paralleled by increases in the overall size and number of functional subdivisions of neocortex and the complexity of internal organization of the subdivisions, and reduplication of circuitry may form the basis for differential evolution of copies of a given system, with differing connectivities to serve a variety of functions (Kaas, 1993; Striedter, 2004).

Recent work (see Arbib, 2005, for a review and commentaries on current controversies) has elaborated the MSH in two main ways: by refining the above three stages and by extending the anatomy of the mirror system. We consider these in turn.

4.25.3 Elaborating the Stages for the MSH

Arbib (2005) extended the evolutionary progression from three to seven stages:

- *S1*. Cortical control of hand movements.
- *S2*. A mirror system for grasping shared with the common ancestor of human and monkey.
- *S3*. A simple imitation system for grasping shared with common ancestor of human and chimpanzee.
- *S4*. A complex imitation system for grasping.
- *S5*. Protosign, a manual-based communication system, breaking through the fixed repertoire of primate vocalizations to yield an open repertoire.
- *S6*. Protospeech, resulting as protosign mechanisms evolved to control a vocal apparatus of increasing flexibility.
- *S7*. Language – the development of syntax and compositional semantics.

The argument for the transitions from S2 to S3 and on to S4, and their suggested timing, is given by:

1. The claim (Visalberghi and Fragaszy, 2002) that imitation plays a major role in learning by human children and a very limited role, if any (the topic is open to debate), in social learning in monkeys.
2. The observation (Myowa-Yamakoshi and Matsuzawa, 1999) that in a laboratory setting, chimpanzees typically took 12 trials to learn to 'imitate' a behavior, and in doing so paid more attention to where the manipulated object was being directed, rather than the actual movements of the demonstrator.
3. Humans, however, are capable of complex imitation. This has two parts: the ability to perceive that a novel action may be approximated by a composite of known actions associated with appropriate subgoals and the ability to employ this perception to perform an approximation to the observed action, which may then be refined through practice.

The transition from S4 to S5 is argued to involve two components:

1. The transition from the use of hand movements for praxis to their use for pantomime, with communication being the primary and intended effect of an action rather than a side effect. A grasping movement that is not directed toward a suitable object will not elicit mirror neuron firing in macaque (Umiltá *et al.*, 2001). By contrast, in pantomime, the observer sees the movement in isolation and infers what nonhand movement is being mimicked by the hand movement, and thus the goal of the action. The transition to pantomime does seem to involve a genuine neurological change.
2. The use of conventional gestures to disambiguate pantomime. This may also require further neurological change.

The power of pantomime is that it provides open-ended communication that works without prior instruction or convention (Stokoe, 2001). However, even signs of modern signed language that resemble pantomimes are conventionalized and are thus distinct from pantomimes. Pantomime *per se* is not a form of protolanguage; rather, it provides a rich scaffolding for the emergence of protosign, a manual-based communication system that broke through the fixed repertoire of primate vocalizations to yield an open repertoire of communicative gestures.

It is not claimed that S5 (protosign) was completed before S6 (protospeech) or that protosign attained the status of a full language prior to the emergence of early forms of protospeech. Rather, once hominids had come to employ pantomime and discovered how to use conventional gestures to increasingly augment, ritualize, and in some part replace the use of pantomime, then S6 followed naturally as vocal gestures entered the mix.

MSH implies that the role of F5 in grounding the evolution of a protolanguage system would work just as well if our ancestors had been deaf. However, primates do have a rich auditory system (Ghazanfar and Santos, 2004). The protolanguage perception system could thus build upon the existing auditory mechanisms in the move to derive protospeech. However, it appears that considerable evolution was needed to yield a vocal-motor system able to accommodate an ever-expanding protospeech vocabulary.

The final stage, S7 – the transition from protolanguage to language – is claimed to involve little if any biological evolution, but instead to result from cultural evolution (historical change) in *H. sapiens*. Pinker and Bloom (1990) espouse the view that universal grammar is innate, and argue for its possible evolution through multiple stages, but their definition of universal grammar is incomplete, and some of their stages seem as amenable to cultural as to biological evolution. Nonetheless, the debate over the nature of protolanguage and thus of what constituted the transition to language remains hotly contested.

4.25.4 Extending the Anatomy of the Mirror System

4.25.4.1 Multimodal Mirror Neurons

A particularly controversial aspect of MSH is that it sees protosign, based on the recognition of hand actions, as providing crucial scaffolding for speech. Many would argue that the vocalization system of nonhuman primates provided the complete basis for the evolution of the language-ready brain. The debate is still very much open, but here are some relevant data.

It has been found that macaque F5 mirror neurons are not limited to visual recognition of hand movements. Kohler *et al.* (2002) studied mirror neurons for actions that are accompanied by characteristic sounds (e.g., breaking a peanut in half), and found that a subset of these are activated by the sound of the action as well as sight of the action. However, monkeys do not use their vocal apparatus to mimic the sounds they have heard, thus weakening any case that these neurons might serve vocal

communication but demonstrating that mirror neurons do receive auditory input that could be relevant to the protosign–protospeech transition.

Ferrari *et al.* (2003) studied orofacial motor neurons in F5 and showed that about one-third of them also discharge when the monkey observes another individual performing mouth actions. The majority of these mouth mirror neurons become active during the execution and observation of ingestive actions. However, there are other neurons active during the execution of ingestive actions for which the most effective visual stimuli in triggering them are communicative mouth gestures – one action becomes associated with a whole performance of which one part involves similar movements. The observed communicative actions for such neurons include lip-smacking, lip protrusion, tongue protrusion, teeth-chatter and lips and tongue protrusion – all a long way from the sort of vocalizations that occur in speech.

Of course, these observations do not address the issue of whether the changes that molded F5 for the language-ready brain were driven by a multimodal system for coordinated control of manual and orofacial movements or by changes focused on the vocalization system itself, perhaps even by interactions between the two. Further analysis must address data on comparative analysis of neural pathways underlying vocal control (Jürgens, 2002). Human speech needs input from the ventral premotor and prefrontal cortex, including Broca's area, for motor planning of longer purposeful utterances, as well as input from the supplementary motor area (SMA) and pre-SMA, which give rise to the motor commands executed by the motor cortex. The crucial fact for evolutionary analysis is that SMA has been found to produce vocalization only in humans, not in other mammals. The anterior cingulate gyrus (ACG), in contrast, produces vocalization in non-human mammals, such as the rhesus monkey, squirrel monkey, cat, and bat, but not in humans. Jürgens (2002) argues that this difference in cortical representation might reflect the far greater role that motor learning plays in the vocal behavior of humans as compared to other mammals.

In humans, lesions that invade ACG as well as SMA and/or pre-SMA usually yield initial akinetic mutism followed eventually by transcortical motor aphasia – the patient can again produce well-articulated, grammatically correct sentences but makes very few spontaneous utterances, and cannot provide emotional intonations (Jürgens and von Cramon, 1982). This reminds us that MSH addresses the parity problem – how meaning may be shared between speaker and hearer – but not the problem of what motivates the speaker to say something, and the hearer to listen. It would seem that the anterior cingulate cortex (ACC) plays here the crucial motivational role. Paus (2001) makes it clear that ACC involves many areas and that these have differential involvement in motor, cognitive, and affective functions. In any case, the roles of ACC appears to be complementary to that of the F5–SMA–pre-SMA system and its parietal extension, to which we now turn.

4.25.4.2 Bringing in the Parietal and Temporal Lobes

4.25.4.2.1 A broader view of the macaque mirror system We now look at data implicating parietal areas in the mirror system of macaque. We distinguish the mirror neurons of macaque F5 from the canonical neurons of F5, which are active when the monkey itself performs a grasp but not when the monkey observes that grasp performed by another. The populations of canonical and mirror neurons appear to be spatially segregated in F5 (Rizzolatti and Luppino, 2001). Both sectors receive a strong input from the secondary somatosensory area (SII) and parietal area PF (Brodmann area 7b). In addition, canonical neurons are the selective target of the anterior intraparietal sulcus (AIP). Perrett *et al.* (1990) and Carey *et al.* (1997) found that STSa, in the rostral part of the superior temporal sulcus (STS), has neurons that discharge when the monkey observes such biological actions as walking, turning the head, bending the torso, and moving the arms. Moreover, some of these neurons discharged when the monkey observed goal-directed hand movements, such as grasping objects (Perrett *et al.*, 1990) – though STSa neurons do not seem to discharge during movement execution as distinct from observation. STSa and F5 may be indirectly connected via the inferior parietal area PF (Matelli *et al.*, 1986). About 40% of the visually responsive neurons in PF are active for observation of actions such as holding, placing, reaching, grasping, and bimanual interaction. Moreover, most of these action observation neurons were also active during the execution of actions similar to those for which they were observers, and were thus called PF mirror neurons (Fogassi *et al.*, 1998).

Computational modeling has explored some interactions observed experimentally and hypothesized others to make the system work. The FARS model (Fagg and Arbib, 1998) shows how prefrontal cortex may modulate AIP–F5 interactions in action selection through task-related analysis of object identity by the 'what' path of inferotemporal (IT) cortex, through

working memory, and through selection cues in conditional tasks. The MNS model (Oztop and Arbib, 2002) shows how STS and PF inputs may be associated with activity in F5 canonical neurons to train F5 mirror neurons so that they can respond to data on the actions of others and not just during action execution. These models challenge us to further anatomical and neurophysiological analysis of the macaque, to 'lift' these models to the human brain, to extend the resulting models to models of language performance, and then test the result in part through more rigorous analysis of macaque–human homologies (Arbib and Bota, 2003).

4.25.4.2.2 Imaging the language-performing brain Jürgens (2002) reviewed human imaging studies, showing that spoken utterance of a single word involves activation of the sensorimotor cortex, SMA, insula, and temporal cortex, but that utterance of a longer sequence of syllables or words involves the additional activation of the inferior parietal cortex and Broca's area. However, lesions of Broca's area alone do not cause Broca's aphasia for more than a few days (Levine and Sweet, 1982) but do cause a persistent reduction in verbal fluency. Imaging studies have demonstrated that Broca's area has the mirrorish property of being active not only during production of speech, but also during listening to speech (Price *et al.*, 1996).

Lesions in the inferior parietal cortex also affect speech, yielding phonemic substitutions (paraphasia) and neologisms (Cappa *et al.*, 1981). Electrical stimulation of the inferior parietal cortex during ongoing speech interrupts speech or causes phonemic errors. On this basis, Jürgens (2002) suggests that inferior parietal cortex employs proprioceptive feedback information from the articulatory organs to transform word representations stored in Wernicke's area into commands that can be used by the motor cortex to control verbal articulation.

4.25.4.2.3 The riddle of Wernicke's area Given that the F5 mirror neurons are part of a larger network that includes parietal and temporal cortex, one must consider the extension to MSH to include the hypothesis that the evolutionary changes that lifted the ancestral F5 homologue to yield human Broca's area also lifted other regions to yield Wernicke's area and other areas that support language in the human brain. Arbib and Bota (2003) sought to integrate MSH with the account of Aboitiz and García (1997) in expanding the account of relevant macaque–human homologies. Aboitiz and García (1997) hypothesize that multimodal concepts in Wernicke's area are mapped into phonological sequences as follows: the system of long temporoparietal–prefrontal connections serves to integrate sensory and mnemonic information from the temporoparietal lobes with the organization of behavior, both short and long term, by the frontal systems. They further hypothesize that selective pressure to learn complex vocalizations through imitation and repeated practice was a key aspect in establishing a phonological working memory system. On this view, a prefrontal system in which information from other sensory modalities was integrated and coordinated with the representation of complex vocalizations was developed concomitantly.

Since frontal projections from macaque area Tpt (≈human Wernicke's region) do not terminate massively in macaque areas 44–45 (≈human Broca's area), Aboitiz and García (1997) propose that in human evolution, area Tpt may have become increasingly connected with inferoparietal regions such as the supramarginal gyrus (area 40), thus feeding the latter with auditory information to be used in the phonological loop. Some neurons in area 40 may then project to Broca's region, thus establishing a neuronal circuit for the phonological-rehearsal system. However, macaque data suggest the importance of an alternative route for auditory input from temporal cortex to Broca's area. The anterior superior temporal (aST) region, just caudal to Tpt, projects to the inferior frontal gyrus (IFG) and other parts of the ventrolateral prefrontal cortex (VLPFC). Together, aST and IFG seem to form a 'what' stream for the recognition of auditory objects (Rauschecker, 1998; Romanski *et al.*, 1999), perhaps related to the role of IT cortex in visual object identification mentioned earlier for the FARS model. Moreover, neurons in macaque aST are quite selective for species-specific vocalizations. Rauschecker (2005) argues that aST in nonhuman primates is a precursor of the same region in humans and that nonhuman primate vocalizations are an evolutionary precursor to human speech sounds. However, recognizing speech sounds is just one small component of being language-ready. It is quite consistent with MSH that as protospeech spirally evolves with protosign, it would co-opt available resources rather than invent a new periphery. Following Rauschecker (2005), one might view the projection from aST to IFG via the uncinate fascicle as serving a similar role for auditory objects to that served by IT cortex for the visual system. Wernicke's area might then be seen as providing an input stage to parietal cortex in an auditory dorsal pathway – but further empirical data are needed to test these hypotheses.

4.25.5 Prospects

In conclusion, we see that the MSH appears to provide rich insights into certain of the distinctive characters of the human brain that make it language-ready, with special attention to the parity property of language and the fact that language performance is multimodal, integrating the use of hand, face, and voice. However, it is not a standalone hypothesis, and future research will increasingly move beyond the mirror to meld it with analysis of the evolution of articulation and working memory, to explicate the linkage between neural mechanisms for scene perception and action planning with those for the perception and production of language, as well as to integrate the symbolic and emotional meanings of utterances.

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4.26 Visual Cortex: Evolution of Maps and Mapping

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Glossary

cortical magnification factor (CMF) The distortion in the size of a given representation in cortex, relative to the geometry of corresponding points in the visual field. In primates and some other animals, the largest cortical representation is devoted to the center (fovea) of the visual field, with progressively less cortex devoted to regions increasingly further (more preipheral) in the visual field.

functional magnetic resonance imaging (fMRI) Magnetic resonance imaging is a noninvasive technique in which the subject is placed in a steady magnetic field, that field is systematically perturbed, and images of the brain (or other target) are obtained by systematic analysis of the induced magnetic field changes. 'Functional' MRIs are obtained when analysis is focused on the hemodynamic changes accompanying changes in neural activity.

hemodynamics Generally, circulatory changes. As applied to neuroimaging, this normally includes changes in blood flow, blood volume, oxygenation, or related parameters which accompany local changes in neural activity.

lissencephalic Roughly, smooth-shaped, when applied to the external shape of the cerebral cortex. The term is generally opposed to 'gyrencephalic', referring to a cortical surface which included multiple folds (gyri and sulci).

retinotopy The topographical projection of the retina onto the cortical surface. Because the projection of tretina reflects the geometry of the visual field, the cortical retinotopy is often related directly to the geometry of the visual field.

4.26.1 Introduction

The goal of understanding the evolution of primate visual cortex is by necessity an imaginative extrapolation from extant species, because decomposing brains leave no fossil record (unless one counts endocasts of the skull). Complicating this, any information about visual cortical maps is available from less than 10 of the ~230 living primate species. Among that 10, comprehensive information is limited mostly to macaques, humans, and a few New World species. Even more problematic, the techniques used to map visual cortex differ across different species. Thus, it has been difficult to define a common map of 'primate visual cortex'.

Vision is the primary sense in most primates, and visual cortex is correspondingly enlarged (occupying more than 50% of the cortical surface in some species) and elaborated (into 10–32 distinct areas). Visual cortical areas are typically distinguished from each other based on four main criteria: (1) retinotopy, (2) function, (3) histology, and (4) connections. In well-accepted, lower-tier visual areas such as V1, V2, and MT, unambiguous information is available from most or all of these criteria, and the area borders defined by all criteria agree perfectly with each other. However, in higher-tier (hypothetical) areas, information about area location and function is often based on only one or

two criteria, and this information can be frustratingly subtle and/or incomplete. In the latter cortical regions, uncertainty about the number and location of areas is rife, and controversies abound accordingly. Areas V3 and V3A represent intermediate levels (of information and consensus), in which recent discussions of area boundaries and differentiation appear to be converging on a common primate model.

Retinotopic criteria are rich in quantitative detail, often conserved across different species, and retinotopy can be measured using multiple techniques. Hence, retinotopic criteria tend to be most credible for distinguishing cortical visual areas. Functional differences between areas are also used as a criterion, and the prime example of this is the motion- and direction-selective area 'MT', also known as 'V5'. However, even though this area appears to be present in all primates tested, some functional differences in 'MT' appear to exist across the primate order. Moreover, the degree of motion- and direction selectivity in other visual areas (e.g., V3 relative to V3A) appears to vary across primate species.

Recent studies have begun to clarify the development of different cortical visual areas, and the nature of connections between them. Thus, despite the overwhelming difficulties of studying a dynamic property (evolution) from a limited endpoint perspective, progress continues to be made, and the broad pattern of organizational principles is starting to emerge.

Among primates, evolutionary differences have been documented at many levels of the visual system, from retina through the lateral geniculate nucleus through the visual cortex (for reviews see Kaas, 1997a; Tootell *et al*., 2003; Orban *et al*., 2004; Preuss, 2004a, 2004b; Sereno and Tootell, 2005). However, much of the variation (and similarity) across species is simply unknown. There are ~230 known species of primate (Fleagle, 1999), but most 'primate' visual research has been conducted on just a few of those species, principally humans and macaques. Thus, any conclusions about the evolution of the visual system require enormous extrapolations between the few species mapped, plus assumptions about genetic predecessors who are completely unknown (and currently unknowable).

Another thorny problem in this analysis is that many of the techniques used to study humans are different from those used to study monkeys. Thus, it has been difficult to directly compare data across those two primates. In response to this problem, it has been increasingly common to use a common technique (e.g., histology or fMRI) to directly compare the visual system in humans and nonhuman primates. This can eliminate the need to worry about technical differences; although in practice some minor technical differences can remain in both the functional magnetic resonance (fMRI) approaches (e.g., Tootell *et al*., 2003; Orban *et al*., 2004) and the histology of humans versus macaques (Tootell and Taylor, 1995; Preuss, 2004a, 2004b).

As such evidence has accumulated, it has become correspondingly more challenging to 'keep a blind eye' to the evolutionary diversity between humans, macaques, and other primates. Some studies embrace this evolutionary diversity, presuming that it will help reveal underlying mechanisms of neural development – and progress in this realm has been made (see below). Other studies have focused on visual processing *per se*, in which it is optimal to show that a given feature is 'fundamental' (i.e., evolutionarily conserved, present and unchanged across multiple species). As eloquently emphasized by Preuss (2004a, 2004b) and others, in such cases there are strong pressures to 'emphasize the similarities and downplay the differences' between humans and the monkey 'model'.

Only one conclusion is certain. As more information accumulates, both differences and similarities will be increasingly found in comparisons of the visual system of humans and other primates. This 'glass half empty/full' situation may be frustrating to both 'splitters' and 'lumpers', but it may be the logical expectation from comparisons of primates separated by ~50 million years of independent evolutionary divergence.

4.26.1.1 Cortical Maps

There are many excellent reviews of cortical visual areas, describing how the areas have apparently evolved among different primates, and how scientific data about them have evolved in different laboratories during the past several decades. The reader is referred to those reviews (Felleman and Van Essen, 1991; Kaas, 1997b; Rosa, 1997; Preuss, 2000, 2004a, 2004b; Kaas and Lyon, 2001; Krubitzer and Kahn, 2003a, 2003b; Orban *et al*., 2004; Gattass *et al*., 2005; Rosa and Tweedale, 2005; Sereno and Tootell, 2005) for detail beyond the scope of this review. One way in which such maps have changed during the last decade is that fMRI is increasingly used to reveal the maps in human and nonhuman primates, to supplement data based on classical, invasive mapping methods. That data are incorporated in the present review.

Cortical maps offer a well-constrained system in which to study evolutionary diversity. Basically,

cerebral cortex is a two-dimensional 'sheet' of enormously variable extent (in hominid primates, ranging from ~7 cm^2 in the marmoset to ~275 cm^2 in humans) but relatively constant thickness (1.5–4 mm), including numerous topographical features which can be 'tracked' (assuming homology) across different species. In some cases, these topographic features can even be directly manipulated to test ideas about cortical architecture and development (for reviews see Grove and Fukuchi-Shimogori, 2003; Krubitzer and Kahn, 2003a, 2003b). To more easily visualize and measure topographic variations in the cortical sheet, it has become increasingly customary to study cortex in 'flattened' format, after physical (Tootell *et al.*, 1981; Olavarria and Van Sluyters, 1985; Tootell and Silverman, 1985; Strominger and Woolsey, 1987; Krubitzer and Kaas, 1993; Sincich *et al.*, 2003) or computational (Van Essen *et al.*, 2001; Sereno *et al.*, 1995; Engel *et al.*, 1997; Dale *et al.*, 1999; Fischl *et al.*, 1999) unfolding of the gyri and sulci. Without such flattening transformations, it is difficult to make sense of the cortical maps, except in species with lissencephalic brains.

Vision is the primary sense in primates, and primate visual cortex has been elaborated accordingly. In macaques, visually driven regions occupy more than half the surface area of cortex. Some authors have distinguished as many as 32 distinct visual cortical areas (Felleman and Van Essen, 1991), although other investigators (e.g., Kaas, 1997a; Rosa and Tweedale, 2005) suggest a smaller number (e.g., 12–15). Moreover, the number of distinct visual cortical areas may well vary across primate species.

Human visual cortex is 'physically' larger than macaque visual cortex. However, visual cortex occupies a 'proportionately' smaller part of cortex (~30%) – presumably reflecting the even greater expansion of nonvisual regions in human cortex (reviewed in Kaas, 1997a; Preuss, 2000; Van Essen, 2004; Rosa and Tweedale, 2005). Relative to macaque, the increase in the absolute size of human visual cortex partly reflects increases in the size of each comparable area, for those areas that can be safely assumed to be homologous (e.g., V1, V2, V3, and MT) (see The Evolution of Visual Cortex and Visual Systems, Evolution of Color Vision and Visual Pigments in Invertebrates).

Some areas vary in proportionate size more than others. For instance, visual cortical area V3 is much thinner in macaque than in human, and area V2 is significantly smaller in the nocturnal aotus monkey, compared to the diurnal monkey species which have been tested so far. Though such variations have prompted some teleological speculation, the reasons for those disproportionate increases are ultimately unknown. Part of the absolute increase in human cortical size (relative to macaque cortical size) is due to co-variation with body size (e.g., Preuss, 2000, 2004a, 2004b; Sereno and Tootell, 2005). However, even in primates that are of equal body size (e.g., chimpanzees and humans), cortex is much larger in humans.

Because human cortex has expanded so much relative to cortex in other primates, it has been suggested that additional areas may exist in human cortex (including visual cortex) which have no counterpart in macaques or other monkeys. However, no such human-specific visual cortical area has yet been identified with certainty (but see Denys *et al.*, 2004).

Before proceeding, it may be helpful to discuss one possible source of confusion in these comparative studies, which arises from evolutionary changes in body posture (see Figure 1). The terms 'dorsal' and 'ventral' are coordinates related to the spine, referring to the back and belly, respectively. In macaques and other quadripeds, these axes coincide with 'superior' and 'inferior' axes in brain, respectively – and they have been used interchangeably (and accurately) in that context. However, humans walk upright while facing forward, so brain axes are rotated accordingly, by 90°. In humans, 'dorsal' and 'ventral' axes coincide instead with the caudal/posterior and rostral/anterior brain axes, respectively.

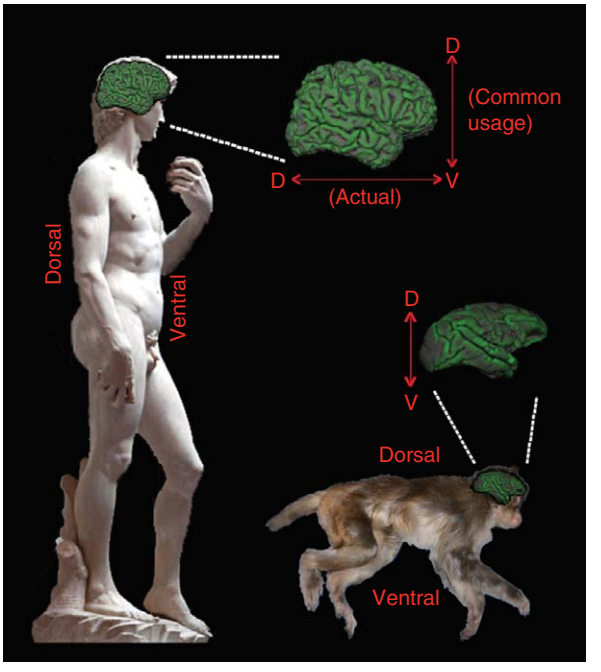

Figure 1 'Dorsal' and 'ventral' axes are rotated in human brain, relative to corresponding axes in macaque brain. D, dorsal; V, ventral.

This discrepancy can lead to confusion if the terms 'dorsal' and 'ventral' are taken literally. For instance, the well-known 'dorsal stream' and the 'ventral stream' (Ungerleider and Mishkin, 1982) are dorsal and ventral to each other in macaque brain, but not in human brain (in which they should be called the 'superior stream' and 'inferior stream'). For the same reason, the names given to some inferiorly located human brain areas are technically incorrect. For example, 'VO' (Liu and Wandell, 2005) is not really located in 'ventral occipital' cortex. In response to this, the neuroimaging field has apparently generalized the terms 'dorsal' and 'ventral' to indicate equivalent brain axes in both humans and quadripeds.

4.26.2 Retinotopy

It is often said that visual cortical areas can be defined based on four different criteria: (1) retinotopy, (2) histological properties, (3) cortical connections, and (4) functional differences (e.g., Felleman and Van Essen, 1991; Kaas, 1997a). Areas that are most certainly defined (e.g., V1, MT, and V2) are based on all four of these criteria, and all the data converge to a common area definition. For instance, area V1 (primary visual cortex) includes one (and only one) complete map of the visual field, thus satisfying the first criterion. V1 is also distinguished from surrounding cortex by a prominent band of higher myelination running throughout layer 4B (the stria of Gennari), which ends abruptly at the border of V1 (i.e., the representation of the vertical meridian based on retinotopy); this satisfies the second criterion. Retinotopically corresponding regions of V1 all project in a point-to-point fashion to V2 and other areas; this satisfies the third criterion. Throughout V1, there are consistent variations in function, some of which are in turn related to laminar and topographic (i.e., cytochrome oxidase blobs) variations within V1; this satisfies the fourth criterion.

However, most cortical areas are based on data from only one or two of these criteria, so that the borders (and even the existence) of these areas are much less certain (e.g., Felleman and Van Essen, 1991). For many areas, histological measures of cortex are indistinguishable, based on current markers. Higher-tier areas often lack retinotopy, and global functional differences between areas can be frustratingly subtle. Studies of cortical connections sometimes lead to ambiguous conclusions because it can be difficult to distinguish between cortical areas and columnar subdivisions within areas. Additional complications of tracer studies are reviewed in Salin and Bullier (1995).

If only one criterion could be picked, many researchers would have most confidence in retinotopic data, because such retinotopic information includes so many informative subdimensions. These retinotopic subdimensions include cortical representations of visual field eccentricity, polar angle, field sign, magnification factor, receptive field size, half- versus quarter-field representations, ipsilateral versus contralateral distinctions – in addition to area size and shape, which are deterministically linked to the global retinotopy in each area.

Retinotopic criteria are also an attractive criterion because the corresponding data are relatively straightforward to interpret across both fMRI and single unit data, in humans and monkeys, respectively. This is not the case for the other criteria. For instance, it is well known that hemodynamic (fMRI) and electrophysiological (single unit) data do not correlate completely, because their biological sources are so dissimilar (e.g., Logothetis *et al.*, 2001; Toth *et al.*, 2001). It is easy to imagine that data based on other criteria (e.g., 'functional' or 'functional connection' data) might not coincide when compared across fMRI versus single unit realms, for a given cortical area. However, because retinotopy is based on multiple, locally systematic representations of spatial location (each of which can be independently verified with reference to the visual field, and with respect to neighboring points in the map), retinotopic maps should be essentially identical, whether based on fMRI or electrophysiology. This conclusion has been generally confirmed (Brewer *et al.*, 2002; Fize *et al.*, 2003; Tootell *et al.*, 2003; Orban *et al.*, 2004).

4.26.2.1 Improbable Retinotopy

By the same token, the many different subdimensions in the retinotopic realm give rise to their own set of expectations, and some of these expectations are made explicit only rarely. What happens when those expectations are not met? Many of the current controversies in the retinotopic realm are of this type.

For instance, it is expected that each retinotopic area will include a complete representation of the contralateral visual field. Proposed areas which do not meet this expectation have been called 'improbable' (e.g., Kaas, 1993) or 'faith-based' visual cortical areas. Though nearly everyone would agree that this whole-visual-field representation is the most parsimonious expectation, often the

empirical data drive investigators to propose such areas at least as a temporary measure: the list of proposed partial representations is not small. One of the most well known of these controversies is whether macaque area 'V3' is comprised of two physically separated representations of the upper and lower contralateral visual fields ('V3d' plus 'V3v': Ungerleider and Desimone, 1986; Boussaoud *et al*., 1990), or whether the upper field representation is really an entirely different visual field ('VP' instead of 'V3v'), based on differences in connectional and functional criteria (e.g., Maunsell and Van Essen, 1983; Felleman and Van Essen, 1991; Burkhalter and Van Essen, 1986; Felleman *et al*., 1997). Increasingly, investigators have adopted the former assumption because it is simpler and it is most similar to the more recently described human fMRI data. However, many of the originally reported differences in macaque VP versus V3 have not been refuted. An analogous controversy has arisen with respect to the human homologue of macaque area 'V4', based largely on fMRI comparison of functional and retinotopic criteria (McKeefry and Zeki, 1997; Hadjikhani *et al*., 1998; Tootell and Hadjikhani, 2001; Wade *et al*., 2002).

Based largely on electrophysiology, other authors (reviewed in Gattass *et al*., 2005) have reported that many extrastriate areas in macaque represent only the central portion of the visual field – for example, from 0 through 30–50°, not the full visible portion of the contralateral visual field (0 through ~80°). By this model, some visual information from the peripheral visual fields would be processed only by areas V1 and V2. A related (but less radical) model suggests that the cortical magnification factor (and thus, the relative emphasis on central versus peripheral visual fields) varies systematically in extrastriate visual cortex. According to this model (Buffalo *et al*., 2005), visual cortical areas in the 'ventral stream' have a relatively larger central representation, whereas the peripheral visual field is represented more prominently in areas of the dorsal stream. This model is supported by earlier tracer results, reporting a projection from foveal V1 to foveal V4, without a corresponding projection from peripheral V1 to V4 (Zeki, 1971; Ungerleider *et al*., submitted).

Another assumption in studies of retinotopy is that visual areas are subdivided along the representation of either vertical or horizontal meridian(s). Reported counterexamples include subdivision between macaque V4v and V4d, which is reported to lie along a polar angle within the lower visual field (Gattass *et al*., 1988). Though unexpected, such an arrangement would rationalize the larger surface area of V4d in comparison to V4v. A similar situation has been suggested in one exceptional report from the V1–V2 border of owl monkey (Blasdel and Campbell, 2001).

Recently, the relative value of the four mapping criteria has become a more pressing issue, following the demonstration that one pair of adjacent cortical visual areas (V3 and V3A, based on retinotopy) has 'switched' functional properties, in comparisons between macaques and humans. In macaques, area V3 is moderately motion selective (Felleman and Van Essen, 1987; Vanduffel *et al*., 2001), whereas adjacent V3A is not; in humans this functional relationship is reversed (Tootell *et al*., 1997; Orban *et al*., 2003). Except for a change in the relative size of V3, the retinotopy of areas V3d and V3A appears to be relatively unchanged in macaques and humans – although the retinotopic definitions of areas V3 and V3A are themselves subject to a new debate (e.g., Rosa and Tweedale, 2005; see below).

4.26.2.1.1 Additional caveats Among the primates, detailed retinotopic maps of visual cortex have been acquired in macaques, humans, owl (Aotus) monkeys, marmosets, and Cebus monkeys. Such maps have been acquired on a variety of techniques, including single unit recording, neural tracers, optical recording, deoxyglucose mapping, and/or fMRI. With all this information, one might hope for a clear consensus about visual cortical maps, and how they evolved. Unfortunately, this goal has proven elusive, although progress has been made.

One major problem is that measurement noise and/or technical differences can significantly obscure the (presumably real) features in maps in each species. Even in macaque, the most thoroughly mapped monkey, different investigators come to quite different conclusions about the cortical maps (cf. Boussaoud *et al*., 1990; Felleman and Van Essen, 1991; Stepniewska and Kaas, 1996), at least in some cortical regions. The situation is similar in humans and other primate species. It is fair to say that complete agreement on cortical mapping features is the exception rather than the rule, except in areas V1, V2, and MT (also known as V5).

4.26.2.1.1.(i) Common areas: V1, V2, and MT Generally, investigators agree on the location and retinotopy of lower-tier visual areas, but disagree more with respect to higher-tier (extrastriate) areas. This generality arose for multiple reasons. First, lower-tier areas have smaller receptive fields, so the retinotopy is easier to define in those areas. Also,

some lower-tier areas can be defined unambiguously based on histological features, as well as the presence of subcortical inputs. In conventional hierarchies of macaque cortex, areas V1 and V2 are the sole first- and second-tier areas, respectively. Area MT is considered to be fifth tier, based on laminar terminations (e.g., Felleman and Van Essen, 1991), but in some respects its hierarchical position is instead 'lower tier' because it also receives direct input from V1 (Maunsell and Van Essen, 1983), and from subcortical nuclei (O'Brien *et al*., 2001). Like V1 and V2, MT is histologically distinctive, and apparently present in all primates tested.

It has been suggested that V1 and MT serve as stable 'molecular anchors' that develop before other cortical visual areas (Rosa and Tweedale, 2005). According to this idea, higher-tier areas are intrinsically more variable because they develop later, from the borders of previously developed areas, based on more diffuse molecular gradients. However, as described above, uncertainties persist in the cortical visual maps reported in different species, using different techniques, from different investigators, and even in different studies from the same laboratory. Thus, it is difficult to know whether additional higher-tier cortical visual areas are fundamentally more variable, or whether they are biologically stable but difficult to map accurately.

It has been suggested that visual cortical areas develop in a manner that minimizes the mean length of axonal connections (e.g., Kaas, 1997a; Van Essen, 1997). A striking example of this can be seen in the topographical relationship of areas V1 and V2, in several species of monkey. In these monkeys, the ubiquitous 'point-to-point' connections between retinotopically corresponding points in V1 and V2 (comprising perhaps the most prominent intracortical connections in both V1 and V2) are demonstrably as short as possible, or nearly so. This produces to a unique folding pattern in the gyri and sulci of these areas, such that most of the border between areas V1 and V2 lies on or very near the crown of the gyral edges of the cortical operculum (see Figure 2). This distinctive folding pattern is found in some species of both New and Old World

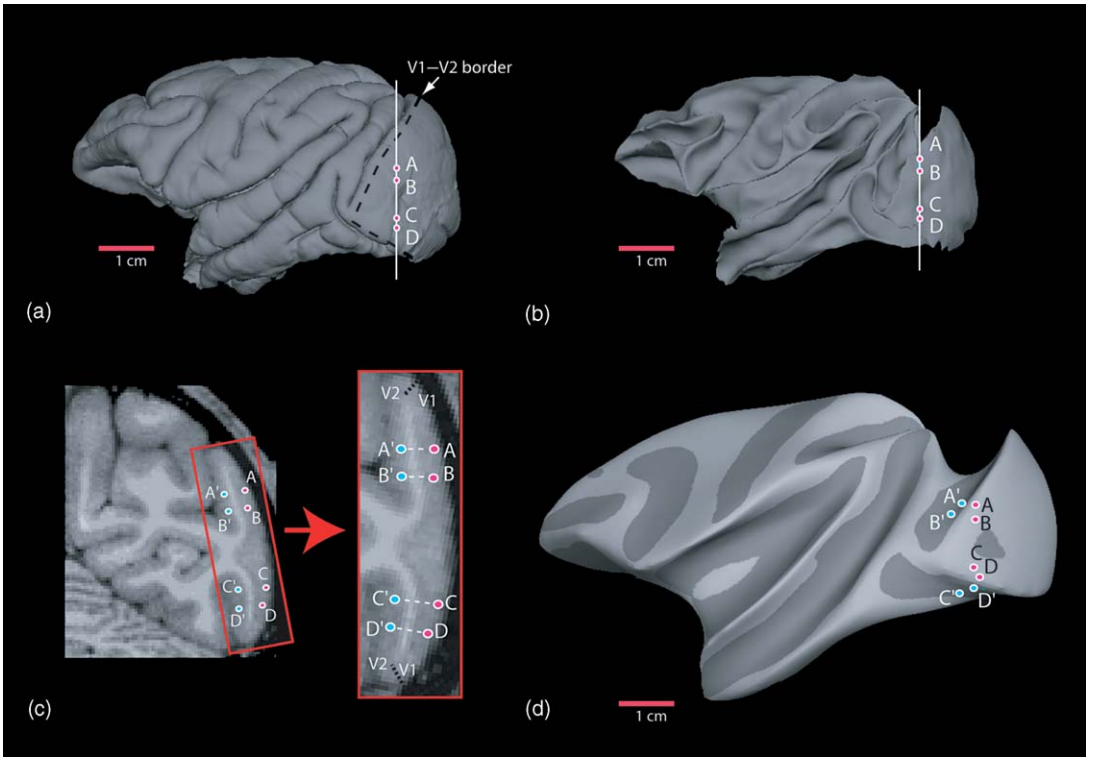

Figure 2 a, A lateral view of the left visual hemisphere, as seen in a normal dissection. An arbitrarily positioned coronal slice plane is indicated in white. Along that slice, two points in the upper primary visual cortex (V1) (A and B) and two points in the lower primary visual cortex (C and D) are indicated on the surface. b, A lateral view of the white matter surface, underlying the view shown in panel (a). The position of these four points are projected onto the white matter surface. c, Now these four points are projected onto the coronal slice. Their retinotopically corresponding projections (A′–D′) on V2 can be seen. d, All of eight points from V1 and V2 are presented on the inflated format of cortex. This figure shows how the retinotopically corresponding points in V2 are found almost immediately underlying their counterparts in V1. Without this particular form of cortical fold, the axons connecting these cortical points would be much longer. This folding pattern connects points within the central half of V1 and V2.

primates (e.g., macaque, *Cercopithecus*, and *Cebus*), spanning ~70 million years of independent cortical evolution. On the other hand, other species with more (e.g., humans) or less (e.g., *Aotus, Saimiri*, and *Galago*) cortical folding do not show this distinctive 'fold' along the V1–V2 border.

4.26.2.1.1.(i).(a) V3 and V3A In the next-most anterior area (V3 or its namesakes), the history of mapping conclusions has been surprisingly convoluted – though recently it has been converging (reviewed in Kaas and Lyon, 2001; Rosa and Tweedale, 2005). In macaque, this 'third' retinotopic map is typically subdivided into dorsal and a ventral halves (as in adjacent area V2), representing ventral and dorsal quadrants of the visual field, respectively (Zeki, 1978; Maunsell and Van Essen, 1983; Ungerleider and Desimone, 1986; Boussaoud *et al.*, 1990; Felleman and Van Essen, 1991). However, unlike the organization in V2, the two halves of V3 were reported to be shorter than their counterparts in V2, separated by many millimeters at the foveal representation, and they do not extend as far into the periphery. Thus, V3 was assumed to be roughly but not truly mirror symmetric to its neighbor V2, located immediately posterior (see Figure 3). If true, these failures to match the mirror symmetry of V2 are not consistent with the 'shortest possible connection' model described above.

Anterior to macaque V3, an additional retinotopic area was subsequently described. Though it is a completely independent area, it was given a misleadingly diminutive name (V3A), because it was discovered after V3 and V4, but located between them on the cortical sheet (Zeki, 1978; Gattass *et al.*, 1988). V3A is considered to include a contiguous map of the entire contralateral visual field.

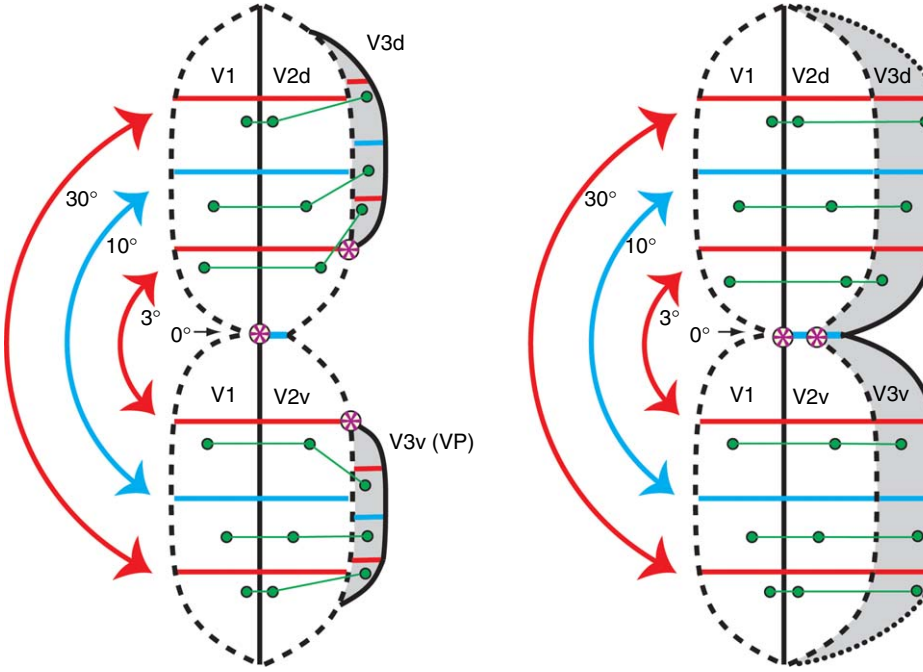

Figure 3 The model of retinotopic organization for human 'V3' is simpler than that for macaque V3. a, Current model for macaque visual areas (V1–V3). The foveal representation is marked as a star, the vertical meridian representation is indicated with solid lines, and the horizontal meridian is represented with dashed lines. The horizontal meridian representation in V1 has been artificially 'split' in this diagram (indicated with double-headed arrows). In all areas, the representation of 3°, 10°, and 30° eccentricities is indicated with red, blue, and red lines, respectively. Retinotopically corresponding points in V1, V2, and V3 are indicated in green. In macaque, the representation of corresponding eccentricities in V3 (V3d, VP/V3v) is shifted, relative to that in V1 and V2. b, Current model for human visual areas (V1–V3). Diagram format as in panel (a), except that short-dashed lines indicate the presumptive (unknown) peripheral representation of V3. The representation of eccentricity in human V3 (V3d and V3v) is topographically aligned relative to that in V1 and V2, unlike that in macaque. This creates shorter connections to/from V3, relative to inputs and feedback connections with V2 and V1.

In human fMRI, the apparent homologue of area V3 is much larger than it is in macaque, both objectively and proportionately (see Figure 3). This has made it much easier to measure V3 in humans, despite the spatial resolution limits of the fMRI (Sereno *et al*., 1995; De Yoe *et al*., 1996; Engel *et al*., 1997; Tootell *et al*., 1997). Topographically, human V3 appears to be essentially a mirror-symmetric replica of V2, continuous through the foveal representation, with contiguous representation of eccentricity throughout its length. Thus, in human V3, the representation of retinotopic eccentricity does satisfy the 'shortest possible connection' rule (Kaas, 1997a; Van Essen, 1997), with respect to the topography of its prominent inputs from V2 (and from V1, for that matter) (see Figure 3). Anterior to human V3, an apparent human homologue of macaque V3A has also been described (Tootell *et al*., 1997).

Because V3 is easiest to measure in humans, and because the topography of V3 is simplest in that primate species (i.e., a simple mirror-symmetric duplication of the previous area, V2), it brings up the question whether the simpler V3 topography also applies to other primates. For instance, recent fMRI maps from macaque (Brewer *et al*., 2002; Fize *et al*., 2003) allow for the possibility that macaque V3 is instead continuous through the foveal representation, as in human V3. However, this cannot yet be confirmed: macaque V3 is very thin (~2–4 mm), and near the limits of spatial resolution of the fMRI technique.

In the New World Aotus (owl) monkey, an analogous, strip-shaped third visual area ('V3') was not found initially (Allman and Kaas, 1975; Kaas, 1997a). Instead, as many as five different visual areas were reported to lie immediately adjacent (and anterior) to V2. One of these anterior areas was a contiguous representation of the contralateral field ('DM'), which was superficially similar to V3A. However, the most recent accounts now do describe a V3 analogue between V2 and DM in this species (Kaas and Lyon, 2001). Thus, current maps of owl monkey are more similar to macaque and human maps – due partly to technical improvements in mapping of New World monkeys (e.g., Lyon and Kaas, 2002).

Recently, it was proposed that a quite different model of the V3/V3A retinotopy is common to all primates (including Cebus monkeys, macaques, and humans). This new 'unified V3' model is generalized from maps of marmoset (Rosa and Tweedale, 2005), including a 'V3' which extends through the foveal representation. However, this new model also eliminates V3A entirely from all primate species; in that sense it is a radical departure from previous models. However, because this retinotopic model is proposed to extend across all primates, it would be unifying if confirmed. Retinotopic mapping based on macaque fMRI can be construed to support this new model (e.g., Brewer *et al*., 2002; Fize *et al*., 2003) – although (as stated above) the 2–4 mm width of macaque area V3 makes it difficult to resolve the underlying retinotopy using fMRI.

4.26.3 Functionally Defined Areas

Here we consider a second criterion for defining cortical areas: functional properties. To the extent that functional properties are distinctive in different cortical visual areas, those properties can be used to 'track' whether that area is present, and/or functionally unchanged, across evolution. Classically, area MT (also known as V5 in macaques and humans) is the visual area considered functionally most distinctive, compared to other visual areas. Single units in MT/V5 respond best to visual stimuli which are moving in a specific direction, in all primates tested (e.g., macaques, Aotus monkeys, and humans, based on fMRI). However, even in MT, there is some evidence for evolutionary variation in function across species. For instance, optical recordings suggest that stimulus orientation is three times as significant as stimulus direction in area MT of owl monkeys (Malonek *et al*., 1994). This contrasts with the profound selectivity for stimulus direction, based on single units from macaque MT (e.g., Dubner and Zeki, 1971; Maunsell and Van Essen, 1983).

A more direct example of evolutionary differences in MT is shown in Figure 4. The left-most panels show fMRI activity acquired from human MT(+) during viewing of a motion aftereffect, along with that produced during control stimuli which do not produce such an aftereffect (Tootell *et al*., 1995; He *et al*., 1998; Culham *et al*., 1999; Huk *et al*., 2001). Such stimuli produce a clear increase in fMRI amplitude with a time course essentially identical to the psychophysical effect (squares in panel (c)), and a 'memory' effect also similar to the psychophysical effect (Culham *et al*., 1999). Subsequent fMRI studies (Huk *et al*., 2001; Nishida *et al*., 2003) suggested that this 'fMRI motion aftereffect' is strongly gated by attention, but those studies do not affect the interpretation of the (passive viewing) results we discuss here.

Subsequent studies initially showed very little correlate of the positive-going fMRI motion aftereffect (in humans) in single units from macaque area MT

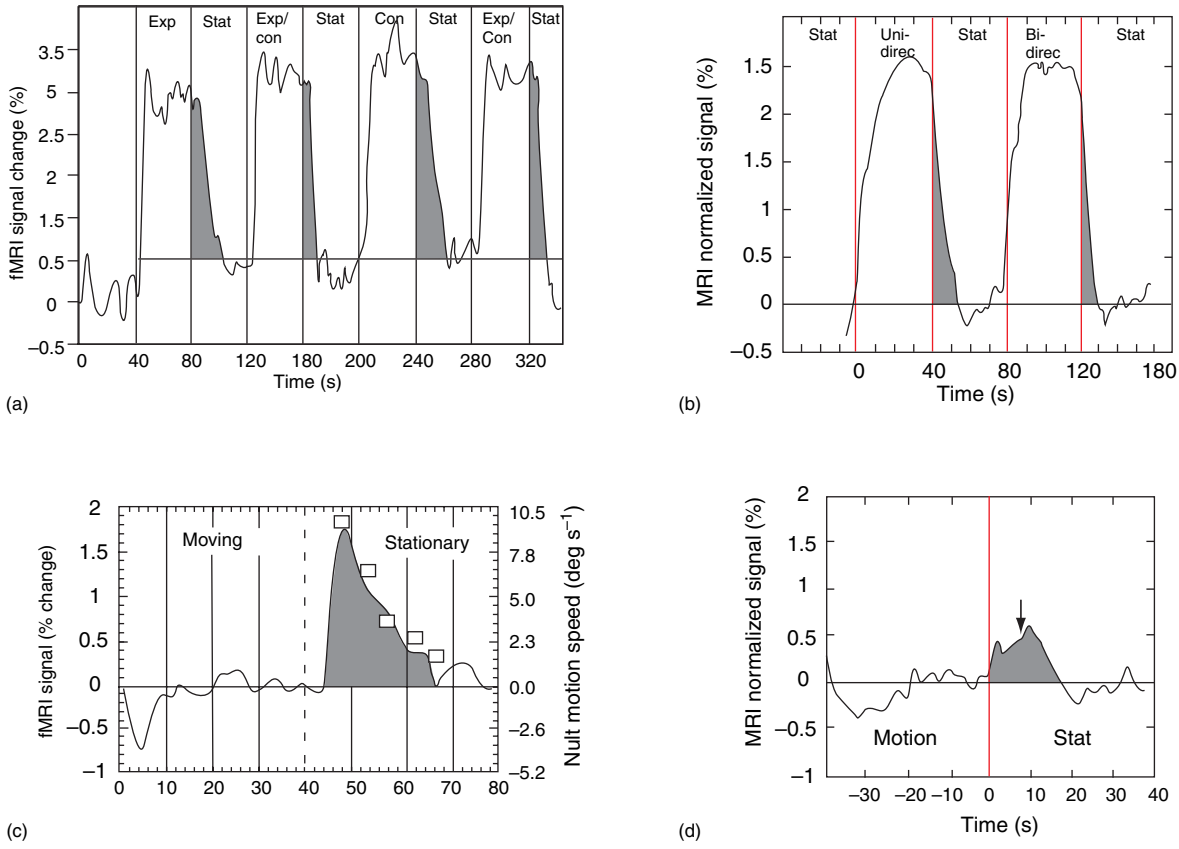

Figure 4 fMRI-based 'motion aftereffect' in both human and monkey. Time course of fMRI activity from area MT (or MT+) from human (a) and monkey (c) subjects, when both were awake and passively viewing, during blocks of unidirectionally moving, bidirectionally moving, and static stimuli. Analogous elevated response to static stimuli are found after seeing unidirectionally moving stimuli (i.e., during perceptual motion after effect) are observed in both humans (b) and monkeys (d). However, this fMRI-based 'motion aftereffect' is smaller in macaques compared to humans.

(Van Wezel and Britten, 2002), but more comprehensive single unit studies did show something analogous (Kohn and Movshon, 2003). In these macaque single unit results, attention was uncontrolled, as in the initial fMRI studies in humans. Also, both single unit studies implicitly assumed that macaques perceived a motion aftereffect like that seen by humans. This is a classic example of using macaque monkeys as a 'model' for human vision.

To test some of the assumptions underlying this across-species comparison, we tested whether an 'fMRI motion aftereffect' is present in macaques as well as humans. The fMRI data were acquired from awake fixating monkeys, with the same stimuli presented earlier to human subjects, using the same MRI scanner and analysis. Surprisingly, these stimuli produced only a very small fMRI-based 'motion aftereffect' in macaques – though it was of the same polarity as that seen in the human experiments (see Figure 4). Although further controls would be useful to assess the effect of attention (in both the macaque single units and the fMRI), the direct comparison in Figure 4 demonstrates that similar processes operate in both humans and macaques in the processing of such stimuli – though differences in the amplitude of this effect are also significant. Other fMRI data (Vanduffel *et al.*, 2002; Denys *et al.*, 2004; Sasaki *et al.*, 2005) also reveal clear differences in functional emphasis between presumably homologous cortical areas and processes in humans compared to macaques.

Other functionally defined regions appear to be amazingly stable evolutionarily. Following many fMRI reports for an apparently face-selective region in human visual cortex ('FFA'; Kanwisher *et al.*, 1997, 1998; Tong *et al.*, 1998; Haxby *et al.*, 2000; Heekeren *et al.*, 2004), a presumably homologous set of face-selective patches was revealed by fMRI in the corresponding location of macaque visual cortex (Tsao *et al.*, 2003; Pinsk and Kaster, 2005). Analysis in flattened cortex, at high spatial resolution, revealed that these macaque 'modules' take the form of small isolated patches (akin to sparsely

distributed cortical columns) rather than a contiguous classical cortical 'area' (Tsao *et al.*, 2003). Prompted by this insight in macaque, reanalysis of fMRI suggests that human FFA can also be described as a set of functionally similar patches rather than a classical cortical 'area'. Presumably, any patchiness in previous fMRI results from humans was less obvious because most of those scans used voxels which were much larger (~25–50 times the volume), compared to those used in the macaque fMRI. This is a nice example of how insight from macaque can 'feed back' to clarify results in human cortex.

This is also a nice counterexample for the generality that higher-tier cortical features are evolutionarily more variable than lower-tier features. The fMRI-defined face-selective patches appear essentially identical in both humans and macaques, though they are distributed widely throughout the higher-tier cortex.

The near-columnar size and irregular topography of the face-selective patches also supports the speculation of Rosa and Tweedale (2005) that classical cortical 'areas' are not really present in higher-tier visual areas such as inferotemporal (IT) cortex. These investigators suggested instead that IT cortex may be divided into column-like modules, separated by gradual rather than abrupt functional transitions. This generality may be simply another example in which area borders are considered to be gradual when they are merely invisible using the techniques available at the time. This is historically common: recall how the extent of 'associative cortex' shrank as more reliably defined cortical areas were revealed within it. On the other hand, the model of Rosa and Tweedale (2005) could rationalize why there is so little consensus in published maps of cortical areas in IT cortex drawn by different investigators (cf. Boussaoud *et al.*, 1990; Felleman and Van Essen, 1991; Stepniewska and Kaas, 1996). Irrespective of whether area borders are exact or gradual in IT cortex, there is increasing evidence for column-sized functional patches in this region (Vanduffel *et al.*, 2000; Tanaka, 1997; Tootell *et al.*, 2004).

4.26.4 Additional Criteria

The final two criteria for defining cortical areas in human and nonhuman primates (cortical connections and histology) are reviewed in detail elsewhere (Kaas, 1997a; Tootell *et al.*, 2003; Preuss, 2004a, 2004b). It remains impossible to definitively trace connections in human visual cortex, though diffusion imaging (Conturo *et al.*, 1999; Le Bihan *et al.*, 2001; Tuch *et al.*, 2001) and other techniques (Paus *et al.*, 1997; McIntosh, 1999; Brandt *et al.*, 2001) may eventually provide supplementary information. Some progress has been made documenting histological differences between human visual cortical areas in postmortem tissue (e.g., Galuske *et al.*, 2000; Eickhoff *et al.*, 2005), though histological differences between cortical areas are usually subtle and probabilistic rather than definitive, outside of a few areas such as V1 and MT.

4.26.5 Development of Visual Cortical Areas

The rich array of architectural features in primate visual cortex, and the intriguing balance of similarities and variation across species in the cortical maps, naturally raises the issue of how these features develop (for reviews, see Grove and Fukuchi-Shimogori, 2003; Krubitzer and Kahn, 2003b). Changes in the size of the cortical sheet could arise relatively simply via changes in the cell-cycle kinetics, via upregulation of genes such as beta-catenin. In retinotopic areas, one possibility is that neurons and local connections are organized based on the local correlation of electrical activity, arising from spontaneous and/or driven activity in the retina. Another idea is that chemical factors such as Wnt, Shh, Fgf8, and BMP govern gross cortical gradients. Another intriguing and plausible idea is that new cortical areas could arise evolutionarily from modular subdivisions within existing areas (Krubitzer, 1995).

4.26.6 Conclusions

After more than half a century of detailed retinotopic mapping in primate visual cortex, it is somewhat sobering to realize how little agreement there is on specific details of the maps in different species. However, some current evidence suggests a more common plan for the organization of areas in different primates; if confirmed, this will simplify the subsequent analysis of area topography. At the very least, there is an increased awareness that systems research needs to take into account differences and similarities in the cortical maps in different species.

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4.27 Evolutionary Specializations for Processing Faces and Objects

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Glossary

amygdala An almond-shaped structure buried in the temporal lobe in the primate brain, consisting of three sets of nuclei of diverse origin. Of the cortical nuclei, the basolateral complex, and the centromedial nuclei, the latter two are implicated in emotional learning.

anterior medial temporal sulcus (AMTS) Sulcus lying in the ventral aspect of the temporal lobe, running in an anterior–posterior direction.

configural processing Visual object recognition strategy that makes use of precise metric information in the stimulus, including the spatial relationships between different parts.

critical period A period of time during which the brain must receive appropriate stimulation from the environment in order to be capable of acquiring a specific ability. A more stringent type of sensitive period.

face-inversion effect Poorer performance in identification of faces that are presented upside-down compared to upright; typically a larger cost is found with inversion of faces than that of other objects.

featural processing Visual object recognition strategy that codes information for individual parts, such as their shape and color. Eyes, nose, and mouth are considered features of the face.

fusiform gyrus Gyrus in the ventral aspect of the human temporal lobe, which contains a smaller face-selective region referred to as the 'fusiform face area'.

homologue Feature that is similar in two species because it is inherited from a common ancestor.

homoplasy As opposed to homology. Any characteristic found in two species whose similarity is not due to common descent.

inferotemporal (IT) cortex Temporal cortex running from the fundus of STS to the bank of the rhinal sulcus, and from the temporal pole in the posterior direction to the OTS marking the occipital lobe.

innate releasing mechanism The process by which only certain innately specified stimuli come to elicit or 'release' appropriate behaviors with exposure.

MT (V5) Motion-sensitive area in the posterior portion of the superior temporal sulcus of the primate brain.

other race effect	Poorer performance in identification of faces from an unfamiliar race compared to faces of a familiar race.
pulvinar	Part of the thalamus, receiving visual inputs from other thalamic nuclei and projecting to the cortex.
sensitive period	A restricted temporal window in development during which experience or learning has a much stronger effect than learning outside the window.
superior colliculus	Subcortical relay station for visual information arriving from the retina, involved in head and eye orienting.
superior temporal sulcus (STS)	Large sulcus running along the anterior–posterior length of the lateral aspect of the temporal lobe. Contains MT/MST/FST in the posterior section, TE on the anterior part of the lower bank, and TPO along the length of the upper bank.
temporal area TE	Lying in the gyrus and banks between the AMTS and the STS.

4.27.1 Introduction

The title of this article illustrates a common misphrase, 'faces and objects', since faces are one type of object. Yet faces are considered as a special case of objects on numerous grounds. Here, we will use faces as an example object class, one that we consider a good candidate for receiving evolutionary specializations in neural circuits.

4.27.1.1 Why Faces?

Across human cultures, social dominance hierarchies exist, making the identification of an individual advantageous for appropriate behaviors. Moreover, common facial expressions and vocalizations indicate the current social climate, again facilitating appropriate responses. Several brain regions respond more vigorously to the presentation of faces than to that of most other objects. The fusiform gyrus, posterior superior temporal sulcus (STS), and amygdala are among the most consistently implicated regions in face processing. Moreover, both acute brain damage as well as congenital and developmental disorders are associated with deficits in processing faces despite relatively spared object recognition abilities (Behrmann and Avidan, 2005; Farah, 1990; Schultz, 2005). Cross-cultural similarities in face processing, together with consistent and discrete neural regions of activation, suggest that this object class may be the target of evolutionary specialization (see Evolution of the Neural Circuitry Underlying Laughter and Crying, Evolution of the Amygdala in Vertebrates).

4.27.1.2 How to Differentiate Innate Mechanisms from Common Developmental Paths (Nature vs. Nurture)

One possibility is that face processing is the product of mostly innate mechanisms. Processing abilities that are unique to faces compared to other objects and present at birth would be the most unambiguous evidence for innate face-processing skills; however, numerous traits that are genetically specified do not appear until later in development. Thus, another marker of innate face mechanisms would involve normal face processing following deprivation during development. An alternative possibility is that face processing develops as the result of an interaction between environmental and innate paths. An innate releasing mechanism or sensitive period in which the right stimulus (or the right stimulus at the right time) must be present for full processing capabilities is exemplified by some types of birdsong learning. Correct song production does not occur in isolation, thus the song is not completely prespecified; yet the wrong tutor call, or the right tutor call presented in the wrong time window, will not lead to correct song productions either, suggesting an innate sensitivity for a particular song during a particular developmental window (Gardner *et al.*, 2005; Marler, 1997). Considering that song learning and production is a fairly complex neural process, it nicely illustrates the ways in which face processing may contain evolutionary and developmental components. Along these lines, one current model of face processing (Nelson, 2001) suggests that specialization for faces is due to experience-expectant processes, whereby early experience with faces drives the development of this neural system. If true, inspection of face processing in infants may provide one means of separating evolutionary from developmental components.

A different approach to studying the evolutionary nature of a skill is comparative: homologous structures in extant primate species would signify a common evolutionary history. Unfortunately, among the social primates, only a few have been studied with respect to face-processing abilities, and even fewer have been examined for possible neural homologues of face processing. As such, it is difficult to separate homology (common evolutionary source) from homoplasy (convergent or parallel evolution) in putative common brain structures. Nevertheless, common neural structures in primates with similar

face-processing abilities would suggest an evolutionary neural specialization.

4.27.2 Evidence from Human Development

4.27.2.1 Newborns

Speculation that face processing is innate dates back to at least the 1930s (Bowlby, 1969; Bühler, 1933). Testing for recognition of faces versus other patterned objects has produced a myriad of results over the last five decades, but the more recent experiments, with more refined controls, reveal some surprising face-processing abilities beyond a mere face preference (Johnson and Morton, 1991; Morton and Johnson, 1991; Nelson and Ludemann, 1989). Newborns can recognize a mother's face from that of strangers (Pascalis and de Schonen, 1994), and preference for gaze towards versus away (Farroni *et al*., 2002). The face preferences in newborns, however, can be subtle, and the state of the infant and exact measurement parameters can make or break an effect, leading to a diversity of results. Newborns can only be tested with a small number of stimuli in any one experiment, and the nature of the control stimuli is crucial. Schematized face-like stimuli are often used (dark spots for eyes and mouth in a tapered simplified head shape on a contrasting background) in efforts to cater to the limited sensitivity of the visual system at that age. Control stimuli have typically been the same pattern upside-down, and no other 'object' control class is generally used. In fact, increasing evidence from studies using more nonface categories suggests that, in days-old newborns, the 'face' preference is captured by any stimuli with top-heavy asymmetry, even when parts are arranged in an otherwise 'non-face-like' configuration (Turati *et al*., 2002). More robust face preferences are obtained in infants older than 2 months, perhaps owing to increased visual acuity and contrast sensitivity (de Schonen and Mathivet, 1989; Simion *et al*., 1998), or to different processing mechanisms, or both. A recent study measured preferences of newborns and 3-month-olds by pitting a natural face stimuli against a top-heavy scrambled face pattern (Simion *et al*., 2006). While newborns preferred the top-heavy nonface stimulus, the opposite preference emerged by 3 months of age. Unfortunately, 3 months of visual experience is quite a bit; thus, one cannot make claims of innate mechanisms. Nevertheless, understanding what aspects of face processing are normally functioning at different stages of development can help narrow down the underlying neural processes, including those aspects that may be innate from those that require experience.

4.27.2.2 The Young Infant – 6 Months of Experience

Within the first 6 months of life, face-inversion effects can be seen (Fagan, 1972), gender can be discriminated (Cohen and Strauss, 1979), and, at 7 months, facial expressions are also distinguished. The other race effect, better discrimination of faces of a familiar race, can be observed as early as 3 months of age (Sangrigoli and De Schonen, 2004), but can still be reversed in adulthood (ethnic Koreans who moved to France as children 3–9 years old show an other race effect for Koreans (Sangrigoli *et al*., 2005)). So it appears that much of a fully developed face-processing system occurs in the course of a few months' development, with some features remaining modifiable well into childhood.

4.27.2.3 Early Deprivation

The inconclusive newborn data, as well as complex developmental changes taking place early in life, leave open the issue of the extent to which developmental processes are required for normal face processing. Are these abilities innately specified, or do they instead result from visual experience? Individuals with congenital cataracts were not exposed to normal visual input in the first few months of life. They represent an interesting test for whether the first few months of development reflect a critical period for face processing. Although able to discriminate faces based on features and contours (Le Grand *et al*., 2001), and to match facial expression and gaze direction (Geldart *et al*., 2002), these patients show deficits in their 'expert' processing of faces, specifically in holistic processing of faces (Le Grand *et al*., 2004) and discriminating faces based on configural changes, leading to an absent face-inversion effect for configurally modified faces (Le Grand *et al*., 2001). Moreover, visual stimulation to the right hemisphere in the first months after birth appears to be necessary for normal development of configural face processing (Le Grand *et al*., 2003). Thus, early visual experience is not required for most face-processing skills to develop, but it appears to be necessary for configural face-processing skills that are useful to individuate faces. This argues against an extreme innate account postulating that the entire face recognition system is hard-wired before birth, but also suggests that certain aspects of face processing exhibit sensitive periods early in life, after which the system will not develop normally.

In sum, it appears as though there are some innate preferences in newborns that lead them to look preferentially at faces, or at least at stimuli sharing some geometrical properties with faces such as a top-heavy part configuration. Whatever the initial preferences, the time from birth to 2–6 months of age appears to be important in the normal development of the full spectrum of face-processing skills, with at least two aspects of face processing, configural and holistic sensitivity, requiring normal visual inputs to the right hemisphere during this time period.

4.27.3 Evidence from Nonhuman Primates

4.27.3.1 Face Recognition in Monkeys

The biological advantages that might be conferred from an evolutionary specialization for face processing in humans also apply to many nonhuman primate species that share social features such as dominance hierarchies, vocal communication, and communication through facial expressions. Thus, neural specialization for face processing may have been present in common ancestors that can be viewed in homologous structures in extant primates. One of the best tests for this would examine the social demands of various primate species, their phylogenetic differences, and the corresponding neural substrates mediating face processing. Unfortunately, very little is known about potential neural substrates for face recognition across primate species. As a consequence, our discussion is limited to macaques, the genus for which we have the greatest collective knowledge about behaviors and neural structures related to face processing (see Preuss, 2000, for a discussion of phyletic analysis and cortical specializations).

Like humans, monkeys are able to identify conspecifics based on their faces (Rosenfeld and Van Hoesen, 1979); they recognize kin relationships, discriminate facial expressions (Haude and Detwiler, 1976; Kenney *et al*., 1979; Redican *et al*., 1971; Rosenblum and Alpert, 1974) and gender (Kenney *et al*., 1979; Rosenblum and Alpert, 1974), and are sensitive to changes in gaze direction, while maintaining an ability to generalize across viewpoints when recognizing an individual (Heywood and Cowey, 1992). They show signs of face 'expertise', as measured by their subordinate-level classification of monkeys but not other animals at the individual, or subordinate, level (Humphrey, 1974), and by the presence of a face-inversion effect (Bruce, 1982; Parr *et al*., 1999; Perrett *et al*., 1988; but see Rosenfeld and Van Hoesen, 1979; Swartz, 1983; Vermeire and Hamilton, 1998).

Although the onset and development of these abilities is largely untested, viewing patterns show gaze sensitivity in 1-week-old monkeys, the youngest age tested (Mendelson *et al*., 1982). Face preferences have been seen at the earliest time points tested, in 2.5–10-week-old macaques (Lutz *et al*., 1998), and species discrimination is possible in 2–3 month-olds (Swartz, 1983). More compelling is evidence coming from the sole study of the effects of complete social isolation on responsivity to faces. Isolated monkeys with no prior exposure to faces, yet not subject to sensory deprivation, responded differentially to the presentation of threatening monkey pictures, but not to fearful, sexual, or neutral images of monkeys, nor to other objects (Sackett, 1966). Changes were observed both in natural behaviors and in lever-pressing to view images. This unambiguously innate behavior was detected at 2.5 months of age. So, at least for macaques, there is an innately specified component to some aspect of face processing, namely, the recognition of threatening emotional expressions, though its interaction with normal developmental processes is still not understood.

4.27.3.2 Face-Processing Regions in the Macaque Brain

In the macaque, neural responses to faces have been commonly observed throughout a large territory of temporal lobe, including the upper and lower banks of the STS, extending along the lateral convexity of the inferotemporal (IT) cortex to the anterior medial temporal sulcus (AMTS), including areas TPO, TEa, TEm, TE1, TE2, and TE3 (Bruce *et al*., 1981; Desimone *et al*., 1984; Gross *et al*., 1972; Perrett *et al*., 1982, 1992). In addition, face-selective cells have been reported in orbitofrontal cortex and the amygdala (Leonard *et al*., 1985; Nakamura *et al*., 1992; Rolls, 1996; Thorpe *et al*., 1983). Face responses are almost exclusively excitatory, and tend to be selective to facial expressions and gaze/head direction changes in STS, and identity in TE (Eifuku *et al*., 2004; Hasselmo *et al*., 1989; Perrett *et al*., 1985). Face cells appear to cluster together, as reported from electrophysiological investigations, and suggested from optical and functional magnetic resonance imaging (Logothetis *et al*., 1999; Pinsk *et al*., 2005; Tsao *et al*., 2003; Wang *et al*., 1998). Temporal lobe regions implicated by these fMRI studies are consistent across monkeys and macaque species (long tailed and rhesus). Subcortical regions such as the amygdala, thalamus, superior colliculus (SC), and ventral occipito-temporal regions have also

shown activation in a subset of studies (Hoffman *et al.*, 2005; Logothetis *et al.*, 1999), perhaps owing to the nature of the stimulus set, the gradient of signal intensity of the coil (i.e., use of surface rather than volume coils), or degree of susceptibility artifact near the structures mentioned.

In contrast to deficits associated with prosopagnosia in humans, selective face-processing deficits in the monkey have remained elusive. Damage to the STS region produced deficits in discriminating gaze direction, but not view or identity (Heywood and Cowey, 1992). Cooling of the STS, or the gyrus above or below it, produced face discrimination deficits, without affecting stripe orientation discrimination (Horel *et al.*, 1987). Interestingly, there was no difference in the face discrimination deficits induced by cooling anterior or posterior parts of STS/TE, suggesting a distribution of critical regions in the anterior and posterior portions of the temporal lobe (Horel, 1993). Facial expression recognition was not tested in either experiment, and though experimental manipulations produced significant deficits, animals showed some preserved face-processing ability, with performances above chance. More extensive lesions in IT that disrupt face processing are nonselective, producing deficits with other objects as well (Gross, 1978). In addition, deficits in socioemotional behavior in 2- and 6-month-old infants follow from TE or amygdalo-hippocampal lesions that are made by 3 weeks of age, but only the amygdalo-hippocampal damage leads to deficits that persist into adulthood (Bachevalier *et al.*, 2001; Malkova *et al.*, 1997). The face-processing abilities in these monkeys has not been tested, thus STS/TE lesions are the only lesions known to affect face processing, and, even there, only gaze perception and configuration deficits are observed. These results could reflect a less modular, less discrete face representation in macaques compared to humans, or that the STS region in monkeys is not a homologue to the human fusiform gyrus but rather to the posterior STS in human.

4.27.3.3 Visual System Development in the Macaque

How are the above-mentioned structures interconnected, and how do such connections develop? Some striking patterns of visual system development occur with no prior visual experience, such as the striation of ocular inputs in the lateral geniculate nucleus (LGN) (Shatz, 1990, 1996). Clearly, then, some components of visual system anatomy are innate. Yet, primates stand out in their delayed neural maturation, specifically for postnatal cortical development.

As in human infants, visual acuity in the monkey at birth is limited: only low spatial frequencies are perceived. Visual acuity in infancy is not limited by retinal receptors or optics, nor by behavioral response deficits, but instead appears to be a more central limitation, perhaps due to delayed development of the visual neocortex, particularly the parvocellular system (Boothe, 1982; Teller, 1983). Indeed, many neocortical areas show very different distributions of projections prenatally and in infancy than those seen in the adult. One striking feature of this development is the change in the relative distribution of supra- and infragranular projections among cortical areas. In general, there is a reduction in the relative proportion of supragranular 'feedback' projections among occipital and temporal regions from embryonic days 112–140 that can continue after birth (Batardiere *et al.*, 2002). The STS, unlike other visual areas tested, has a prominent supragranular projection to V1 in infancy that disappears by adulthood (Kennedy *et al.*, 1989). Moreover, this transient supragranular projection pattern is not seen in the cat, whose cortical associational projections appear to develop and stabilize much sooner (Bullier *et al.*, 1984; Luskin and Shatz, 1985; Price and Blakemore, 1985). Other transient projections are seen between the amygdala/surrounding rhinal cortices, and visual areas TE and TEO (Webster *et al.*, 1991a), projections which can be maintained if area TE is damaged early in development (Webster *et al.*, 1991b). Thus, cortico–cortico projections between striate cortex and other regions of visual cortex appear to be in flux both pre- and postnatally. Consistent with this postnatal plasticity, lesions to striate cortex made early in development have more modest effects than lesions made in adulthood, suggesting striate cortex is part of a developmental visual process, but that other visual areas are somehow able to compensate.

One candidate circuit that could process visual information in the absence of striate cortex is comprised of the SC, which receive retinal input, and which project to the amygdala and cortical visual areas via the pulvinar. This superior colliculus route has two features important for early visual processing: it is already developed in newborn monkeys (Wallace *et al.*, 1997) and is biased towards low-spatial frequency stimuli. Evidence for a compensatory role of this circuit includes virtually unaltered responses in middle temporal (MT) and superior temporal polysensory area (STP) as a result of striate or SC lesions alone, but an elimination of visual processing in MT for combined lesions (Bruce *et al.*, 1986; Girard *et al.*, 1992; Rodman *et al.*, 1990). In contrast, striate

lesions alone are sufficient to eliminate V2, V4, and IT visual responses (Girard and Bullier, 1989; Girard *et al*., 1991; Rocha-Miranda *et al*., 1975; Schiller and Malpeli, 1977). In light of these findings, the most likely route through which cortical activity can be maintained when striate cortex is damaged is from the SC–pulvinar pathway.

What might an SC–pulvinar route to the amygdala or STS have to do with face processing? Aside from the general responsivity to faces in these areas, which could be entirely due to input via LGN-striate cortex, there is evidence from human fMRI and lesion data suggesting that the SC, pulvinar, and amygdala are active in processing emotional or dangerous stimuli (Liddell *et al*., 2005; Morris *et al*., 1999). In particular, all three regions prefer the low-spatial frequency band for emotional face stimuli (Vuilleumier *et al*., 2003). Studies from blindsight patients suggest this subcortical route is active for processing threatening faces (Morris *et al*., 2001). Finally, one recent study of a patient with unilateral pulvinar damage suggests that the pulvinar is required for the rapid processing of visual information about threatening stimuli (Ward *et al*., 2005). Taken together, key attributes of this system across studies seem to include sensitivity to threatening stimuli, low-spatial frequency sensitivity, and fast perception/transmission of stimulus information. Recall that there was maintained neural activity in STS (but not in IT) following striate lesions that suggests some, but not all, regions of neocortex would be responsive to this alternate route (Bruce *et al*., 1986; de Gelder *et al*., 1999). Previous studies have suggested that the STS, compared to adjacent areas of IT, is particularly responsive to facial expressions (Eifuku *et al*., 2004; Hasselmo *et al*., 1989). The face-selective responses in STP also commonly occur at very short latencies, peaking in the range of 50–80ms, though the source of visual input is unknown (Hoffman, unpublished observation). The above observations hint at a role of the SC–pulvinar pathway in face processing in the adult monkey. But consider that infant monkeys, who only process low spatial frequency information, had an innate response to threatening monkey faces. These coincidences aside, there is no hard evidence that processing of emotional face stimuli occurs in the STS through a SC–pulvinar route at the time of birth.

4.27.4 Models of Face-Processing Acquisition

One popular conceptual model of newborn face preferences postulates two separate mechanisms, one of which corresponds to a subcortical pathway, such as the one described above. This mechanism, termed CONSPEC, would be in effect for the first months of infancy, and would only be responsible for the preference for a basic face configuration observed in newborns. At around 6 weeks, it would be replaced by CONLERN, a more flexible cortical system taking over the charge of learning what distinguishes individual faces (Johnson and Morton, 1991). Other models propose even more systems to explain further changes taking place during infancy (de Schonen and Mathivet, 1989). Whereas the CONSPEC/CONLERN model incorporates some of the developmental features of the brain, the distinction between the two systems may be somewhat artificially imposed. A subcortical system need not be completely inflexible nor supplanted by a cortical system.

A complementary view, requiring only one system, attempts to explain not only the postnatal effects, but, importantly, how some aspect of sensitivity to faces could begin earlier, in the absence of visual experience (Bednar and Miikkulainen, 2003). Internally generated activity appears to play a role in the development of other neural systems such as LGN segregation, auditory cortex, and retinal organization; likewise, the visual cortex might receive spontaneous activity waves to form connections in the absence of external input, specifically through ponto-geniculo-occipital (PGO) waves occurring during rapid-eye-movement (REM) sleep. A formal model is proposed to demonstrate how a simple input pattern could propagate via the PGO wave through the LGN and visual cortex, to provide a face-detection response consistent with the schematic face-like stimuli used in several newborn studies (Johnson *et al*., 1991). Though interesting and simple in design, there is little independent evidence to support this theory. The role of PGO waves must be explored, and the layers themselves (labeled retina, LGN, V1, and face-selective area) may actually be implemented through other structures. Further research should test the behavioral and functional predictions of their model.

Finally, a more general model extending from faces to other objects incorporates externally generated activity as inputs to two competing modules (Dailey and Cottrell, 1999). The mere addition of low-spatial frequency information to one of the modules and high-spatial frequency information the other is sufficient to bias the former toward individuating faces and the latter toward classification of objects at the superordinate level. Although not explicitly a developmental model, it is interesting that LSF information leads to face individuation,

since LSF processing is more commonly associated with threatening stimuli including faces. Furthermore, this model provides an explanation of how other (dissimilar) objects may come to be represented in separate circuits from those involved in face processing.

4.27.5 Conclusions

Supposing that face recognition develops in a similar fashion in humans and macaques, there is some evidence for the existence of a neural system that contains both innate and sensitive-period elements. It appears that such a system:

1. supports recognition of threatening facial expressions with no prior experience;
2. requires normal visual experience in the first postnatal months to support configural and holistic processing; and
3. under normal circumstances, acquires many of the adult face-processing abilities within the first few months after birth, beginning with a preference toward low-spatial frequency images with a top-heavy bias.

The term 'neural system' is meant to include any brain region or structure that contributes to any of the numerous aspects of face processing, including recognition of the individual, threatening expressions, or gaze sensitivity. There is evidence that these processes occur at different points in development, and may involve largely independent regions in the brain. We nonetheless consider these as components of a larger system. The CONSPEC/CONLERN model proposes independent components that are useful at different stages of development. In contrast, the pattern-generating wave model proposes largely interacting components. By way of illustration, consider the amygdalar modulation of the fusiform gyrus activation for fearful faces (Vuilleumier *et al*., 2004) . The amygdala is not currently thought to help in the individuation of faces, but the fusiform region is. Yet, amygdalar activity can modulate face responses in the fusiform gyrus. These two areas may have separate developmental histories and primary functions, and nevertheless interact under the appropriate conditions of face processing. It is in this sense that they may be better conceived as parts of a larger neural system for face processing.

Understanding which neural components subserving face processing are most closely linked in function and development will require further research. Studying the specialization of structures before birth, and the mechanisms underlying the rapid changes in ability after birth, could benefit from the intersection of two lines of research: (1) cross-primate comparisons of face-processing abilities and the corresponding neural specializations, and (2) neural development occurring around the time of birth. Hopefully, such future research will clarify the function behind our innate, early, and protracted face-processing abilities.

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4.28 The Evolution of Human Emotion

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Glossary

homology	A trait or behavior in two related species that is attributable to a common ancestry.
social emotions	Emotions such as shame, embarrassment, guilt, and pride that require self-consciousness and an understanding of social rules and their transgressions.
theory of mind	The ability to understand the mental states of others.

4.28.1 Introduction: Emotion Is Necessary for Survival

> Human emotions, rooted though they are in our animal nature, are nevertheless run through, as mere animal emotions are not, with thought and belief, wish and want, fantasy and imagination, as should be expected of language-using, concept-exercising creatures (Bennett and Hacker, 2005, p. 31).

Emotion has long been viewed as an important phenomenon underlying animal behavior. Emotion helps individuals attend to and extract relevant information from the environment and organize physiological, motivational, and cognitive action patterns that facilitate adaptive responses to aid the survival of the organism. As such, emotion can be thought of as a process that facilitates appropriate responses to a wide range of both internal and environmental situations. Emotional reactions are critical in avoiding predation, finding mates, nurturing young, caring for the elderly, and engaging in behavior that is consistent with social and cultural rules. Some researchers refer to these as drives, innate reflexes, or unconditioned responses. No matter the term, each of these phenomena involves emotion.

Emotion is not only an elusive concept by definition, but the investigation of emotion is fraught with difficulty. One of the main reasons is that emotion influences, and is influenced by, so many facets of the mind and biology. Emotions involve cellular and hormonal processes that in turn affect physiological and neural systems. These collectively affect behavior, the interactions between individuals and other species, and ultimately the integration of the individual within its society. In part because of its interdisciplinary nature, it is becoming a more common practice for researchers to examine emotion as a multidimensional construct, incorporating multiple levels of analysis that cross basic evolutionary, biological, behavioral, social, and cultural boundaries (Cacioppo and Gardner, 1999; Frijda, 1986; Kappas, 2002; Keltner and Haidt, 1999). Whereas this componential approach has a progressive structure, ranging from basic biological to social processes, it has been argued clearly by others that this progression is anything but linear or hierarchical (Cacioppo and Berntson, 1992). In fact, social factors can influence biological factors and vice versa, with no one level of analysis having any greater role than another for understanding emotion. Many have argued that the only way to fully understand emotion is to examine each level, not restricting the analyses to any single component (Kappas, 2002; Leventhal, 1991). Only through the collective integration of ideas and findings across disciplines will researchers begin to identify the quantitative and qualitative features leading to the expression of emotion in animals

and ultimately to identify and understand what is unique about human emotion.

4.28.1.1 Directions for Understanding the Evolution of Emotion

This article will focus on three broad developments in the emotional behavior of animals with reference to human behavior. The first is that emotion involves innate, reflexive, and adaptive behavior that shares commonalities in its underlying mechanisms of production in all animals. Studies from both nonhuman primates and human neonates suggest, for example, that some facial expressions are produced from birth with little or no social experience. Additionally, the underlying mechanisms for these behaviors have a common signature in animals due to the role of various brain structures, particularly the limbic system, and the recruitment of physiological processes that serve to increase the survival of the organism (Panksepp, 1982). These survival responses are aided by the nervous system's bias for processing negative information at high levels of arousal (Cacioppo *et al.*, 1999). These reflexive and adaptive behaviors are arguably the building blocks from which more complex and flexible forms of emotional responses evolved and, even in advanced species such as humans, they are ever vigilant.

The second development is that socially living species have means to communicate with one another about emotion, through visual, auditory, tactile, or chemosensory signals. The communication system and the signals therein need not be specific or referential, or even self-conscious, for that matter. There need not be a specific association, for example, between the signal, e.g., a facial expression, and its emotional meaning, e.g., fear, although this has been suggested in humans (Ekman, 1992). Rather, these signals are predictive of the general motivation and/or tendency of the organism to engage in a series of actions given a set of social and environment conditions (van Hooff, 1973). The signals, however, must be easy to recognize and discriminate from other signals and/or noise in the environment, or their information content and predictive value will be lost. Thus, in addition to the signal being discrete and distinctive, the receivers of the signal must be tuned to its detection. Because of the strong relationship between facial expressions and emotion in humans, much of this section will focus on evidence for homologous facial expressions in humans and related species and introduce the development of a behavioral coding system, similar to that used for humans, that will prove extremely influential in identifying the underlying meaning of these signals.

Finally, greater flexibility in communication systems, more complex social environments, and individual social relationships fueled by an expanding neocortex contributed to the evolution of socially derived emotions, or self-conscious emotions, such as guilt, shame, pride, and embarrassment. These emotions are rooted in a basic awareness of the self and the unobservable mental states of others, a concept known as theory of mind. The absence of these socially derived emotions is, in many cases, associated with various forms of psychopathy and neurodevelopmental disorders such as autism (Heerey *et al.*, 2003; Hillier and Allinson, 2002; Levenston *et al.*, 2000). Though there is little conclusive evidence for theory of mind in species apart from humans, it will be argued that a precursor to theory of mind must have been an awareness of others' emotions, a basic form of empathy, which appears to be present in rudimentary forms in mammals, including our closest-living primate cousins (Parr, 2001; Preston and de Waal, 2002; Povinelli and Preuss, 1995).

4.28.1.2 Emotion Underlies Adaptive Behavior

4.28.1.2.1 Subconscious evaluative processes
Emotion involves behavioral reflexes that represent adaptive responses by an organism to environmental challenges. These basic reflexes can be deconstructed to include approaching things judged to be positive, or appetitive responses, and avoiding things judged to be negative, or aversive responses. This basic bivalent system was derived from research on unconditioned reflexes in animals, with appetitive motivational systems including behaviors such as ingestion, copulation, and nurturing young and aversive motivational systems including behaviors such as withdrawal and the rejection of noxious stimuli. Furthermore, there is evidence that these basic evaluative systems are implicitly associated with motor responses that aid in the adaptive responding of the organism. Studies have found, for example, that muscle extension is more often associated with negative affective responses, such as withdrawal and avoidance, than with muscle flexion, which is associated more with positive responses, or approach (Cacioppo *et al.*, 1993). In this experiment, stimuli were judged more pleasant when individuals flexed their arms as if pulling an item toward them than when their arms were extended, as if pushing the item away (Cacioppo *et al.*, 1993). These stimuli consisted of neutral Chinese ideographs, ruling out the possibility that subjects had preconceived affective attitudes toward these stimuli that subconsciously affected their evaluations. Additionally, these effects were not found when subjects simply observed someone engaging in

muscle flexion and extension, indicating that the motor action itself was necessary for differential evaluative responses. Similarly, response latencies to either push or pull a lever when presented with affective words were faster when the flexion action of pull was paired with a positive emotional word-stimulus and when the extension action of push was paired with a negative emotional word-stimulus than when push and pull were incongruently paired with positive and negative stimuli, respectively (Chen and Bargh, 1999). These associations were not the result of any conscious processes, in that subjects did not report any awareness of the association between their behavior and their affective evaluations. In addition, when asked to form intentional, conscious judgments about certain stimuli, such as semantic characteristics compared to evaluative characteristics, these responses weakened (Bargh *et al.*, 1996). Finally, these processes were found for novel, abstract stimuli that were rated as being either positively or negatively valenced (Duckworth *et al.*, 2002). This provides further support for the influence of unconscious, affective evaluative processes on the automaticity of adaptive behavioral responses (Murphy and Zajonc, 1993).

At this primitive level, appetitive and aversive behavioral responses are modulated by specific neural circuits in the brain that share a common neuroarchitecture among mammals. These brain systems are genetically hard-wired to enable animals to respond unconditionally to threatening, or appetitive, stimuli using specific response patterns that are most adaptive to the particular species and environmental condition (Berridge, 1996; Panksepp, 1982). When aversive stimuli are detected, for example, receptors in the sensory cortex and thalamus are activated. These sensory afferents activate the amygdala directly, leading to a cascade of responses that enable the organism to escape or reject the current situation (LeDoux, 2000). The same information travels via a slower route to the cortex and then back to the amygdala, providing the organism with an opportunity for postcognitive emotional evaluation. Appetitive systems, on the other hand, have a rich history in studies of the nucleus accumbens and mesolimbic dopamine system that are involved in regulating the body's response to reward and pleasure (see Berridge, 2003). Although it is still common for the neural control of emotional behavior to reside solely on the shoulders of the limbic system (MacLean, 1949; Panksepp, 1982), studies have implicated a variety of brain structures in both cortical and subcortical regions including the amygdala, cingulate cortex, hypothalamus, insular cortex, orbitofrontal and prefrontal cortices, and even brainstem sites and cerebellum (Allman *et al.*, 2001; Berridge, 2003; Lane *et al.*, 1998; Parvizi *et al.*, 2001). Studies in both humans and animals using a variety of techniques are now showing that a single brain area subserving emotion is drastically simplistic, in fact, just plain wrong. Rather, the brain appears to have distributed connectivity among regions that individually play key roles in the experience of emotion, but only collectively provide the color for emotional experience (for excellent reviews, see Berridge, 2003; Buck, 1999).

4.28.1.2.2 Emotions are negatively biased The evaluations described above are, however, not without influence and/or interindividual variability. Stimuli are evaluated not only with respect to environmental change but are weighted in relation to the individual's current motivation within that environment. To determine which response is most adaptive given a set of internal and environmental conditions, animals, like humans, rely on a basic system of appraisals that evaluate environmental events according to their self-relevance. Appraisals refer to implicit, rapidly occurring evaluations made about the significance of an object or event and are likely to differ depending on the individual, his or her previous history, and his or her current condition (Scherer, 2000). In terms of emotion, similar patterns of appraisals may emerge across individuals, resulting in similar emotional experiences. When objects and events are appraised as being conducive to achieving an important personal goal, happiness may result, whereas events that are appraised as occurring suddenly, with uncertain and potentially threatening consequences, are likely to result in emotions such as fear (Scherer, 2000). Whereas appraisals are considered a form of cognition, they are not necessarily conscious, although conscious awareness and control of behavior may occur later in the processing stage in some species.

From an evolutionary perspective, species should arguably be more invested in aversive, defensive response systems in some situations than appetitive, positive response systems. If an individual does not safely avoid the dangers of predation, he or she will not be able to seek out mates and reproduce, and thus contribute his or her genetic investment to future generations. Thus, individuals might be in a constant state of vigilance, with defensive systems ready to respond if dangers are detected. Moreover, positive and negative motivational systems may be co-activated, having nonreciprocal associations rather than occur along a single bivalent dimension. Thus, an animal may choose to approach a food source in the presence of a potential predator if its feeding motivation is high, co-activating appetitive and defensive systems. Similarly, a human may refrain from eating his or her favorite dessert when on a diet. Thus, an

improvement in the traditional bivalent system, where positive and negative motivations lie at opposite ends of a continuum, Cacioppo and Berntson (1994) have proposed that attitudes and emotions are better viewed according to a model of evaluative space. Evaluative processes refer to the neural circuitry and subsequent behavioral adaptations involved in processing the affective significance of a stimulus. This is opposed to nonevaluative processes that refer to the discrimination and categorization of such stimuli (Cacioppo *et al.*, 1999). According to the evaluative space model, positive and negative responses are under the regulation of separable motivational substrates that can act independently of one another (Cacioppo and Berntson, 1994). Therefore, dimensions of positivity and negativity can be activated reciprocally or individually or they can be uncoupled with each dimension either co-activating or co-inhibiting the other.

According to the evaluative space model, the positive motivational system is characterized by greater activation when the arousal level of the system is low. So, at low levels of arousal, individuals tend to respond with greater positive responses than negative responses. This also makes sense from an evolutionary perspective. Positive emotional behaviors, such as copulation, maternal care, and grooming, are all highly social interactions that are best served when individuals are calm and not likely to be easily driven into a heightened state of defensive arousal. In contrast, the negativity bias, as it has been termed, is defined as greater output in the negative motivational system than in the positive motivational system, given the same degree of activation, or level of evaluative input. Evidence for these two dimensions has been found in a number of studies. Negative stimuli, for example, elicited larger event-related potential responses in humans than positive pictures, even when subjects were not required to make any affective evaluations of the stimuli, evidence of the negativity bias (Ito and Cacioppo, 2000). In addition, Ito *et al.* (1998) used regression analyses to assess people's arousal ratings for a variety of emotional stimuli. As predicted by the regression parameters, a shallower slope and higher value at low levels of arousal were found for positive arousal ratings (higher intercept value), whereas a steeper regression slope with greater ratings of negativity at higher levels of arousal was found for negative arousal ratings (negativity bias). Thus, the model helps to explain the evolution of distinguishable motivational systems in animals that allow for the co-occurrence of exploration and self-preservation (Cacioppo *et al.*, 1999). These models are particularly useful for developing theories about the evolution of human emotion because they lay a framework for understanding the behavior of animals in an ever-changing and increasingly complex social and ecological environment. Basic drive systems are constantly present, but control over these basic reflexes creates a myriad of potential outcomes that have helped to shape the evolution of animal behavior and emotion. These concepts will become important for the discussions in the final section of this article.

4.28.1.3 The Innate Expression of Internal Arousal Systems

4.28.1.3.1 Hedonic facial reactions to taste One of the earliest and most conspicuous behavioral manifestations of these arousal systems is the production of facial expressions. Although numerous studies have examined both the perception and production of facial expressions in human infants (Izard, 1971; Nelson, 1987), fewer studies have focused on these distinctions in nonhuman primates. However, evidence to date suggests that some facial expressions may have strong innate components, occurring in primates soon after birth, with little or no prior social experience with conspecifics.

One set of evidence for the innate production of facial expressions comes from a series of elegant studies in humans and nonhuman species on the faciogustatory response to various tastes and smells. Sweet, sour, and bitter stimuli, for example, produced highly similar patterns of spontaneous facial displays in both adult humans and neonatal infants; i.e., neonates were less than 16 h old and were tested before their first feeding experience (Steiner and Glaser, 1995). These authors found very stereotypical facial reactions in all subjects in response to these gustatory and olfactory stimuli that could be divided according to basic hedonic valence. Sweet stimuli produced facial relaxation, retraction of the mouth corners into a basic smile, and licking and sucking movements (see The Evolution of the Sweetness Receptor in Primates). Bitter tastes produced depression of the mouth corners and elevation of the upper lip, whereas sour tastes produced pursing of the lips (Steiner, 1974). Based on these facial response patterns, stimuli could be divided into two broad hedonic or motivational categories: one of acceptance, consummation, and pleasure, and the other suggestive of rejection, repulsion, and aversion (Steiner and Glaser, 1984).

To determine the evolutionary commonality of these innate facial reactions, Steiner and colleagues examined facial responses to tastes in numerous

species of nonhuman primates including prosimians, New World monkeys, Old World monkeys, and hominoids (Steiner and Glaser, 1984, 1995; Steiner *et al.*, 2001). Facial reactions were coded using detailed ethograms of facial movements, including components of the facial action coding system (FACS) developed by Ekman and Friesen (1978) to characterize human facial movements according to their underlying facial musculature (see Section 4.28.2.2.2.1) and for ethological descriptions of primate facial expressions (Andrew, 1963). Also important to these studies is the concept of homology described in the second section of this article (see Section 4.28.2.1). In brief, it is important to show that the pattern of facial reactions and accompanying behavior is homologous across the species tested, thus enabling researchers to conclude that the behavior is the result of similar affective evaluations mediated by similar neural substrates. Results found that facial reactions and behaviors in nonhuman primates could be grouped according to positive and negative hedonic dimensions and that these affective behavioral reactions were similar across species and taste categories (Steiner *et al.*, 2001). Reactions observed in all species shared universal components, including a rhythmic pattern of tongue protrusions in response to the sweet stimuli and an open-mouth gape with head shake in response to the bitter stimuli. Furthermore, across taxonomic groups, taste-elicited affective reactions were more similar among closely related species. Hominoid-specific expressions, for example, included actions involving the lips and middle part of the face. A smile in response to sweet stimuli, for example, was observed only in apes and humans. Old World monkeys showed characteristic brow elevation in response to the sweet stimuli, a pattern not observed in the other species. Reactions specific to New World species included a tongue protrusion pattern that categorized bitter versus sweet stimuli. Therefore, these gustofacial responses are innate, stimulus-dependent communication signals common among related primate species that reflect the organisms' ability to differentiate afferent sensory stimuli along basic hedonic dimensions (Steiner, 1974; Steiner *et al.*, 2001). As such, these facial patterns can be used to measure and compare emotional responses across species and provide evidence for an innate and phylogenetic relationship between facial expressions and emotional experience.

4.28.1.3.2 The role of social experience One of the first psychologists to speculate about the relationship between emotion and social behavior was Harry Harlow at the Wisconsin Regional Primate Research Center in Madison, Wisconsin. His research focused on the emotional needs of young rhesus monkeys (*Macaca mulatta*) during their early stages of development. Although these studies did not explicitly emphasize the importance of facial expressions or emotional communication, this research was pivotal in documenting the severe emotional and behavioral consequences that result when young rhesus monkeys are deprived of social contact with their mother from birth and are raised in social isolation (Harlow *et al.*, 1971). The attachment of an infant to its mother was not simply based on the need for food, but on the even more fundamental need for what Harlow termed love and affection. These studies also provided an experimental format for examining nature versus nurture issues, such as which behaviors are expressed innately and which require social experience. Regardless of the specific application, these studies raised a critical awareness within the primatological community, and far beyond, as to the importance of early social experience for normal social and emotional development (Harlow and Mears, 1983).

During this period, Gene Sackett, a student of Harlow, demonstrated that socially naïve monkeys produced appropriate facial expressions and behavioral responses when shown photographs of threatening conspecifics compared to photographs of conspecifics with playful or fearful expressions, monkeys engaged in sex, or control photographs depicting nonsocial stimuli (Sackett, 1966). The key conclusion from these studies was that social experience was not necessary for the production of species-typical facial expressions; rather, these expressions were the product of an innate releasing mechanism and could be elicited in a morphologically intact form in the complete absence of social experience (Sackett, 1965). When these isolated monkeys were later introduced to a social group, however, they were unable to respond appropriately to the range of facial displays and complex emotional messages produced by their new group mates. It seemed that although the appropriate production of facial expressions may be the result of innate mechanisms, the comprehension of facial displays made by other individuals in a dynamic social context required a period of normal social and emotional development.

Sackett's studies suggest that the subtle context-dependent meaning of facial expressions can be learned only from direct social experience. A direct gaze, for example, is interpreted by rhesus monkeys as being threatening; hence an important component of a threat face is to stare directly at the opponent (Hinde and Rowell, 1962; van Hooff, 1962). But do monkeys really understand these

cues from birth or must they see others engage in these interactions before they understand their meaning? To study the development of how infant rhesus monkeys respond to a direct open-mouth threat, 1-, 3-, and 7-week old rhesus infants were tested on their responses to the faces of conspecifics with direct or averted gaze (Mendelson *et al.*, 1982). These faces were presented using projection slides. No differences in viewing were detected in the 1-week-old infants. By 3 weeks of age, however, infant monkeys looked for a longer time at the faces with the direct gaze than at those with the averted gaze. This increased viewing was accompanied by negative emotional behaviors; i.e., the individuals showed submissive squealing, grinned, and lip-smacked at the direct-gaze faces. By 7 weeks of age, the infants avoided looking at the direct-gaze faces altogether. Instead, these infants preferred to look at the faces with the averted gaze and no longer produced emotional responses in their presence. These results suggest that by 3 weeks of age, rhesus monkey infants understand the social implications of the direct stare and respond submissively to this threatening stimulus. These infants, however, do not as yet have the social competence or knowledge to avoid looking at these faces. By 7 weeks of age, the infants' behavior changed dramatically. They now learned to respond to this socially threatening stimulus by looking away, a response that is critically important for integration into a rhesus macaque social group.

Further evidence for the specific role of facial expressions in social communication was provided in a series of studies by Izard, one of the founders of facial expression research in humans and infants (Izard, 1971). In a series of studies, Izard and colleagues transected the facial nerve (cranial nerve VII) of 3-year-old monkeys and examined the behavioral and emotional consequences when these individuals were physically unable to make facial expressions. How would individuals get by when the primary tool for social and emotional communication was not at their disposal? Although these studies were never published in a peer-reviewed format, preliminary results suggest a number of important findings. (The preliminary report was published in Izard's seminal book *The Face of Emotion*, 1971, pp. 385–391.) First, the animals that received facial nerve transections displayed more aggression, mounting, and general activity than their cage mates. Second, when returned to their social group, experimental subjects showed greater displacement activities, threats, and attacks than group mates and eventually dropped in rank. Third, the relationship between the operated females and their infants was compromised. Infants appeared not as strongly attached to their mothers and spent more time apart from her interacting with other, unoperated group members. Results suggest that communicating through facial means is an important, and perhaps necessary, ability for social integration and the expression of typical social behavior.

A series of intriguing studies was performed by Robert Miller and his colleagues. They demonstrated that rhesus monkeys could communicate affective information to one another using facial expressions. Specifically, Miller *et al.* (1959) exposed monkeys to a photograph of a familiar group mate with a neutral face. The presentation of this conditioned stimulus (CS) was paired with a shock that subjects could avoid only by pressing a lever in their test cage. Subjects quickly learned to press the lever when this face appeared, thereby avoiding the shock, i.e., standard operant conditioning. Next, subjects were shown another photograph of the same CS individual, but this time making a fearful facial expression. When subjects were shown this new stimulus, they produced spontaneously significantly more avoidance responses than during the acquisition of the conditioned response when the CS was the same monkey with a neutral face. This suggests that a negative facial expression is much more effective in communicating an upcoming aversive event, i.e., the shock, than a neutral face, illustrating that at some level the monkeys understood its inherent negative emotional meaning. Similar findings have been reported in humans using implicit priming studies. Winkielman *et al.* (1997) demonstrated that when humans were presented with photographs of smiling faces for 10 ms, too quick for conscious perception, they rated neutral stimuli as being more pleasant than if primed with frowning faces or blank slides.

In a second study, Miller *et al.* (1963) tested whether a stimulus monkey and a responder monkey could cooperate in producing an avoidance response. In this paradigm, only the stimulus individual could see the CS that signaled the upcoming shock, but unlike the previous situation, this individual had no access to the avoidance lever. In another room, the responder monkey could access the lever, but it could not see the projection screen that displayed the CS. The responder monkey could see only a live video feedback of the face of the stimulus monkey in the other room. The idea was that changes in the facial expression of the stimulus individual as it viewed the CS would be sufficient to communicate the upcoming danger to the responder monkey, who in turn would press the lever so that

they both would avoid the shock. The result of this cooperative avoidance paradigm was that the responder monkey produced more lever presses during the period when the CS was visible to the stimulus monkey, indicating that the stimulus monkey's facial expression was sufficient in communicating the appropriate affective information to the responder monkey.

These studies serve to demonstrate several important characteristics of affective facial expressions in nonhuman primates. First, these expressions have an innate component. This includes both the production of specific facial expressions and the type of information they communicate. Threatening facial expressions are better for communicating negative events, congruent with their emotional quality, than neutral faces. Interestingly, this was not found for positive facial expressions, supporting the concept of a negativity bias in the evolution of affective communication (Ito *et al.*, 1998) and perhaps the fact that these monkeys were in a heightened state of arousal in this aversive conditioning paradigm. Second, they provide an excellent reminder of the importance of normal social development and social experience for learning the appropriate meaning of facial expressions and how to use them with sufficient skill when interacting with conspecifics.

4.28.2 Assessing Emotion through Facial Movement

Emotion, to this point, has been described primarily as an automatic, adaptive process with a strong innate component. Its social expression and utility in maintaining social cohesion, however, require experience and learning. Emotion is also an experiential process that includes a strong subjective component unique to each individual. Because this process may never be fully understood or achieve direct comparison both within and between species, emotion must be studied at a more concrete, observable level. The human literature has provided a rich history for the study of observable emotion through facial actions and expressions. From the early empirical work of French neurologist Duchenne de Boulogne, who was the first to electrically stimulate facial muscles to create emotional expressions, to more contemporary studies by Paul Ekman and others, the association between facial movement and emotion provides a means for identifying and measuring emotions in naturalistic and experimental contexts (Duchenne de Boulogne, 1862; Ekman and Friesen, 1975; Izard, 1971; Tomkins and McCarter, 1964).

Additionally, facial expressions provide a robust level of comparison for understanding the evolution of emotion for several reasons. First, the facial expression repertoires of related species, despite highly varied patterns of social organization, are very similar. Common facial expressions have been described in many different species of nonhuman primates, suggesting that they were important behaviors in our evolutionary history (see The Evolution of Parallel Visual Pathways in the Brains of Primates). Furthermore, some of these expressions are elicited in similar social contexts, which is suggestive of, although not evidence for, a common function or meaning. Second, the mimetic facial musculature that forms the structure of facial expressions is highly conserved across primate species. This indicates that, with few exceptions, the facial behavior of related species can be compared directly in a manner that reinforces evolutionary continuity in the form of expressive behavior. Third, a large body of multidisciplinary research, as has already been discussed, strongly supports the association between facial expressions and emotion in primates. Humans in many diverse cultures are able to identify basic facial expressions using emotion labels, leading to the claim that facial emotions are biological universals, not culturally derived behavior patterns. Finally, this cross-cultural research has been aided considerably by the development of an objective, highly detailed coding system for analyzing facial movements according to their units of production, the mimetic facial musculature. Therefore, facial expressions represent a highly salient and empirically robust unit of measurement for understanding the evolution of emotion in primates. These different points are discussed below.

4.28.2.1 Are Primate Facial Expressions Homologous?

4.28.2.1.1 Facial expression repertoires are similar among closely related species Although the order of Primates can be characterized by striking differences in social organization and behavior across species, the appearance of facial expressions has remained remarkably similar. Contemporary research over the past half-century has considerably advanced our understanding of the communicative repertoire of nonhuman primates. This includes detailed descriptive reports of facial expressions in a variety of primate species including chimpanzees, bonobos, macaques, and capuchin monkeys (de Waal, 1988; Hinde and Rowell, 1962; van Hooff, 1962; Andrew, 1963;

Goodall, 1968; Parr *et al.*, 2005; Weigel, 1979). The two main goals of this research have been to document the range of communication systems present in our closest ancestral lineage and examine whether these facial expressions are similar, both morphologically and functionally, to our own.

This similarity in the appearance of facial displays is due, in part, to a general conservation of the facial muscles that underlie these expressions (discussed below), which show a similar basic plan across mammals (Huber, 1931), and to ritualization, a process by which a formerly adaptive, unspecialized behavior becomes divorced from its originally adaptive context to take on a different and typically more communicative meaning. The result of this ritualization process has been that signals become easily recognizable, highly conspicuous, and often stereotypical in their movement, helping to ensure that they are easily detected and understood by members of the same species.

Due to the association in humans between facial expressions and emotion, comparing the characteristics of facial expressions between related species would be insightful for making general assumptions about the emotional quality of nonhuman primates' facial behavior. That said, determining homology in behavior is a particularly daunting task because there are many levels on which to draw such comparisons. Behavior can be homologous due to its genetic basis, ontogenetic development, morphological structure, nervous innervation, and/or behavioral function (Lauder, 1994; Redican, 1982). Indeed, a character may be continuous throughout evolution on one of these levels, but shift from the ancestral character on another level. This can make the identification of homologous traits both difficult and controversial (Cartmill, 1994). In determining the most optimal unit of analysis, researchers are often self-serving; i.e., primatologists might use behavioral appearance, whereas molecular biologists might use protein structure. In addition, a behavior might be strikingly similar between species at one level of analysis, i.e., such as the appearance of facial expressions, but closer examination of other levels including the ontogeny, underlying musculature, and social function, could reveal dissimilarity. This raises an important point with regard to understanding the homology of primate facial displays: visual appearance alone is insufficient to make assumptions about the homologous nature of facial displays because different species have different facial shapes that may influence the appearance of facial actions made by the same muscle, or the facial actions may look similar but the muscular basis for these movements might be different. Thus, for the comparison between human and nonhuman primate facial expressions, appearance and musculature together provide a better and more evolutionarily stable unit of analysis for understanding the evolution of emotion than appearance or social function alone.

Several expressions have been addressed in terms of human and nonhuman primate homology, particularly the silent bared-teeth (sbt) display, often called the fear grin, and the relaxed open-mouth face, or play face. These have been compared to the smile and laughter displays of humans, respectively (Preuschoft and Van Hooff, 1995; Waller and Dunbar, 2005). The sbt consists of a retraction of the mouth corners, pulling the lips away from the teeth while the mouth remains closed. The evolutionary origin of this movement is suggested to be a protective response in drawing the lips away from noxious stimuli (Andrew, 1963). Through ritualization, this movement has come to signal social submission and/or appeasement, although its use is quite varied. In the chimpanzee, for example, the sbt has been observed in aggressive contexts and more positive emotional contexts, such as affinitive behavior and play, and thus seems to have diverged considerably from its association with fear (Parr *et al.*, 2005). Van Hooff (1973) recognized this important deviation and suggested that in some highly developed taxa, the sbt might have a reassuring function associated with attachment tendencies. The muscle action that accomplishes this movement in both humans and chimpanzees is contraction of the zygomaticus major, although there has been debate about whether the same visual appearance can be achieved through the contraction of the risorius, which pulls the mouth corners laterally, or the platysma, which pulls the mouth corners downward on the jaw (Redican, 1982). These phylogenetic comparisons are important to fully understand the homologous relationship between underlying structure and observable function and how these have been shaped by evolution to fit the specific needs of each species.

4.28.2.1.2 Primate facial expressions share structural elements The mimetic muscles involved in the production of facial expressions have changed little over the course of primate evolution. It has even been argued that they have been shaped by evolution specifically to aid visual communication (Huber, 1931). The facial muscles in all primates are formed from two basic sheets – the sphincter colli superficialis and the sphincter colli profundus (Huber, 1931). The latter provides the material for the majority of the facial muscles, particularly

those that are involved in expressive movements, and the former develops into the platysma, a broad sheath of muscle fibers in the neck region. In general, the complexity and subtlety of the facial musculature as a whole are features that are unique to the order of Primates, but the relative size and differentiation of these two muscle sheets do vary between primate species. There is greater differentiation observed in the sphincter colli superficialis and, moreover, this differentiation is attributed to the more highly expressive species (Huber, 1931). Another interesting feature of this muscle sheet is that the individual muscles that arise from it often attach directly to the skin, which is in contrast to other skeletal muscles that attach to bone and connective tissue. This provides confirmatory evidence that the primary function of the muscles derived from the sphincter colli superficialis is to produce movement of the facial landmarks, i.e., skin, providing the basis for visual communication. There is also evidence linking the evolution of facial expressions and elaboration of the facial musculature to brain function. Sherwood *et al.* (2004) reported phylogenetic differences in the organization of neurons enriched with neurofilament protein in the primary motor cortex among nonhuman primates. Species with more elaboration in the mimetic facial muscles and greater repertoire of facial expressions, such as great apes and humans, showed a greater density of neurons that might contribute to greater dexterity and more voluntary control of the facial region. In addition, the volume of the facial nucleus in the pons of the brainstem was larger than would be predicted based on standard phylogenetic regression in great apes and humans, again suggesting greater spontaneous control of the facial musculature in hominoids than in other nonhuman primate species (Sherwood *et al.*, 2004). Thus, there appears to be a phylogenetic relationship between facial expression repertoire and neural control of the facial musculature.

An understanding of the phylogenetic nature of the facial muscles can inform us about interesting species differences in facial movements by tracing the origin of these movements back to their muscular basis. Prosimians, for example, an evolutionarily older group of primates, having split from the ancestors of anthropoids 40–45 Mya, have very fine control of their ear movements through action of the postauricular muscles. Contraction of this muscle retracts the posterior portion of the scalp and results in the action of ear flattening. This muscle is also present in the cercopithecine primates; however, it has less influence over ear movement and instead serves primarily to retract the scalp in collaboration with frontalis. Thus, in one species, contraction of this muscle produces noticeable differences in ear movements, but in other species, it acts mostly to change the appearance of the scalp. In humans and some species of great apes, further differentiation between the actions of the postauricular and frontalis muscles has resulted in a separation between the actions of the ears and scalp. As a result, ear movement is not associated with brow or scalp movement (Burrows *et al.*, 2006; Waller *et al.*, 2006). Some have suggested that because of the previous connection between the actions of the postauricular and frontalis muscles, the brow movements of humans are vestiges of ear perking movements in lower mammals, for example, when orienting attention (Andrew, 1963). It has been suggested that brow movements can act as conversational signals of emphasis and attention, invoking the phylogenetic origin of these muscle actions (Ekman, 1977).

An examination of the buccinator muscle provides an illustration of how muscles can be released from their original adaptive function and through ritualization can come to play important roles in social communication. The buccinator is a muscle that lines the buccal pouch in many primates and aids primarily in the manipulation of food in the mouth. In humans, the buccinator is used during mastication and when contracted it also dimples the cheek fat, an expressive movement associated with contempt and, in contrast to other emotional expressions, does not appear to be present in primate species other than humans (Matsumoto and Ekman, 2004). Thus, although some interesting differences between species can be discussed, primates appear to share displays that are similar in appearance (expressions), in their structural elements (underlying musculature), in their use in particular social contexts (function), and in their ability to communicate information to conspecifics (meaning). Thus, facial expressions provide a robust unit of behavior for comparing emotional communication across related species.

4.28.2.2 Assessing Similarity in Human Facial Expressions

4.28.2.2.1 Facial expressions as biological universals

Numerous reviews have been written regarding the ability of humans to recognize emotion from facial expressions (Ekman, 1992; Izard, 1994; Russell, 1995). The two main claims from this work have been that facial expressions of emotion are described by people in similar ways and are displayed in response to similar emotional

experiences regardless of culture. Considerable debate still surrounds these premises, largely due to the presence of well-known display rules and various methodological issues that arise when conducting cross-cultural research (Russell, 1995). Display rules represent culturally specific social norms that dictate when and when not to express certain emotions and/or behaviors in social contexts. One of the most well-cited studies is that of Ekman and colleagues (1972). They showed stress-inducing films to college students in the United States and Japan when the subject was either alone or in the presence of an experimenter from the same cultural group. When alone, the students in both groups made facial expressions that differed only minimally; however, in the presence of an experimenter, the Japanese students inhibited their negative facial expressions more often and showed more smiling than the American students (Ekman and Friesen, 1971, cited in Fridlund, 1994). This is presumed to be the result of the collectivistic Japanese culture to control negative emotional expression in public as a way of facilitating a harmonious community (Shioiri *et al.*, 1999).

Since this study, display rules have become an accepted mechanism for the regulation of emotion, although these studies have largely viewed regulation of emotion as occurring through the inhibitory control of facial expressions. Matsumoto *et al.* (2005) have developed a new approach that goes beyond simple measures of inhibition or suppression. According to the display rule assessment inventory (DRAI), individuals may express emotion with no inhibitions, amplify or deamplify the intensity of the expression, mask the true expressions with either a neutral face or another expression type, or qualify their expression, which involves showing the true expression together with a smile. In another study, these authors requested American, Russian, and Japanese subjects to try and feel a set of basic emotions in four hypothetical social settings: interactions with family members, with close friends, with colleagues, or with strangers. Americans were found to have the greatest amplification scores, whereas Japanese had higher deamplification and qualification scores (Matsumoto *et al.*, 2005). Thus, the DRAI provides a more sensitive and detailed tool for the measurement of cultural variation in the use of different display rules.

Cultural differences are also present in the ratings of facial emotions. Izard (1971), for example, tested the ability of American, European, Japanese, and African individuals to identify basic facial emotions from photographs of Americans and found some cultural variation in emotion recognition accuracy. American and European subjects showed the best overall recognition, whereas Japanese and African subjects showed quite poor recognition. Indeed, some expressions, i.e., anger, seem to be poorly recognized across cultures, whereas other expressions, i.e., happiness, are almost universally recognized. Several studies have attributed such discrepancies to in-group expertise effects. In other words, individuals are best at identifying facial emotion when posed by members of their own race, or at least of races for which they have considerable experience; i.e., Japanese-Americans would be good at discriminating Caucasian facial expressions, but Japanese students who have lived only in Japan would not. This phenomenon is very similar to the other-race effect described for general face processing and cannot be accounted for by simple motivational differences at judging faces of another ethnic group (Tanaka *et al.*, 2004). Elfenbein and Ambady (2003), for example, found that recognition rates for six basic emotions, happiness, fear, anger, disgust, sadness, and surprise, were significantly better when the judges were of the same ethnic origin as the posers of the facial expressions. Similar results were found when the ethnicities of the rater and the poser were different, but the rater had had considerable experience within the culture of the poser's country, i.e., Tibetan raters living in China or African raters living in the United States (Elfenbein and Ambady, 2003). Thus, emotion recognition judgments were influenced by the degree of exposure individuals had with the ethnicity in question. No such investigation has been made into potential cultural or regional differences in expressive behavior in nonhuman primates. That primates might show cultural variation in their use of communicative facial behaviors has not been suggested up to this point, despite evidence showing cultural variation in other types of behavior. Such an investigation, however, would be extremely interesting and informative.

4.28.2.2.2 An objective coding system links expressive movement to muscle action

4.28.2.2.2.(i) The FACS In one review, Paul *et al.* (2005) suggested that the activity of the facial muscles would provide a useful endpoint for comparing emotion in animals and humans if related facial expressions in animals could actually be identified. Due in part to the issues mentioned above, such as problems in identifying the homologous characters of facial behavior, very few studies have attempted to directly compare particular facial expressions in human and nonhuman primates. Darwin's (1872) seminal book *The Expression of the Emotions in Man and Animals* remains one of the most detailed

comparisons of facial expressions across species and it claims that, to understand human emotional expressions, one must first understand emotional expressions in animals. Even with renewed interest in this question, contemporary studies suffer from many of the same problems that Darwin encountered, namely, what unit of measurement to use and how to directly compare behavior across species without relying on anthropomorphic interpretations.

In humans, the development of an objective and extremely detailed coding system for measuring facial movements has virtually eliminated potential emotional biases that can arise when interpreting facial expressions. The FACS, developed by Ekman and colleagues, identifies a series of action units (AUs) that represent the movements of underlying facial muscles (Ekman and Friesen, 1978). This enables researchers trained in this technique to describe faces in terms of visible action units rather than emotions. When a person is happy, for example, he or she typically smiles. However, smiles can be posed, insincere, and even deceptive (Ekman and Friesen, 1982; Ekman *et al.*, 1988). Using the FACS system, however, smiles associated with genuine enjoyment, or so-called felt smiles, were found to be associated with contraction of the zygomaticus major, which retracts the lips over the teeth (AU12), and contraction of the obicularis oculi (AU6), which produces slight wrinkles around the eyes, sometimes called crow's feet (Frank *et al.*, 1993; Krumhuber and Kappas, 2005). The AU6 smile is often referred to as the Duchenne smile, after the seminal work on facial expression measurement by the neurologist Duchenne de Bologne (1862), which predated Darwin's book. Subjects rated smiles associated with genuine enjoyment, compared to those associated with false enjoyment, more accurately when those smiles contained contraction of the obicularis oculi and these individuals were viewed as being more positive. Researchers also found that there was less variability in smiles that contained AU6, suggesting that the presence of an AU6 is a reliable indicator that the smile is associated with genuine enjoyment (Frank *et al.*, 1993).

Various studies have validated the usefulness of the FACS system for identifying universal facial emotions and the unique involvement of individual AUs in their categorization. Kohler *et al.* (2004) used certified FACS coders to rate human facial expressions using action unit codes and then analyzed whether each facial emotion was associated with unique action unit identifiers. These FACS ratings revealed different patterns of action units for expressions of posed and elicited happy, sad, angry, and fearful expressions. Though there was some overlap in action units recorded for each facial expression category, both the presence and absence of unique units, or unit combinations, enabled an identifying pattern to be found for each emotion type – happiness: unique AU6 (cheek raiser) and AU12 (lip corner puller), absent AU4 (brow lower), and AU20 (lip stretcher); sadness: unique AU17 (chin raiser), absent AU26 (jaw drop); anger: unique AU9 (nose wrinkler) and AU16 (lower lip depressor), absent AU1 (inner brow raiser); fear: unique AU5 (upper lid raiser) and AU2 (outer brow raiser), absent AU7 (lid tightener) and AU10 (upper lip raiser) (Kohler *et al.*, 2004).

4.28.2.2.2.(ii) The chimpanzee FACS Our understanding of the evolution of emotion would be considerably advanced if such a rigorous and objective system could be applied to comparisons of human and nonhuman primate facial expressions. An FACS system for identifying chimpanzee facial movements based on the underlying facial musculature has been developed (Vick *et al.*, 2003). This involved cataloguing thousands of videos and still photographs of naturally occurring chimpanzee behavior and identifying recurring facial movements. The muscular basis for these movements has also been verified through an extensive review of the comparative anatomy literature and through direct intramuscular stimulation of both humans and chimpanzees to verify the appearance of facial movement when muscles are stimulated (Waller *et al.*, 2006). Additionally, facial dissections have been performed to identify the presence or absence and precise location of facial muscles in the chimpanzee to help in validating the behavioral and physiological approaches described above (Burrows *et al.*, 2006). These studies confirm the homologous nature of chimpanzee mimetic facial muscles and the action they produce when contracted. These studies are critical for comparing emotional expressions in chimpanzees and humans.

The development of a chimpanzee facial coding system will be extremely valuable for understanding similarities and differences in the facial movements of chimpanzees and humans. The use of such a system could, for example, provide a direct assessment of whether sbt displays differ among species where this expression has an appeasing, reassuring function versus a fearful, submissive function. One might assume that the emotional differences would also be associated with different muscle action patterns. The chimp FACS has already validated the subjective categorization of nine chimpanzee facial expressions: the bared-teeth display (bt), play face (pf), hoot face (ho), relaxed-lip face (rl), neutral (n), scream (sc),

Table 1 Predicted classification of nine chimpanzee facial expressions using chimp FACS by discriminant functions analysis

	Chimpanzee facial expressions assignment category								
	bt	*pf*	*ho*	*rl*	*n*	*sc*	*al*	*po*	*wh*
bt	78.9	13.2	0.0	0.0	0.0	7.9	0.0	0.0	0.0
pf	0.0	87.5	0.0	0.0	0.0	6.3	6.3	0.0	0.0
ho	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
rl	0.0	0.0	60.0	0.0	40.0	0.0	0.0	0.0	0.0
n	0.0	0.0	16.7	0.0	75.0	0.0	0.0	8.3	0.0
sc	5.6	2.8	0.0	0.0	0.0	91.7	0.0	0.0	0.0
al	20.0	20.0	20.0	0.0	20.0	0.0	20.0	0.0	0.0
po	0.0	0.0	84.6	0.0	0.0	0.0	0.0	7.7	7.7
wh	11.1	0.0	0.0	0.0	11.1	0.0	0.0	0.0	77.8

al, alert face; bt, bared-teeth; ho, hoot face; n, neutral; pf, play face; po, pout face; rl, relaxed-lip face; sc, scream; wh, whimper.

alert face (al), pout (po), and whimper (wh). One hundred and seventy-eight individual chimpanzee expressions from 63 different individual chimpanzees were FACS coded using 10 action units in the chimp FACS (by two trained chimp FACS coders), AU6, AU9, AU10, AU12, AU16, AU17, AU22, AU25, AU26, and AU27. These were then subject to a discriminant functions analysis, similar to the approach used by Kohler *et al.* (2004). According to this analysis, over 74% of the expressions were categorized correctly after cross-validation using chimpanzee FACS action units. The classification matrix can be seen in Table 1. Expression types that were not accurately classified included the relaxed-lip face, the alert face, and the pout. Based on this kind of analysis, prototypical action units that best characterize each expression type can be identified, a tool that will be extremely useful when comparing directly the homologies between emotion expressions in chimpanzees and humans.

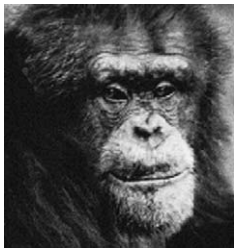
Table 2 provides a comparison of prototypical facial expressions in chimpanzees and humans and gives their collectively coded action units according to the human FACS and the newly developed chimp FACS. These are matched not only according to their morphological similarity and involvement of similar muscle movements, but according to their functional similarity as well. It is not possible to provide a detailed assessment of the proposed function of chimpanzee facial displays in this article. Readers are referred to numerous excellent reviews and ethograms for detailed descriptions (Goodall, 1968; van Hooff, 1967, 1973; Marler, 1965; Parr *et al.*, 2005). In brief, the bulging-lip face is typically displayed by males after a dominance display, suggesting its association with displaying status and superiority. The bared-teeth display can be silent or can be accompanied by a scream, squeak, or bark vocalization. It is used in many different situations including times of uncertainty, to signal appeasement and/or friendly contact, when fearful, and during times of extreme excitement, such as feeding time. No study to date has attempted to determine whether the bared-teeth displays used in each of these contexts can be distinguished morphologically, as in the case of the Duchenne smile. Chimpanzees scream in many contexts, including during aggressive/assertive situations, after receiving aggression, and in protest of another's actions. The pant–hoot is used to signal excitement over long distances and most often precedes a dominance display, or bluff display, and is often accompanied by piloerection. The pout is given when individuals are denied access to something and are most typically seen in weaning infants and during times of distress. The relaxed open-mouth display, or play face, is used almost exclusively during playful interactions with other individuals.

4.28.3 The Evolution of Social Emotions

4.28.3.1 Building Blocks for Complex Emotions

Group living offers many advantages to the individual, including access to resources (mates, food, and shelter), predator defense, and the ability to defend resources from intergroup encounters. Yet, group living also incurs costs, for although animals in groups are able to access and defend a greater number of resources, they also experience increased intragroup competition for these valuable resources. Many animal species have evolved strategies for dealing with these cost–benefit conflicts, but many primates have mastered a particular quality of behavior that allows complex, interactive groups to form and remain stable for long periods of time. We refer to this as sociality. Sociality reduces the

Table 2 Chimpanzee and human facial expressions characterized by the chimpanzee and human FACS systems

Chimpanzee display		*Prototype AUs*	*Equivalent human facial expression?*	
	Bulging-lip display	*AU17* + *AU24*		Angry face
	Silent bared-teeth display	AU10 + *AU12* + AU16 + *AU25*		Smile
	Scream	*AU10* + *AU12* + AU16 + *AU25* + *AU27*		Scream
	Pant–hoot	AU22 + AU25	?	
	Relaxed open-mouth display	*AU12* + *AU25* + *AU26*		Laughter

AUs in italics are found in the chimpanzee display and the proposed human equivalent.
Human images from Matrinez, A. M. and Benavente, R. 1998. The AR Face Database. CVC Technical Report No. 24.
Taken from Waller, B. M. 2005. Facial Expressions in Chimpanzees and Humans: Measurement and Meaning. PhD. thesis, Department of psychology, University of Portsmouth.

costs associated with group living by facilitating the formation of flexible dominance hierarchies and assisting in the formation of long-lasting relationships, including alliances, coalitions, and cooperative behaviors (Boesch, 2002; de Waal, 1989).

Arguably, the difference between groups of animals that exist with sociality and those that exist without sociality is the presence of a complex, yet flexible system of communication. It is in this capacity that the relationship between distinctive, observable facial movements and underlying emotional states might be more central for understanding sociality than previously considered. Once emotional states are expressed, interpreted by group members, and responded to in socially appropriate ways, requiring a prolonged period of social learning during early development, they become part of a dynamic, interactive social network, or society. In addition, the cognitive processes necessary for both the effective production and efficient processing of signals within such a communicative system are also likely to have undergone selection and may have even co-evolved within this system (i.e., see social brain hypothesis in Dunbar, 1998). The end result is that animal relationships and societies are hinged on emotional communication and the more complex the society/relationship, the greater the need for the expression, perception, and perhaps most importantly, the experience of emotion. The presence of nonlinear hierarchies, intragroup and intergroup encounters, and complex social organizations replete with dynamic and reciprocal social relationships have contributed to the advancement of social emotion/motivation systems beyond the innate, reflexive survival mechanisms described above. Here we will describe several unique qualities of the social organization of chimpanzees and how this may have placed demands on their communication system leading to the evolution of social emotions.

4.28.3.1.1 Complex social environments and social relationships The ability to recognize and monitor not only one's own relationships, but also the relationships among other individuals, though striking in chimpanzees, is not a skill unique among anthropoid primates. Many other primate species show complex forms of social knowledge (e.g., Cheney and Seyfarth, 1990). Social knowledge is critical for navigating a social environment in which knowing who is your friend and, perhaps more importantly, who is your aggressor's friend is crucial to avoiding conflict and maintaining access to resources. Valuable social and physical resources (e.g., grooming bouts, food sharing, and alliances) are traded between individuals in a 'biological marketplace' where traders must not only keep up with their own trading partners and transactions, but also keep track of commodity flow among others (Henzi and Barrett, 2002; Noe and Hammerstein, 1995). Primates, and chimpanzees in particular, are known to share food in exchange for grooming bouts (de Waal, 1997), engage in cooperative hunting (Boesch, 2002), and form coalitions (Watts, 1998). Such mental record keeping in complex societies typically involves triadic interactions, where individuals monitor and manipulate the behavior of others, not only in reference to themselves, but with reference to others. Triadic reconciliations have been reported in a number of species, where the former aggressor in a conflict will engage in affiliative interactions with kin members of the victim and not the victims themselves (Aureli *et al.*, 1992; Call *et al.*, 1996). Moreover, individuals who witness conflicts have been found to increase affiliative behavior with each other, despite having played no direct role in the conflict itself (Judge and Mullen, 2005).

The result of these complex interactions is that many societies have developed rules for social behavior that function to maintain order within the group. Some rules are basic and are likely to be present in many animals (i.e., do not bite the alpha male, do not kill infants), but others are more complex (i.e., the tit-for-tat reciprocity described among chimpanzees; de Waal, 1997). These require that group members recognize one another at the individual level, remember interaction partners, keep track of the details of the interaction, including the type of commodity involved, remember these events for some duration of time, make the decision to respond or not respond at some point in the future, and subsequently adjust their knowledge about the interaction partners for future reference. These details are specifically challenging for individuals that live in dispersed social systems in which individuals are not always in visual proximity and can travel individually or in small subgroups for periods of time (see Section 4.28.3.1.2). The basic assumption underlying social emotions, described below, is that they require individuals to understand that social rules and norms apply to their behavior and that violating these rules and norms will have social consequences. Among nonhuman primates, social transgressions are typically addressed by the alpha male of a group, who may physically punish those individuals who break social rules, but other group members, including females, may also play pivotal roles. De Waal (1982; 1989) has elegantly provided numerous descriptions of such interventions. Among

captive animals, humans may become entangled in such triadic interactions involving transgressions of behavioral norms that we have come to impose on our simian colleagues. The following anecdote was observed by the lead technician in L.P.'s laboratory:

> I had positioned the computer cart in front of the animal's cage for testing and loaded the software program. While holding the joystick panel up to the cage, Patrick (the only male in the group) was messing around with me while I tried to secure the top hook. Julie was sitting calmly and awaiting her turn, but then I noticed she began to fiddle with something below the panel. She used her two index fingers to fish the cord that ran from the back of the joystick to the computer through the caging. When I realized what she was doing, I tried to pull the cord from her, but she grabbed it and bit it at the same time. So this severed the cord from the joystick and she ran off with it. I proceeded to remove the joystick panel and the cage mates were all screaming. I looked into the cage and the older females were screaming at Julie. Lulu (47 years old) was above her on a perch and Wenka (52 years) was positioned at her side. Julie was on the floor below them against the wall. She was screaming with bared teeth and reaching out to Lulu with an open palm, a typical gesture used by chimpanzees when requesting reconciliation or reassurance. A few moments later Julie returned to the front of the cage and handed the cord to me, unsolicited, through the mesh. She then turned back to the older females and continued to gesture for reconciliation. Patrick, the only male in the group, was in the back doorway displaying. Later that afternoon, I went out to their group and Julie presented for me to groom her and allowed this for almost an hour! She was sweet and nonaggressive, quite different from her typical behavior toward me (May 2005).

All behavioral interpretation can follow a high or low cognitive road. The high-level interpretation of this scenario is that the older females in the group, who often take initiative in helping to resolve group disputes, recognized that Julie had done something inappropriate, i.e., grab and break the joystick. This action led the researcher to remove the joystick panel, which, despite the individual who is working on a task, is often associated with everyone getting some kind of food treat. They responded by berating Julie for her actions. Julie's only response was to use her communication skills to request forgiveness, by extending her open hand and baring her teeth, signaling her need for reassurance and for the screaming to end. Some time later, she allowed the researcher to groom her, perhaps in an attempt to re-establish a good social bond with the researcher after her bad behavior. A low-level interpretation might be that the old females were somehow disturbed by the broken joystick and responded with an egoistic bout of screaming, which would reveal that they were each affected in the same manner by the incident as they collectively targeted Julie. As in the high-level interpretation, Julie responded with social cues that she has learned will sometimes terminate conflict. Her grooming tolerance later might have been an attempt to receive some treats she missed earlier.

4.28.3.1.2 Nonlinear hierarchies and fission–fusion societies Many species of primates maintain stable groups through a linear and despotic dominance hierarchy (e.g., rhesus macaques, *Macaca mulatta*; de Waal and Luttrell, 1985). According to such a system, dominance relationships between two individuals are unambiguous and stable: the dominant animal will always win the resource through force and intimidation, and this is not likely to change during the lifetime of the individuals. However, some species have more flexible, egalitarian styles of dominance, in which power relationships can be more fluid and complex (e.g., chimpanzees, *Pan troglodytes*; de Waal, 1982). Contests are won through cooperation and alliances rather than aggression and specific dominance relationships are dependent on the presence or absence of other individuals – alliance partners – and can change often throughout one's lifetime.

Preuschoft and van Hooff (1995) have proposed that the use and function of communicative signals among primates vary in accordance with dominance style, reflecting the specific demands placed on the communication systems of species with different social hierarchies. A full description of this hypothesis (the power asymmetry hypothesis of motivational emancipation) is outside the scope of this article (Preuschoft and van Hooff, 1995, 1997). Briefly, species with strict dominance hierarchies show clear signals of affiliation, appeasement, and submission that can be easily distinguished from one another in both their morphological form and context of use, or social function. The sbt display in rhesus macaques, for example, is a highly ritualized facial expression of submission that is unidirectionally performed by a subordinate individual toward a dominant (de Waal and Luttrell, 1985). In contrast, species with a more relaxed, nonlinear dominance style will show less predictability between display types and their associated social function. The homologous sbt display in Tonkean macaques, for example, might be performed by the dominant individual in some contexts, and its form has become blended with that of an appeasement display, changing its social function in this species (Preuschoft, 1992). The same is true of the chimpanzee sbt display, which is often used during sexual contacts, during play, and during uncertainty (Parr *et al.*, 2005; Waller and Dunbar, 2005). In sum, the architecture of a flexible social environment requires signals to have

meanings that are transferable across different situations and, as a consequence, the demands placed on cognitive flexibility are increased as species must develop strategies for dealing with increasingly flexible communication systems (Parr *et al.*, 2005). If communicative signaling is additionally influenced by emotional state, it follows that species living in complex social groups must also possess well-developed emotional processing skills, as they must be able to interpret the different meanings associated with similar facial displays used in different emotional contexts.

An addition to the cognitive complexity placed on species living in relaxed dominance hierarchies, such as the chimpanzee, is the nature of their society itself. Chimpanzees, as is characteristic of most ape societies, live in a dispersed social system, what is referred to as a fission–fusion society. (Spider monkeys (*Ateles* spp.) are perhaps unique among monkeys in that they have fission–fusion structure.) In contrast to other primate group structures, fission–fusion societies are not characterized by constant, gregarious, group living, where all individuals travel and sleep together, but instead each chimpanzee community is composed of small foraging groups that unite after varying periods of time (Nishida, 1979). The large complete group is, nonetheless, bonded interindividually, and complex, long-term relationships exist among group members (Goodall, 1986). This presents a taxing cognitive challenge for these species as they must be able to keep track of and remember not only their own social relationships, but the relationships among other individuals who they may not see together for long periods of time. During that time, new relationships may have formed and these social changes must be processed in the absence of direct experience, i.e., by directly observing and interpreting behavior with reference to previous knowledge.

A hypothesis to explain the relationship between complex societies, advanced cognitive abilities, and unusually large brain size compared to body size among hominoids has been postulated. The 'social brain hypothesis' proposes that large neocortex volume and superior sociocognitive skills coevolved to deal effectively with the increasing demands stemming from large and complex social groups and more specifically the increased number of individual relationships that one can sustain (Dunbar, 1998). Large brains, such as those present in chimpanzees and humans, equaled better cognitive processing speed and the ability to keep track of many individuals at once, including interindividual relationships, and remember social events over longer periods of time. In fact, the part of the brain that shows the strongest relationship with group complexity is the newer region of the neocortex, not phylogenetically older regions, such as the amygdala or areas of visual cortex (Joffe and Dunbar, 1997). Moreover, Barrett *et al.* (2003) have suggested that maintaining group cohesion in light of such temporal and geographical distance is far more demanding in terms of computational and cognitive load than a group that preserves a constant grouping structure, such as that found among most cercopithecines. Therefore, there is compelling evidence that social cognition and brain evolution are mutually interactive.

In sum, facial cues may provide information about the consequences of social interactions even when group mates do not directly witness all the details of those events. Thus, facial expressions can be used not only to reason about the immediate motivation of the signaler, i.e., they are upset, hurt, or angry, but also to understand something about an event that was not observable, i.e., a change in social status. This distinction is similar to what has been described as knowledge by acquaintance versus knowledge by description. Knowledge by acquaintance is an innate understanding based on direct immediate perceptual experience, has been selected for throughout evolution, and is commonly found in most animals. In contrast, knowledge by description is a more general process whereby experience, conditioning, and cognition collectively provide the means to reconstruct raw data into an internal representation of reality (see Buck, 1999). Thus, this latter skill gives one the ability to reason about events in which the details of the events are not all immediately present. Although this does not indicate that nonhuman primates, such as chimpanzees, are able to attribute or reason about the mental states of other individuals, an ability referred to as theory of mind, this emotional reasoning ability, or emotional awareness, allows them to infer something about the quality of unobserved interactions among group mates. In sum, emotional communication and emotional awareness facilitate social cohesion and increases the quality of social relationships.

4.28.3.2 Social Emotions

Although researchers have described basic or biological emotions for several decades, some theorists have begun describing a new generation of emotions that appear to be specifically involved in social regulatory processes. These are considered secondary emotions, or social emotions, given their function in evaluating social interaction (Buck, 1999;

Keltner, 2003). Moreover, some of these social emotions have been viewed as moral, self-conscious emotions as they occur in response to the violation of social rules and moral behavior (Eisenberg, 2000), require an understanding of the consequences of one's actions and the expectations of others, and function to promote reconciliation and apology (Keltner and Buswell, 1997). These emotions are considered unique to humans and, to date, there is little justification to argue for their existence in other animals (Harris, 2003).

Although there is no general agreement as to the number of discrete social emotions, most theorists agree that they require some degree of self-assessment, or self-consciousness, and an evaluation of others compared to basic, or biological, emotions. Bennett and Hacker (2005) have identified these as pride, shame, humiliation, regret, remorse, and guilt. Others have limited the self-conscious emotions to include embarrassment, pride, guilt, and shame (Keltner and Buswell, 1997; Tangney *et al.*, 1996). Social emotions have also been regarded in terms of their reciprocal contingencies, i.e., whether the goal was achieved or not. These include pride/guilt, envy/pity, joy/sorrow, arrogance/shame, and jealousy/trust (Buck, 1999). Regardless of the number and/or psychological arrangement of these emotions, they share in common a degree of self-consciousness that is not typically invoked when referring to basic emotions such as fear, anger, happiness, sadness, surprise, or disgust. That said, social emotions are not always independent of biological emotions and are often extensions of basic emotions or include emotion blends.

4.28.3.2.1 Facial displays of pride, embarrassment, guilt, and shame Distinct displays have been associated with pride, embarrassment, and shame. Pride, for example, is associated with an upward head turn (AU53) and slight Duchenne smile (AU6 + AU12) with pressed lips (AU24) (Shiota *et al.*, 2003). Pride may be unique among the self-conscious emotions in that it is associated with positive affect, like happiness, whereas most self-conscious emotions are negative. Displays of pride may function to draw social attention to the individual, signal success at a socially valued endeavor, or highlight one's important status within the group (Shiota *et al.*, 2003). Unlike the basic emotion of happiness, however, pride does not appear to be recognizable from the face alone, with greatest recognition rates found in forced-choice tasks involving entire body postures (Tracy and Robins, 2003).

Embarrassment is described by a sequence of gaze aversion and gazing downward, smiling and smile control, a downward head turn to expose the neck, and face touching (Keltner, 1995; Keltner and Buswell, 1997). When these expressive components occur together, embarrassment is recognized up to 92% of the time from its facial expression alone (Keltner, 1995). This is comparable to, if not greater than, recognition rates of basic emotions from their expressive displays (see review by Fridlund, 1994). According to several theories, embarrassment is associated with an acute sense of how others evaluate their behavior and social identity (Tangney *et al.*, 1996). Thus, it can often occur when an individual fails to act in accordance with these socially defined conventions. These conventions must be known by others; for example, one might not experience embarrassment after tripping in a private situation compared to tripping in public (Keltner and Buswell, 1997). Moreover, the experience of embarrassment is less intense in the presence of family and close friends, individuals who would be very forgiving when social norms are violated, than in the presence of strangers (Lewis *et al.*, 1991). Finally, theorists have speculated that embarrassment has evolutionary origins in the appeasement behavior of animals. Appeasement, including bowing the head, gaze aversion, and face touching in nonhuman primates, restores social harmony, decreases the likelihood of future negative interactions and aggression, and reduces the interindividual distance among group members. Furthermore, these displays may have an earlier ontogenetic onset than previously considered, with reports of coy smiles appearing in human infants at approximately 2–3 months of age (Reddy, 2000). Thus, it functions to restore relationships after social transgressions (Keltner and Buswell, 1997).

Shame is typically displayed as a coordinated sequence of downward gaze and head movements and lowered posture. Shame typically occurs when one's actions fail to live up to the expectations of others, when one's personal standards are not met, or when one fears criticism or disapproval from others (Harris, 2003). Thus, shame necessitates an understanding of the expectations of others, the expectations of one's self, and the ability to weigh one's own actions against them. Shame can, for example, occur in the absence of onlookers and appears to be most associated with failure to fulfill obligations either to the self or to others (Tangney *et al.*, 1996). Guilt is similar to shame, but is different in that the transgression involves deviation from a moral rule or norm (Harris, 2003; Tangney, 1992). Therefore, in general, guilt is more society-focused, or focused on the specific behavior involved, whereas shame is more self-focused. Although distinctions

can be drawn between shame and guilt, both of these emotions correlate with feelings of empathy, suggesting a strong relationship between these emotions and perspective taking (Harris, 2003). Although Tangney (1991) found that when controlling for level of shame, guilt was more associated with empathic responsiveness than shame. There is no associated facial expression or postural expressive pattern described for guilt and it often results from the self-appraisal that one has put a low level of effort toward a situation.

These last three emotions do not have known correlates in nonhuman primates. Most of their behavioral elements are similar to those described for appeasement displays (Keltner and Buswell, 1997). Behavioral responses similar to shame, for example, have been observed by the author in the context of cognitive research on captive chimpanzees at the Yerkes Primate Center. Subjects are typically tested in pairs, with one going first while the other waits, and then their roles switch. This sequence has been reinforced for over 10 years of studies and very rarely does the subject waiting misbehave. If such misbehavior occurs, subjects might grab at the experimenter, or attempt to distract the testing subject by grabbing his/her hands away from the joystick, soliciting play, etc. When this behavior is discouraged by the experimenter, the chimpanzee will often lower its face, rubbing it with its hands, close its eyes tightly, and retreat to a corner of the enclosure. Although these are anecdotes and do not occur frequently, they suggest that the subjects understand the rules of the game and when their attempts to disrupt these conventions cause them to be scolded by the experimenter, they express behavior roughly consistent with the shame/guilt descriptions provided above.

4.28.3.2.2 Asymmetrical versus symmetrical facial expressions Another aspect of social emotions that makes them distinctive from basic emotions is that they often involve more exaggerated forms of expression and are often more asymmetrical. These exaggerations, or embellishments, can include winks or brow raises that are typically unilateral in their execution. The wink, for example, involves the eye closing (action unit LAU46), which can refer to a private message, be suggestive, or teasing. A unilateral outer eyebrow lift (LAU2) can be questioning, doubtful, or an expression of interest. The unilateral upper lip raise (LAU10) and the unilateral nose wrinkle (LAU9) are typically involved in expressions of contempt or disgust. More specifically, contempt has been shown to involve a unilateral lip corner raise and tighten (LAU12 + LAU14; Matsumoto and Ekman, 2004). Although contempt is often referred to as a basic emotion, this is debated in the literature (Izard and Haynes, 1988) and studies have shown that scenarios involving contempt can indicate an assessment of another's low social status or one's own demonstration of moral superiority (Keltner and Haidt, 1999). It is unclear, although doubtful, that such embellishments or patterns of asymmetry are present in nonhuman primates despite findings that many of their basic expressions involve asymmetrical components (Fernandez-Carriba *et al.*, 2002). These asymmetries reflect a greater intensity of the displays associated with one side of the face or the other and do not involve the inclusion of unilateral embellishments.

4.28.3.3 Brain Systems Involved in Social Emotions

In a review of neuroimaging studies of emotion, Phan *et al.* (2002) conducted a meta-analysis of emotion studies using both positron emission tomography and functional magnetic resonance imaging (fMRI). This included 55 studies using only healthy human subjects published through the year 2000. They further categorized studies according to whether the task involved a distinct cognitive component, such as emotion naming, emotion identification or ratings, and recall/encoding or recognition, or noncognitive tasks involving the passive presentation of emotional stimuli. The results revealed the most commonly activated region, regardless of the type of task or emotion employed in the study, to be the medial prefrontal cortex (MPFC). Researchers have, for example, consistently reported activation of the MPFC during the processing of photographs designed to elicit either pleasant or unpleasant emotion, compared to neutral photographs (George *et al.*, 1993; Kesler-West *et al.*, 2001; Lane *et al.*, 1997). Specifically, the rostral–ventral area of the MPFC has been shown to respond to individual emotions (BA9 + BA10). This region extends into the anterior, emotion-processing region of the cingulate cortex, which will be discussed below. Moreover, this region is separable from the orbitofrontal region (BA11 + BA12), which, although associated with processing positive and negative emotional images, is thought to play a predominant role in the monitoring and evaluation of the quality of social versus nonsocial information (Damasio *et al.*, 2000; Northoff *et al.*, 2000).

The emotion of fear was found to be strongly associated with activity in the amygdala (Phan *et al.*, 2002). This finding supports data from

numerous areas of psychology and neuroscience that have shown the amygdala to involve a direct, fast-acting pathway for the precognitive and emotional aspects of stimulus processing. The amygdala has been implicated in the processing of fearful facial expressions, whether processed implicitly or explicitly (see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates; Adolphs, 1999; Morris *et al.*, 1998; Whalen *et al.*, 1998), during fear conditioning (Davis, 2000; LeDoux, 2000), and in phobias (Lang *et al.*, 2000). Because of the primacy of fear processing in the amygdala, and its association with implicit autonomic arousal, some researchers have speculated that the amygdala is not so much tied to the processing of basic fearful stimuli, but rather is tuned to detect salient stimuli in the environment, even at subconscious levels (LeDoux, 2000). Therefore, the amygdala is critically involved in aiding the allocation of necessary biological resources to aid the organism's response to potentially threatening environmental stimuli.

The region of the cingulate cortex is interesting for the processing of emotion because it bridges the numerous brain areas including the amygdala and orbitofrontal cortex (OFC). Numerous studies have identified activity in the anterior cingulate in response to emotion processing (Damasio *et al.*, 2000; George *et al.*, 1993; Keightley *et al.*, 2003; Lane *et al.*, 1998). Additionally, the cingulate gyrus can be divided into a rostral–ventral region (BA25, BA33, and the rostral portion of BA24) that is involved in emotional awareness, and a more dorsal region that is involved in cognition and attention (Drevets and Raichle, 1998). Researchers have demonstrated selective activation of both the MPFC and the anterior cingulate when subjects were required to make an emotional appraisal of photographs, but activations were restricted to the amygdala and insular regions when the task involved only passive viewing, suggesting the specific involvement of these regions in more attentive, self-conscious emotion processing (Taylor *et al.*, 2000).

Interestingly, researchers have identified the presence of specialized projection neurons in layer V-b of the anterior cingulate cortex in great apes and humans, but not in gibbons or monkeys (Nimchinsky *et al.*, 1999). These cells, referred to as spindle cells because of their shape, might play a key role in the integration of sensory inputs related to emotion, vocal production, and the recognition of emotional faces, as described above. They lie in a region of the cingulate just superior to the genu of the corpus callosum (BA24), a region shown to be active during the recall of guilt emotions by Shin *et al.* (2000), described below. Thus, the anterior cingulate appears to play an important role in the processing of emotion, particularly when the emotion is explicitly evaluated, or processed with specific reference to the self, and these specializations may be unique to hominoids.

Although numerous studies have examined the neural responses to basic emotions, some of which are described above, fewer studies have examined self-conscious emotions or those that may involve perspective-taking skills (Phan *et al.*, 2002). Takahashi *et al.* (2004), for example, measured brain activity using fMRI as subjects were asked to rate the emotional content of short sentences describing situations of guilt or embarrassment. They revealed greater activation in the MPFC, left posterior superior temporal sulcus (STS), and visual cortex. Embarrassment was also associated with greater activation in the left OFC, anterior temporal cortex, and the anterior right temporal cortex. Distinguishing the two categories of emotion, however, was the finding of greater activation of the right temporal cortex, bilateral hippocampus, and visual cortex for embarrassment and greater activation of the MPFC for guilt (Takahashi *et al.*, 2004). Shin *et al.* (2000) found greater activation of the anterior temporal poles, anterior cingulate gyrus, and left anterior insular cortex during a relived-emotions task involving guilt than during a task involving neutral emotion. Thus, it appears from the associated brain regions that guilt involves more self-conscious emotion processing than does embarrassment.

When comparing responses to self-conscious emotions that involved either intentional or unintentional (embarrassing) violations of social norms using fMRI, Berthoz *et al.* (2002) found greater activity in the left medial and superior prefrontal cortex, anterior cingulate gyrus, left inferior parietal cortex, and left superior occipital gyrus. Specific judgments involving moral issues activated anterior prefrontal cortex (BA9 + BA10), posterior cingulate (BA31), and angular gyrus (BA39) (Greene *et al.*, 2001). Moll and colleagues reported activation in similar regions: the MPFC, anterior temporal cortex, angular gyrus, and globus pallidus; however, in another study, they included an important control for the neural systems activated by emotion judgments by presenting moral and nonmoral emotional stimuli (Moll *et al.*, 2002). Regions activated during the nonmoral condition included the amygdala, lateral OFC, and some areas of visual cortex. Moral judgments, however, activated the medial OFC, left temporal pole, and STS in a region near the angular gyrus. Comparing the two regions revealed selective activation of the medial OFC and STS in the moral compared to the

nonmoral condition. Thus, moral emotion judgments appear to exclusively activate OFC and STS and can be distinguished from self-conscious emotions that additionally activated the medial prefrontal cortex, portions of temporal cortex, and anterior cingulate cortex (Moll *et al.*, 2002).

Self-conscious emotions are, thus far, uniquely human traits and appear to be integrally associated with brain structures that also subserve theory of mind skills, including the medial prefrontal cortex, the anterior cingulate cortex, and the orbitofrontal cortex, as described above. Studies have examined the recognition of self-conscious emotions and their association with theory of mind skills in autistic children. Autism is a pervasive developmental disorder characterized by impaired language function, decreased social responsiveness, deficits in communication and emotional expressiveness, and repetitive or stereotypical behavior before the age of 3 years. Individuals diagnosed with autism spectrum disorder also have poorly developed perspective-taking skills (Dawson *et al.*, 2002) and lack of empathy and theory of mind (Baron-Cohen *et al.*, 2001; Joseph and Tager-Flusberg, 2004; Mundy, 2003). Impairments in joint attention skills in autistic individuals, for example, are more strongly correlated with impairments in tasks that rely on the ventromedial prefrontal cortex, an area that is associated with general affective, motivational, and social behavior (Dawson *et al.*, 2002). Heerey *et al.* (2003) examined the ability of autistic children to identify from photographs both self-conscious emotions, such as embarrassment and shame, and non-self-conscious emotions, such as contempt, anger, disgust, fear, happiness, sadness, and surprise. They found that the children with autism showed no differences in their ability to identify the non-self-conscious emotions compared to the control children, and these scores were uncorrelated with theory of mind skills, but the autistic children were significantly impaired in recognizing the self-conscious emotions. In addition, for both groups of children, their performance on self-conscious emotions was significantly correlated with their theory of mind skills (Heerey *et al.*, 2003). Hillier and Allinson (2002) examined the responses of children with and without autism to several levels of embarrassment: one in which the individual's behavior is conspicuous to others, one in which the individual takes into consideration how the audience evaluates his or her behavior, and one in which the embarrassing event happens to a friend. As opposed to other studies, children with autism showed deficits only when interpreting scenes of empathic embarrassment – the last scenario, in which the embarrassing event happens to the friend of the story's protagonist and the subject was asked to rate how the protagonist felt (Hillier and Allinson, 2002). Thus, their deficits in embarrassment occurred only in the context of empathic awareness.

Self-conscious social emotions, such as those described above, do not take the place of more primitive, basic emotions that are primarily under the control of subcortical structures and their interconnections. As Berridge (2003) has stated, subcortical systems do not lose their basic function in higher organisms, but they may give up some of their autonomy in terms of the degree to which higher cortical systems can modulate their functions or regulate the emotional experience. Emotion regulation is a growing area of research that will reveal important new insights into the role of specific brain regions involved in the cognitive control of emotion. These areas are, not surprisingly, similar to the brain areas involved in self-conscious emotions, most specifically the medial and lateral prefrontal cortices and anterior cingulate cortex (Beauregard *et al.*, 2001). What may fundamentally differ between humans and other animals, despite the evolutionary similarity in the organization of basic emotive brain circuits and connectivity (LeDoux, 2000), is the likelihood that these circuits have more elaborate, reciprocal connections to higher cortical areas involved in both the self-regulation of emotion and the experience of self-conscious emotions. These reciprocal interconnections provide the individual with information as to the nature and meaning of their emotional experiences (Panksepp, 1982).

4.28.3.4 Precursors in the Evolution of Human Emotion: A Final Word

This article has drawn a rough trajectory through various aspects of emotion and its impact on communication and the organization of primate societies, from reflexive, biologically adaptive behaviors to complex social interactions. The important theme therein is that in primates, both human and nonhuman, the ability to understand emotion in others is critical for maintaining social interactions. One way in which individuals are able to learn about the emotional states of others is through the process of emotional contagion. Primitive emotional contagion has been referred to as "... the tendency to automatically mimic and synchronize expressions, vocalizations, postures and movements with those of another person and, consequently, to converge emotionally" (Hatfield *et al.*, 1994, p. 153). Although this definition might be somewhat

limited, it does present a basis for comparative studies on the evolution of emotion across multiple modalities, e.g., face, voice, and posture, in which the presence, absence, or intensity of mimetic convergence across individuals can be empirically measured. Emotional contagion also describes a mechanism for the interpersonal sharing of emotion, or empathy, which features a matching of internal states between individuals, in that individuals will converge emotionally through the mimetic synchrony of behavioral expression. This emotional synchrony can be more specifically referred to as a kind of resonance of physiological arousal between the individuals. Similar changes in arousal between individuals can, under some conditions, result in similar subjective feelings that can significantly affect the subsequent behavior and emotional experience of the observer in the absence of conscious awareness (Dimberg, 1987; Vaughan and Lanzetta, 1980). This type of emotional awareness, or low-level empathy, functions to coordinate activity among group members, facilitate social cohesion, and motivate conciliatory tendencies and is likely to play a key role in coordinating social behaviors in large-brained social primates, such as hominoids.

Evidence for behavioral synchrony is widely reported among numerous animal species from schooling fish to the synchronized flying behavior of birds. Researchers have reported evidence of contagious yawning in adult chimpanzees in response to a video showing a conspecific's yawning compared to conspecifics with open mouths (Anderson *et al.*, 2004). Thus, only the actual behavior and not relevant aspects of the perceptual features of the behavior were important to elicit this behavior, similar to the data on reflexive crying in human neonates, in which only the cries of age-matched infants could elicit crying in subjects (Sagi and Hoffman, 1976). Among primates, behavioral synchrony also takes the form of basic imitation. Infant chimpanzees, like human neonates, have been shown to imitate facial expressions shortly after birth (Bard, 1998). Specifically, Myowa-Yamakoshi (1996) demonstrated facial imitation in an infant chimpanzee between 5 and 11 weeks of age, including mouth opening, tongue protrusion, and lip protrusion, in response to posed expressions by an experimenter. Notably, after 12 weeks, the infant chimpanzee showed a marked decrease in imitative performance (Myowa-Yamakoshi, 1996). A similar study reported facial imitation in two infant chimpanzees reared by their mothers within their first week of life (Myowa-Yamakoshi *et al.*, 2004). These infants imitated a human who demonstrated mouth opening and tongue protrusion, but this ability declined after 9 weeks of age, suggesting that this innate ability seen in neonates might be replaced by more socially communicative behaviors early in development, similar to what is reported in human infants (see Neurological Specializations for Manual Gesture and Tool Use in Humans; Meltzoff and Moore, 1977; Myowa-Yamakoshi *et al.*, 2004).

According to these examples, neonatal imitation is an innate/contagious process that provides not only valuable sensorimotor feedback for matching the behavioral state of another, but also provides an avenue for proprioceptive feedback that may facilitate an understanding of the relationship between facial expression and emotional responses. The importance of this relationship for emotional communication was reviewed in Section 4.28.2. Proprioceptive feedback, or affective resonance, in the form of physiological changes can easily become conditioned to specific internal or external events facilitating the likelihood that an individual, whether human or nonhuman, will show a similar emotional response in a future context. Therefore, one individual's emotional reactions may be considered a conditioned stimulus for unconditioned emotional responses in an observer (Vaughan and Lanzetta, 1980). Through these basic contagion processes, individuals come to learn about their own emotional states and the relationship between these and other's emotions during early ontogeny. As a consequence, emotional contagion and its affective resonance is one of the most powerful mechanisms for transmitting emotional information and facilitating coordinated actions and feelings among social animals.

From an evolutionary perspective, it is not surprising that one of the primary modalities for the transmission of emotional information is through the face. This article has highlighted some of the important issues in understanding the evolution of human emotion. Section 4.28.1 described the adaptive function of emotion in aiding the survival of living organisms. These innate biological reflexes include facial expressions and body postures that appear more tuned to processing negative information rapidly at high levels of arousal. Section 4.28.2 described the need for social animals to communicate about emotion and the importance of the face in affective communication. This section revealed that one of the primary modes for expressing emotion and reading emotional signals from others shares homologous mechanisms in humans and some nonhuman primates. Finally, Section 4.28.3 introduced the presence of self-conscious or social emotions and their relationship to specific brain regions that not only differ from regions involved in basic emotions

but also appear to overlap with brain regions subserving moral problem-solving and theory of mind. These emotions may be unique to humans. Although some have suggested that chimpanzees might share mental reasoning abilities, such as theory of mind, with humans (Hare *et al*., 2001; Tomasello *et al*., 2003), the data are scarce and limited to situations in which individuals compete for food. Thus, though clearly one role of emotion is to help an organism respond rapidly and unconditionally to life-threatening situations with adaptive behavior that increases survival, these emotion systems and the behaviors they produce are also highly dependent on an individual's life history, early experience, and cognitive perceptions in a complex social environment. Thus, one of the key roles of emotions is to free the individual from responding unconditionally. This provides humans, and perhaps related species, with a greater ability to speculate about emotion, engage in emotionally complex relationships, and attribute emotional states to others.

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http://www.ncbi.nlm.nih.gov – National Center for Biotechnology Information (NCBI).

www.chimpfacs.com – Chimp facial action coding system.

4.29 Evolution of the Neural Circuitry Underlying Laughter and Crying

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Glossary

affect induction	The process by which call acoustics elicit emotion-related responses in conspecific receivers.
crying	A distress signal produced by humans; characterized by repetitive, unarticulated vocalizations produced in conjunction with spasmodic breathing patterns and interruptions of vocalization, typically upon inhalation.
laughter	An affiliative signal produced by humans; characterized by repetitive, unarticulated vocalizations produced in conjunction with spasmodic breathing patterns and interruptions of vocalization, typically upon exhalation.
nonreferentiality	A quality of a signal that characterizes its dependence upon arousal, stimuli, and/or context in order to convey meaning.

4.29.1 Introduction

Humans are noisy organisms, persistently communicating with one another by vocalizing. Although speech is the most prominent means of communication among humans, it is not the only type of vocal signal on which we rely. Humans also chuckle, snort, gasp, weep, sigh, and use a variety of other vocal signals. Laughter and crying are two vocal signals that are often described as being uniquely human. Indeed, a number of aspects of these expressions differentiate them from other species' vocalizations. In the functions they putatively serve, however, these signals are not exclusive to humans. Other species produce both homologous and analogous signals, and while these are not acoustic equivalents of laughter and crying, their functional similarities suggest that similar neural substrates might underlie their production across species. Emphasizing comparisons between humans and nonhuman primates (hereafter primates), this article will describe the purpose of laughter and crying from an evolutionary perspective, and use studies of human and primate neural structures to describe the salient circuitry involved in the production of these dynamic signals.

4.29.2 Homologues and Analogues of Laughter and Crying

As is the case with most primate calls, human laughter and crying are species-specific. That is, homologous and analogous calls found in other species are not acoustically comparable to human versions. Laughter has been argued to have emerged during the course of hominid evolution to facilitate the occurrence of cooperative relationships, including those among unrelated conspecifics (Owren and Bachorowski, 2001). Some primates also exhibit such affiliative signals, with the laugh-like vocalizations produced by juvenile chimpanzees seemingly the clearest homologue to human laughter. Human crying, in contrast, is akin to the distress signals that have been documented to occur in a large number of primate species.

According to an affect-induction account of vocal signaling, the function of calling is to utilize the acoustics of vocalizations to influence the behavior of conspecific receivers (Owren and Rendall, 1997; Owren *et al.*, 2003). In the case of affiliative signals such as human laughter, calling serves to strengthen social bonds by forging associations between individually distinctive vocalizations and positive affect on the part of the receivers. Many species of

primates display a play face, an unequivocally positive facial expression that is considered to be a homologue to smiling in humans (Van Hooff, 1972). Only a few species of primates produce an accompanying pant-like vocalization that is most likely to be produced during roughhousing among younger animals in some primate species. This play panting, hypothesized to be the phylogenetic precursor of human laughter (Van Hooff, 1972), is exhibited in common chimpanzees and pygmy chimpanzees, although similar pants are produced during social play in both bonobos and Barbary macaques (de Waal, 1988; Kipper and Todt, 2002).

Species-specific distress calls are ubiquitous and have been shaped by the need to protect oneself against predators and aggressive conspecifics (Edmunds, 1974). Crying is functionally similar to these calls in that it is meant to influence conspecifics by encouraging them to aid the vocalizer. The noxious acoustic features of crying and other distress signals can readily induce negative affect in conspecific receivers (Owren *et al.*, 2003). Receivers typically seek to alleviate the induced discomfort, for instance, by aiding the vocalizer or ceasing an attack on the vocalizer.

Aside from primates, a few other mammalian species exhibit apparent analogues to both laughter and crying. For example, juvenile rats produce both a 50 kHz call that has been interpreted as an affiliative signal (Knutson *et al.*, 1998; Panksepp and Burgdorf, 2003) and a 22 kHz distress call (Anderson, 1954; Brudzynski, 2001). Although studied less frequently, vocal repertoires of the common cat have also been characterized as including both affiliative and distress signals (Zhang *et al.*, 1994; Nicastro and Owren, 2003).

4.29.3 Laughter and Crying Are Distinct from Speech

There are a number of aspects associated with both laughter and crying that indicate they are similar to particular forms of species-specific primate vocalizations, but dissimilar to speech. These features include, but are not limited to, production mode, repetition, innateness, and lack of referentiality.

Laughter and crying are both readily recognized by their distinctive acoustic features. Both are essentially repetitive, unarticulated vocalizations produced in conjunction with spasmodic breathing patterns (Deacon, 1989, 1997; Bachorowski *et al.*, 2001; Ruch and Ekman, 2001). Laughter typically involves interruptions of vocalization upon exhalation, whereas crying involves interruptions of vocalization upon inhalation. Many of the vocalizations produced by various species of primates also consist of this sort of repetitive call structure, spasmodic breathing pattern, and call interruption on inhalation, exhalation, or both. In contrast, a hallmark characteristic of speech is the communication of novel information. It is therefore rarely repeated in quick succession (Deacon, 1997).

Behavioral evidence also supports the homology of laughter and crying to primate calls, and distinguishes both from human speech. For instance, laughter, crying, and arguably most primate calls, with the possible exception of vervet monkey alarm calls, are innate (Seyfarth *et al.*, 1980). Primates reared without hearing their own species-specific calls can still produce those calls (Owren *et al.*, 1993), and humans who are born both deaf and blind still laugh and cry (Eibl-Eibesfeldt, 1989). Language acquisition, on the other hand, involves a critical period during which exposure to language is necessary for its ultimate emergence.

Laughter and crying, as well as the affiliative and distress calls used by primates, lack the referentiality of human speech. Speech is often used to reference objects, events, internal states, and organisms in a way that is not necessarily bound by arousal, stimuli, or context (Deacon, 1989; Hauser, 1996). In contrast, nonreferential signals such as laughter and crying depend on arousal, stimulus, and context. Rather than to communicate information, these nonreferential signals seem to function largely by eliciting affect-related responses in others (Owren and Rendall, 1997).

4.29.4 Neural Correlates in Primates

Two main brain regions have been consistently associated with the mediation of species-specific calls, including homologues and analogues of laughter and crying, in primates: the periaqueductal gray (PAG) and the anterior cingulate gyrus. We also note that many primate species also have a number of other brainstem and limbic areas that have been implicated in vocal production, including the internal capsule, cerebral peduncle, tegmentum, medial lemniscus, substantia nigra, inferior colliculus, pontine nuclei, amygdala, thalamus, ventral hypothalamus, and hippocampus (Deacon, 1989). Most of this neurophysiological research has focused on squirrel monkeys.

The short latency between stimulation of the PAG and a vocal response is one reason this area was first considered important to vocal behavior. Jürgens and Pratt (1979) found that vocalizations elicited from PAG stimulation not only show the shortest

response latency compared to all other vocalization-elicitation loci, but also show the greatest variety of calls produced. The caudal, rostral, medial, lateral, and dorsal sections of the PAG have all been implicated in species-specific call elicitation (Jürgens, 1994). The PAG and adjacent parabrachial and dorsal tegmentum are probably the phylogenetically oldest vocalization centers, and are argued to be the final common pathway for species-specific vocalizations (Deacon, 1989). That is, these areas receive convergent projections from almost all other loci implicated in vocalization as well as from the motor nuclei of the brainstem, which control vocal and respiratory musculature.

Ablation of the PAG results in mutism in monkeys and eliminates the capacity of any other loci to elicit vocalization (Deacon, 1989). Additionally, lesions to the areas of the parabrachial and dorsal tegmentum adjacent to the PAG have been shown to alter various qualities of species-specific vocalizations in many different species (e.g., Jürgens and Ploog, 1970). Lesions to the lateral tegmentum bordering the PAG have been shown to cause very specific deficits in primates. For example, in squirrel monkeys, it is possible (through partial lesioning) to abolish the vocal alarm call while leaving intact the vocal startle-response to being grabbed (Newman and MacLean, 1982; Jürgens, 1994). Jürgens and Pratt (1979) identified efferent projections from the PAG terminating in the parabrachial nuclei, the reticular formation of the lateral pons, the motor nucleus of the trigeminal nerve, and the area surrounding the motor nucleus of the facial nerve and the nucleus ambiguus. These areas are all highly involved in species-specific vocalizations. The motor nucleus of the trigeminal tract controls the mandible, the facial nucleus controls the facial musculature, the nucleus ambiguus innervates the laryngeal musculature, and the rostral nucleus ambiguus controls oral manipulation (Deacon, 1989).

The PAG does not project efferents to the hypoglossal nucleus, the motor nucleus that innervates the tongue. This absence of projections supports the contention that primate vocalizations are largely unarticulated. As noted earlier, this lack of articulation is also characteristic of both laughter and crying. The tongue does, however, play a crucial role in the articulatory gestures involved in speech production. There is thus evidence for a clear dichotomy between the neural pathways involved in species-specific primate calls and human speech, largely stemming from differences in the circuitry at the midbrain-pontine level (Deacon, 1989; see Evolutionary Specializations for Processing Faces and Objects and Premotor Cortex and the Mirror Neuron Hypothesis for the Evolution of Language).

It should also be noted that the areas implicated in primate species-specific vocalizations do not overlap those areas most commonly implicated in human speech, such as the left frontal neocortex. While damaging neocortical motor areas in humans can cause mutism via paralysis of the vocal musculature, lesions in the same areas in nonhuman primates do not appear to significantly disturb the structure of species-specific calls (Sutton *et al.*, 1974; Jürgens *et al.*, 1982; Deacon, 1989).

Early mapping studies of monkey brains (*Macaca mulatta*) demonstrated that species-specific vocalizations could be elicited by electrical stimulation of the anterior cingulate gyrus (e.g., Smith, 1945). Subsequent research revealed that many primate species, such as macaques, squirrel monkeys, rhesus monkeys, gibbons, and marmosets, also have so-called vocalization centers in or near the anterior cingulate cortex. Although the anterior cingulate gyrus has often been held to be a primary locus for species-specific call production, this view is incomplete. Lesions to the cingulate cortex do not abolish all, or even most vocalizations. Lesions to this area do, however, cause serious detriment in the conditioning of call production in macaques, possibly because of interference with volitional vocal production (e.g., Sutton *et al.*, 1972; Aitken, 1981). Furthermore, latency between stimulation and calling can be very long (up to 2.2 s) when the cingulate cortex is stimulated (Jürgens and Ploog, 1970; Jürgens, 1979). These latency data indicate that production of a vocalization might be secondary to other processes in the anterior cingulate gyrus. One hypothesis is that the anterior cingulate gyrus plays a mediating role in the production of affect-related vocalizations.

The research on neural correlates of species-specific calls in primates does not seem to distinguish between areas responsible for affiliative signals, distress signals, and other calls. For instance, different areas of the PAG have been shown to elicit various species-specific calls, but the exact loci vary among individual primates within a species (Jürgens, 1994).

4.29.5 Neural Correlates in Humans

Directly studying human neural circuitry is always difficult, but perhaps even more so with respect to laughter and crying. Aside from the difficulty of obtaining lesion-related data, the neural correlates of laughter and crying must be teased apart from those of the emotions that often elicit these behaviors. Some of the most informative research in this area comes from case studies involving exploratory stimulation and lesions due to pathology.

The neural correlates of pathological laughter and crying are studied far more frequently than those of typical laughter and crying. It is accepted that pathological laughter and crying are usually caused by disinhibition of regulatory circuitry, but the specific nature of this circuitry is still debated. For example, it has been asserted that lesions of cerebro-ponto-cerebellar pathways are responsible for the disinhibition of these behaviors (Parvizi *et al.*, 2001), but lesions that affect prefrontal regulation of limbic circuitry have also been implicated (Tateno *et al.*, 2004).

Still, case studies of pathological laughter and crying are germane to understanding the neural correlates of these vocalizations. A problem in sorting through these studies is that the pervasive neurological deficits that often result from stroke or other brain damage also often result in pathological laughter or crying. Because of the co-occurrence of pathological laughter and crying with other neuropathologies, the research on the neurology of pathological laughter and crying has probably invoked too many areas as being vital to their production. Patients suffering from pathological laughter and crying often self-report that their affective state is not appropriately matched by their vocalizations (although it should be noted that some patients do exhibit mood elevations or depressions that are respectively associated with pathological laughter or crying; Mendez *et al.*, 1999; Okun *et al.*, 2003; Wild *et al.*, 2003). Below are just a few of the case studies that examine the neural correlates of laughter and crying.

In their review of a case involving a 67-year-old man who had laughed unremittingly for 20 years, Mendez *et al.* (1999) proposed that the anterior cingulate gyrus is responsible for "endowing experiences with emotional consciousness." The authors also implicated a feedback loop involving the temporal lobes and amygdala, which both send emotional content back to the anterior cingulate. Lastly, they described a pontomedullary circuit that coordinates the cognitive, emotional, and muscular circuitry involved in laughter. These neural substrates are very similar to those discussed earlier in regard to primate vocalizations.

During exploratory intracranial stimulation of a 16-year-old girl, Fried *et al.* (1998) identified a 4 cm^2 area on the left superior frontal gyrus that was found to induce both laughter and mirth. When asked why she was laughing, the patient attributed humor to various objects or people in the room. Although the stimulated area was spatially close to the anterior cingulate, the results are difficult to interpret as humor and laughter were entangled. Still, this outcome is interesting insofar as it illustrates a top-down influence on laugh production.

Okun *et al.* (2003) described a case study of a 46-year-old woman with Parkinson's disease who demonstrated pathological crying when her subthalamic nucleus was stimulated; her crying was not associated with actual changes in mood. Although limbic cortices are highly involved in emotional processes, this finding supports the idea that these cortices play a role in the elicitation of crying that is separable from their role in the elicitation of negative affect.

Parvizi *et al.* (2001) reviewed the case of a 51-year-old, otherwise healthy, man who showed both pathological laughter and crying after a stroke. MRIs revealed three brainstem and two cerebellar lesions. The authors noted that the descending pathways to the cerebellum originate in areas that include motor and limbic cortices. These pathways then continue through the internal capsule and cerebral peduncle to the nuclei of the basis pontis. The authors thereby concluded that the observed pathological laughter and crying were a direct result of the partial deafferentation of the cerebellum, which caused deficits in the cerebro-ponto-cerebellar pathways responsible for mediating such vocalizations. While the cerebellum does not appear to be directly implicated in the production of such vocalizations in other research, it is possible that it inhibits their production. The other nuclei that Parvizi and colleagues identified as being important in both laughter and crying were all brainstem and limbic centers; these data are congruent with those obtained from studies on species-specific vocalizations in macaques.

Taken together, although very little research has been conducted directly on the human neurophysiology of laughter and crying, the available findings indicate that the neural substrates underlying these vocal behaviors include the same structures that appear to be involved in the production of species-specific calls in other primates. One notable exception is the PAG, which has not yet been directly implicated in the production of either human laughter or crying. There are two likely reasons for this exception. First, case studies involving neural stimulation (usually involving patients with intractable epilepsy) would most likely not probe the PAG. Second, lesions to the PAG would most likely cause widespread vocal deficits and therefore not directly link this area to laughter and crying.

4.29.6 Conclusions

Laughter and crying are examples of species-specific calls that are similar to the affiliative and distress, respectively, signals produced by other primates. The production and function of these vocalizations differentiates them from human speech, but supports the

idea that they are homologous or analogous to similar primate behaviors. Data from primate studies consistently show two main areas as being highly involved in the production of species-specific vocal signaling: the PAG and anterior cingulate gyrus. Along with involvement from other limbic and brainstem regions, these areas are thought to be responsible for coordinating the cognitive, emotional, and neuromuscular aspects of species-specific calls. The available data from case studies suggests that similar neural circuitry is involved in human laugh and cry production.

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4.30 Role of Spindle Cells in the Social Cognition of Apes and Humans

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Glossary

von Economo cells — Large bipolar neurons described by the anatomists Constantin von Economo and Georg Koskinas, in 1925. They are restricted to anterior cingulate cortex and frontoinsula cortex, and present in great apes and humans, but not other primates.

immunohistochemistry — The use of antibodies to recognize and label specific molecules in a tissue specimen.

Nissl stain — A classic cell-body stain used for nervous tissue. Cresyl violet, a typical Nissl stain, stains the somas of neurons and the nuclei of glia purple.

Golgi stain — A metal impregnation technique used to label neurons, glia, or nerve fibers. It is used in some applications to randomly stain the cell bodies and processes of a small percentage of cells in nervous tissue, allowing the dendritic arborization of a few individual neurons to be distinguished from the surrounding cells.

dopamine — A neurotransmitter derived from tyrosine. Dopaminergic cells are located in discrete nuclei in the midbrain, which project heavily to the frontal cortex and the basal ganglia. Dopamine is implicated in motor processes and reward processing, and is also the precursor to epinephrine and norepinephrine.

serotonin — A molecule derived from tryptophan. Serotonin is found widely throughout the central nervous system, where it acts as a neurotransmitter, and in the periphery. In the gastrointestinal system, serotonin causes the peristaltic action of the smooth muscles.

4.30.1 Morphology

4.30.1.1 Background and History

One way to divide neurons into distinct classes is to base the categories on shape, as is evident in the names of the pyramidal and stellate cells (see The Evolution of Neuron Types and Cortical Histology in Apes and Humans). Similarly, when Nimchinsky *et al.* (1999) identified a type of cell that was unique to great apes and humans, its identity was based on its distinctive morphology. At the time, they termed this population the spindle cells, but to avoid potential confusion with other uses of this name we now refer to them as von Economo (VE) cells.

This name is chosen in honor of the neuroanatomist Constantin von Economo, who, with Georg Koskinas, first described this distinctive class of neurons in 1925 (von Economo and Koskinas, 1925). Upon inspection of his Golgi preparations of human cortex, he noted that these large cells were located in layer 5 and restricted to two regions of the human brain: the anterior cingulate cortex (ACC) and in posterior orbitofrontal cortex adjacent to the insula, a region that he termed frontoinsular cortex (FI, Figure 1). Both of these regions lack a granular layer 4; as in motor cortex, this agranularity may reflect a functional specialization.

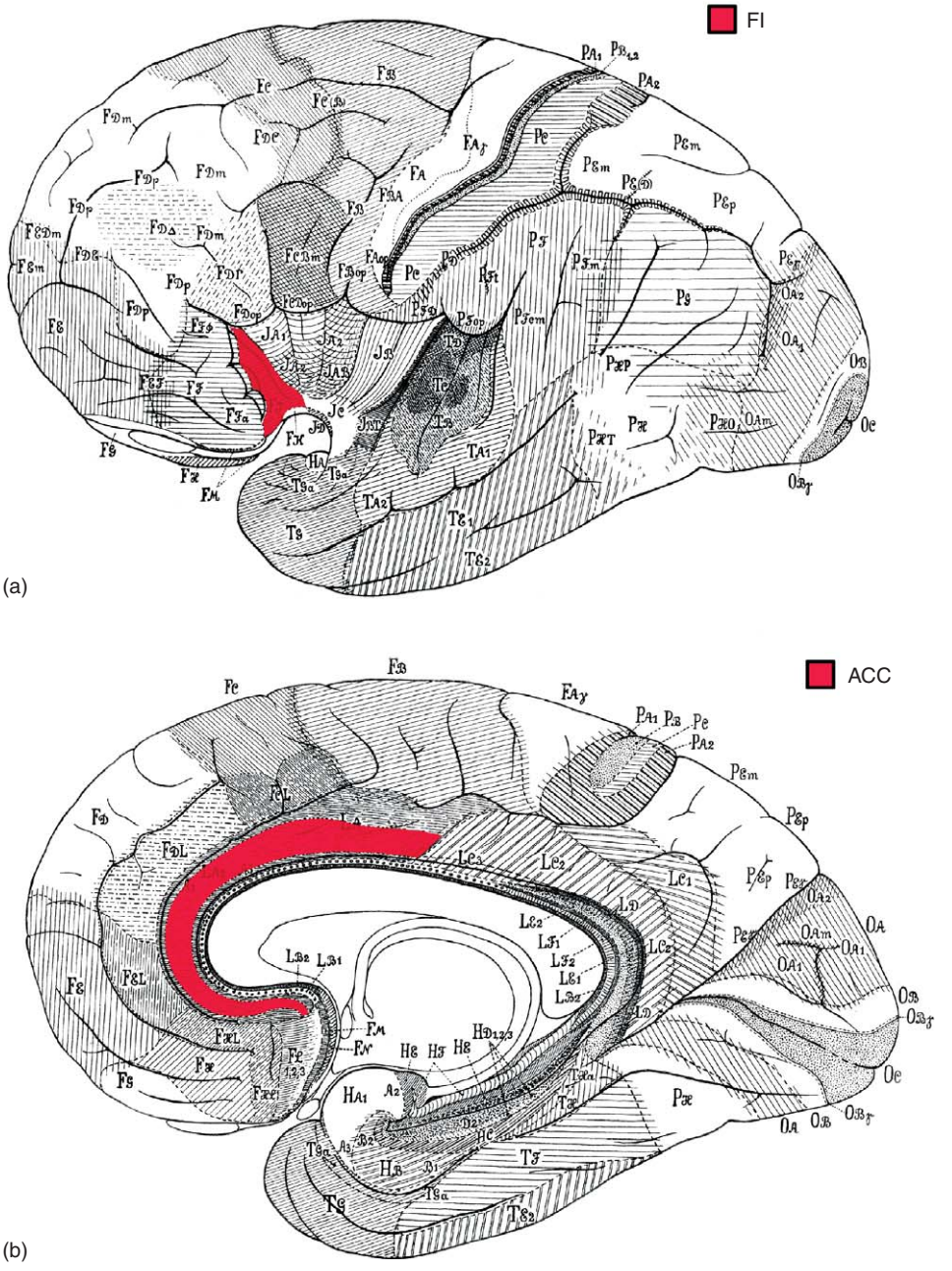

Figure 1 Cytoarchitechtonic divisions of the adult human brain as drawn by von Economo. Lateral view of the brain is shown in (a), with FI indicated in orange. In the medial view (b), ACC is visible and indicated in orange. Drawing modified from von Economo, C. and Koskinas, G. 1925. Die Cytoarchitectonik der Hirnrinde erwachsenen Menschen. Springer, by Atiya Hakeem.

In a cresyl violet-stained sample of human or great ape cortex, these cells may be easily distinguished from the neurons around them due to their symmetric, bipolar soma shape and their large size (Figure 2). Years later, it was discovered that these distinctive-looking cells were only present in the great apes and the humans, which implies that they evolved within the last 15 My (Figure 3). In the case of the VE cells in the FI, they are present only in the great African apes and not the orangutan. This pushes the likely emergence of VE cells in that region to 9 Mya, which in turn predates the rapid expansion of the hominid brain by 3–6 My (Kumar and Hedges, 1998; Wood and Collard, 1999).

Using the Golgi technique, we found that the apical dendrites of the VE neurons are quantitatively very similar to the apical dendrites of the neighboring pyramidal neurons; however, the branching pattern of the basal dendritic trees is much simpler on VE cells compared to the pyramids (Figure 4) (Watson *et al.*,

2006). The somatic symmetry that is apparent in Nissl stains is retained for some distance along the dendritic tree for VE cells. The Golgi technique also revealed spines on the VE neurons of the frontoinsula, which implies that these large cells are excitatory.

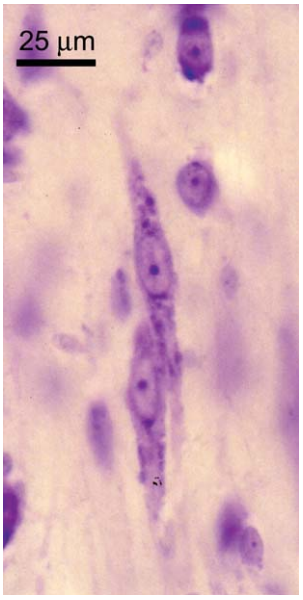

Figure 2 Nissl-stained brain section from a 7-month-old human infant. The somas of two VE cells are stained purple in the center of the image, with the apical dendrite oriented upwards. Note the elongated shape and presence of a single large basal dendrite.

In order to determine the projection targets of neurons, typical tract-tracing methods require invasive applications of the tracing material and the subsequent sacrifice of the animal. This technique would obviously be difficult or unethical in hominoids. However, there are several pieces of evidence to indicate that the VE cells are projection cells. First, they are located in layer V, a classical output later. Second, they are immunohistochemically labeled with SMI-32, a selective antibody for nonphosphorylated neurofilament (NPN) (Nimchinsky *et al.*, 1995). Upon phosphorylation, these molecules are translocated from the soma to the axon, where they increase the diameter of the axon and, consequently, transmission speed. Third, the volume of the VE cell somas is correlated with encephalization, with the largest VE neurons occurring in the largest brains of the primates. This is true also of the Betz cells of the motor cortex, which are known to project long distances (Sherwood *et al.*, 2003).

4.30.1.2 Sterology and Development

VE cells appear in the 35th week after conception in very small numbers. Unlike typical pyramidal cells, the VE cells are still very sparse at birth, suggesting that they differentiate from pyramidal cells or migrate in after birth (Allman *et al.*, 2005). Although we do not at this time know the exact age of proliferation, the population size is very

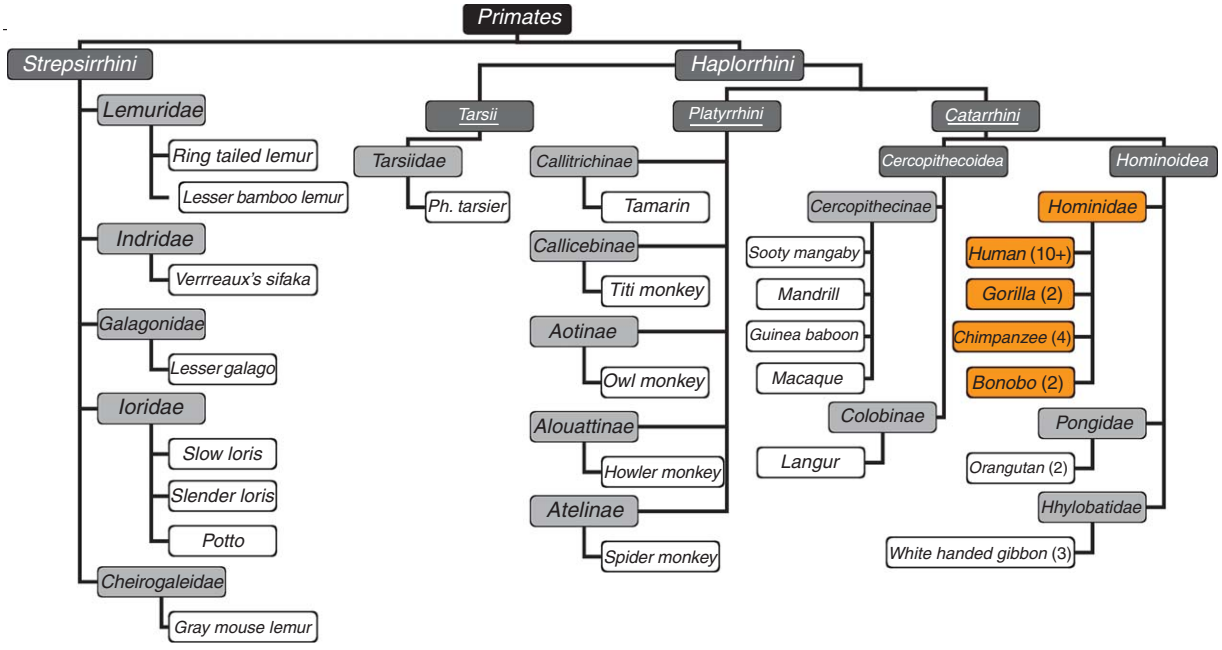

Figure 3 Primate phylogenetic tree. Species that have VE neurons in frontoinsula are indicated in orange. The number of brains that have been inspected is indicated. Based on the Semendeferi, Welker, Yakovlev, and Allman Brain Collections; analyzed by Atiya Hakeem, Nicole Teteault and John Allman. Figure by Atiya Hakeem based on Rowe, D. 1996. The Pictorial Guide to the Living Primates. Pogonias Press.

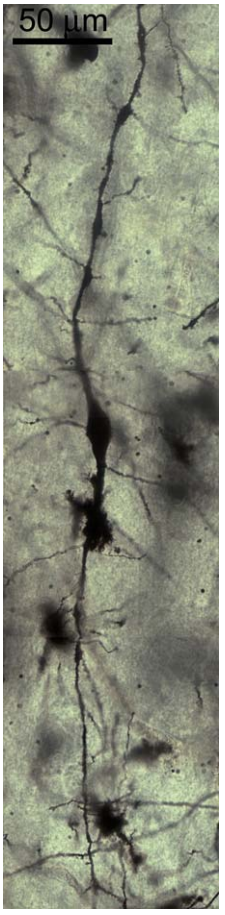

Figure 4 Neurolucida models of Golgi-stained neurons from the FI of a 23-year-old male human. Note the relative absence of dendritic complexity (i.e., sparse branching) on the VE neuron (left) in comparison to the FI pyramidal neuron (right). Neurolucia tracings by Tiffianie Jones.

near the adult level in the single 4-year-old human specimen that we have analyzed.

Humans have far more VE neurons than the great ape species. All species, however, show a consistent asymmetry, with about 30% more VE neurons in the right hemisphere compared to the left.

4.30.2 Immunohistochemistry and Functional Insights

What is the function of the VE cells? The fMRI literature reveals that two major types of paradigms activate the VE cell regions: decision-making in the context of high uncertainty and social paradigms. This in turn allows us to make educated guesses about what sort of molecules, particularly surface receptors, might be expressed by the VE cells, which we could then probe using immunohistochemistry on postmortem human specimens.

4.30.2.1 Uncertainty, Dopamine, and Serotonin

The VE cell regions appear to be strongly activated during periods of high uncertainty. In an fMRI study during which subjects were engaged in a simple gambling task, activation in both FI and ACC got increasingly stronger as the uncertainty in the task increased (Critchley *et al.*, 2001). In a similar vein, both regions were activated during a reversal task, in which a subject attempts to maximize reward during a task that changes contingencies when the subject's behavior stabilizes (O'Doherty *et al.*, 2003). A series of incorrect answers will prompt the subject to switch strategies, at which point both ACC and FI show increased activity.

Recordings from individual dopaminergic neurons in the macaque monkey ventral tegmentum reveals a similar pattern of activation. During trials with high uncertainty of reward, dopamine neurons exhibit a gradual increase in firing rate across the duration of the trial (Fiorillo *et al.*, 2003). Dopaminergic neurons project heavily to frontal cortex and limbic regions, including the VE cell regions. Using D3-specific antibodies on human brain tissue, we found that the VE cells are labeled strongly for this high-affinity dopamine receptor on the somas and apical dendrites (Figure 5). VE cells are labeled in this manner more often than the neighboring pyramidal cells, with 85% of VE cells

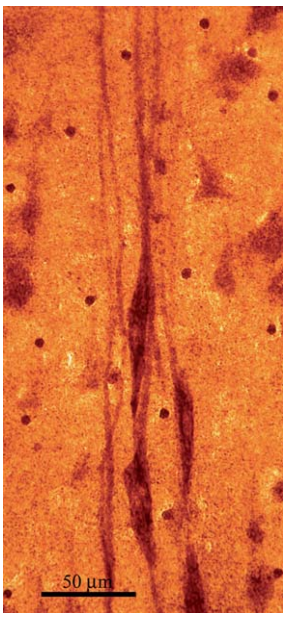

Figure 5 Dopamine D3 receptor labeling on two VE neurons (right) and a pyramidal neuron (lower left) in human ACC. Brown diaminobenzidine (DAB) deposits indicate the presence of the receptor on the soma and the apical dendrites.

being labeled compared to only 50% of the layer V pyramidals.

Taken together, this evidence implies that the VE cells take part in a circuit that is involved in processing uncertainty. Dopaminergic input is known to be associated with learning, signaling the extent to which a reward is unexpected.

Serotonin has been proposed to mediate the aversive component of learning, signaling the presence or absence of punishment in a manner similar to relationship between dopamine and reward (Daw *et al.*, 2002). Immunohistochemistry reveals that the VE neurons express at least two serotonin receptors, including 5HT-1b and 5HT-2b (Figure 6). 5HT-1b is associated with the inhibition of aggression; applying a 5HT-1b antagonist or knocking out the receptor in mice increases aggressive behavior, while applying a 5HT-1b agonist decreases aggression (Bouwknecht *et al.*, 2001; de Almeida *et al.*, 2001). The 5HT-2b receptor, while heavily prevalent in the human gut, is rare in the human central nervous system (Borman *et al.*, 2002). We found that, in the VE cell regions, the 5HT-2b receptor was specific to layer V and present on VE cell somas, pyramidal somas, and short segments of apical trunks in the absence of somatic labeling. In the human gastrointestinal system, this receptor causes peristalsis through the induction of smooth muscle contraction. Interestingly, insular cortex has recently been shown to be involved in interoception, that is, the representation and monitoring of one's internal states (Craig, 2004). The presence of 5HT-2b on both the VE neurons and in the viscera may indicate a functional connection between the two, perhaps a collateral projection that allows the CNS to rapidly process information relevant to the body. The presence of such a connection would be highly relevant to Damasio's (1994) theory of somatic states, which hypothesizes that many decisions are made on the basis of signals arising from regulatory processes that occur in the periphery.

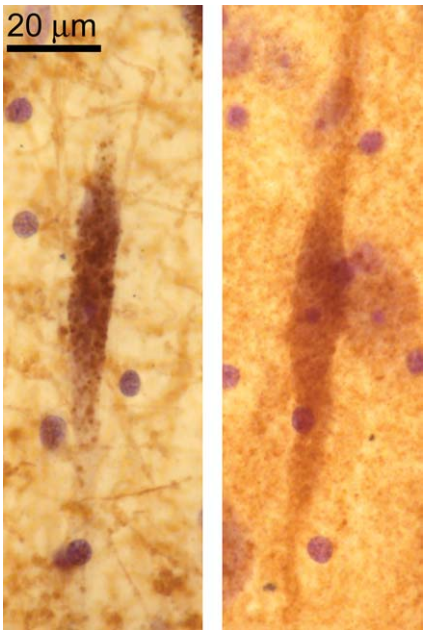

Figure 6 Immunohistochemical (DAB) labeling of the serotonin 1b (a) and 2b (b) receptors on VE neurons in the ACC. Both samples are Nissl counterstained to reveal unlabeled cells. Scale bar applies to both images.

4.30.2.2 Social Behavior and Vasopressin

Functional imaging paradigms associated with social behavior also reliably activate both VE cell regions. For example, both ACC and FI are active during the act of lying (telling untruths), and they are both active when a subject receives an unfair offer while playing the ultimatum game (Sanfey *et al.*, 2003; Spence *et al.*, 2004). Studies by Bartels and Zeki (2000, 2004) show both regions are active when subjects view the face of their love partner or child. Singer *et al.* (2004b) showed that both VE cell regions are active when a person feels empathy for pain, that is, when they know that their loved one, outside of the scanner, is being delivered an electric shock. Interestingly, the extent of activation an individual shows under these conditions is directly correlated to that individuals score on a trait measurement for empathy. Finally, in a separate study, Singer *et al.* (2004a) demonstrated that left FI is specifically active when subjects view faces of individuals who are reported to behave in a trustworthy fashion.

Fortunately, there is an excellent molecular model that allows us to specifically implicate the VE neurons in these various social behaviors. A body of work by Insel and Young indicates that the oxytocin and vasopressin V1a receptors mediate social bonding (Insel *et al.*, 1998; Young *et al.*, 2001; Lim *et al.*, 2004). Insel *et al.* (1998) also suggest that these molecules may interact with dopamine to impart the rewarding aspects of social bonding. Our immunohistochemical results show that the antibodies specific for the V1a receptor label a subpopulation of VE cells, as well as pyramidal neurons in layers 2/3 and 5 of ACC and FI.

4.30.3 The Social Cognition Hypothesis

In light of the above evidence, we hypothesize that the recently evolved VE neurons are a functional specialization of a circuit involved in making appropriate responses during quickly changing, ambiguous circumstances (Allman *et al.*, 2005). Links between the VE cells and interoception – including, literally, gut feelings – could provide the basis for their role in fast decision-making in the absence of explicit reasoning. In apes and humans, complex social interactions between conspecifics provide a forum in which this cognitive capacity would prove to be particularly useful (see Human Cognitive Specializations). This is because participants must rapidly synthesize an enormous number of relevant but often ephemeral informational cues in order to act appropriately. We thus propose that VE cells mediate the rapid assessments and behavioral modifications required for the successful navigation of social interactions.

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4.31 Evolution of the Sexually Dimorphic Brain

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Glossary

androgen Class of hormones that primarily influence the growth and development of the male reproductive system. The main and most active androgen is testosterone, produced by cells in the testes. Androgens produced in smaller quantities, mainly by the adrenal gland but also by the testes, support the functions of testosterone. Females produce trace quantities of androgens, mostly in the adrenal glands, as well as in the ovaries.

concurrent discrimination task Subjects are presented with a list of 20 pairs of objects. In each pair, one of the objects is rewarded and subjects must learn the correct object within each pair. In this particular version of the task, the 20 pairs are presented to the subjects only once a day.

delayed-matching task Subjects are first shown a stimulus and, after a short delay, the subjects are required to make a choice between the familiar stimulus and a new one. In this version of the task, subjects must select the familiar stimulus (match) to be correct.

delayed-response task Subjects are shown a reward being placed in one of two locations on a testing tray. The two locations are then covered with identical plaques and, after a delay that prevents the subject's view of the tray, the subject is required to retrieve the reward.

estrogen Class of hormones that primarily influence the female reproductive system's development, maturation, and function. The three major estrogens – estradiol, estrone, and estriol – are mainly produced by the ovaries and placenta; the adrenal glands and the testes secrete smaller amounts.

object discrimination reversal task Subjects are presented with two stimuli (one rewarded and the other not) and learn to select the rewarded stimulus on each trial. After acquisition of the discrimination problem and, without warning, the reward is now shifted to the previous unrewarded stimulus. Subjects must inhibit a learned response and select a correct strategy. Several reversals are generally given.

spatial span memory task A small circle appears on a particular location of a computer screen and subjects are required to touch the circle to receive a reward. On the second trial, the first circle in the same location and an identical circle in a new location appear and subjects need to displace the circle covering the new location. On each trial, circles are added one at a time over a new location of the screen. Spatial span is the number of circles displaced before the subjects make an error (i.e., displace a circle in a location that had already been selected in the previous trials).

4.31.1 Introduction

Evolutionary forces together with numerous molecular events create individual organisms with great genotypic and phenotypic similarity. Yet, within the common genetic boundaries that define a species, there is wide diversity of individual phenotypes. One of the most striking differences in phenotypes is between males and females of the same species. A large number of studies in birds and rodents have indicated that gonadal hormones act during development to establish permanent sex differences in the anatomy and function of the vertebrate brain. While it is generally agreed that most brain sexual differences are determined by the central action of androgens during development, the relative contribution of ovarian secretions to the full development of female brain organization and function is still a matter of debate (Pilgrim and Hutchison, 1994; Fitch and Denenberg, 1998).

Gonadal steroid hormones are implicated in the development of sex differences in reproductive behaviors at two critical periods (Phoenix *et al.*, 1959; Harris and Levine, 1962; Young *et al.*, 1964; Goy, 1968; Phoenix, 1974). First, the release of testicular (androgens) and ovarian (estrogens) hormones during critical periods of brain development (organizational period) exerts a profound effect on the genesis and survival of neurons in specific brain areas related to reproductive behaviors. They also act during adolescence and adulthood (activational period) to activate and modulate existing neural system pathways in a reversible manner.

Increasing experimental evidence indicates that, as with reproductive behaviors, sex differences in cognitive abilities could similarly be determined by genetic and hormonal factors acting at specific critical periods, i.e., early in development during the organization period to regulate neural events in neural structures mediating cognitive abilities, and later in adolescence and adulthood to activate and modulate the same neural structures. The critical periods of hormone influence vary among species and among the different behaviors. For example, in rhesus monkeys, the critical period for hormonal influences on maternal grooming by the offspring occurs earlier than the critical period of hormonal influences on juvenile play (Goy *et al.*, 1988).

Converging findings from different research areas are supporting this view. First, sex differences have been reported in various cognitive abilities that depend on neural structures other than those involved in reproductive functions. Second, there are sex differences in the morphology of neural structures mediating cognitive abilities. Third, biochemical studies of gonadal hormone binding and metabolism have revealed that androgen- and estrogen-binding sites and metabolizing enzymes are present in neural structures that are known to mediate cognitive functions. Fourth, lesions of neural structures underlying specific cognitive functions can affect males and females differently. Finally, sex differences in cognitive abilities, brain morphology, and/or in the effects of brain lesions can be reversed by manipulations of gonadal steroid hormones in animals. Although most data pertaining to the effects of gonadal steroid hormones on cognitive abilities has been gathered in rodents (for review, see Beatty, 1979; Meaney, 1988), there is a growing corpus of data suggesting that the same may hold true from nonhuman primates and humans. The present article first presents evidence for cognitive sex differences in nonhuman primates and then provides comparisons with recent data in humans. The final section offers tentative speculations on how differences in brain functions could have evolved (see The Development and Evolutionary Expansion of the Cerebral Cortex in Primates, Primate Brain Evolution in Phylogenetic Context, The Evolution of Parallel Visual Pathways in the Brains of Primates, Brain Size in Primates as a Function of Behavioral Innovation, The Evolution of Language Systems in the Human Brain, The Evolution of Hemispheric Specializations of the Human Brain, The Evolution of Human Brain and Body Growth Patterns, Constraints on Brain Size: The Radiator Hypothesis, Evolutionary Specializations for Processing Faces and Objects, Evolution of the Neural Circuitry Underlying Laughter and Crying).

4.31.2 Distribution of Gonadal Hormone Receptors in the Brain

The distribution of androgen- and estrogen-binding sites in the monkey brain is widespread and includes not only brain structures known to mediate reproductive behaviors, but also those that support nonreproductive behaviors and cognitive abilities. Estrogen receptors are found in many regions of fetal and adult brains, such as the medial basal hypothalamus, amygdala, cerebral cortex, and cerebellum. However, no definitive sex differences in the distribution of estrogen receptors were found in any neural structures examined, suggesting that both sexes are potentially susceptible to estrogen influence (Pfaff *et al.*, 1976; MacLusky *et al.*, 1986; Sholl and Kim, 1989). Similarly, androgen receptors are widely distributed in the primate

brain, including the hypothalamus, amygdala, dorsolateral prefrontal cortex, orbital frontal cortex, visual and somatosensory cortex, as well as corpus callosum of the fetus and adult rhesus monkeys (Pomerantz *et al.*, 1985; Roselli and Resko, 1986; Sholl and Pomerantz, 1986; Clark *et al.*, 1988). In addition, Sholl and Kim (1990) noted that, in the medial basal hypothalamus and amygdala, androgen aromatase activity (the enzyme responsible for the transformation of testosterone to estradiol) was higher in male than in female monkeys, whereas the levels of 5α-reductase (the enzyme responsible for the transformation of testosterone to dihydrotestosterone (DHT)) and androgen receptors did not differ between sexes. They proposed that, at an early stage of development, differentiation of the hypothalamus and amygdala of male fetuses may be more susceptible to androgen modification, by way of aromatization to estrogens, than corresponding areas in female fetuses. Furthermore, androgen receptors are differentially distributed between the right and left cerebral hemispheres of the male fetal rhesus monkeys (Sholl and Kim, 1990). Thus, androgen receptor levels were higher in the right than in the left frontal lobe in males but not in females. By contrast, androgen receptor levels were consistently higher in the left than in the right temporal lobe in males only. Together these data suggest a role for gonadal hormones in the modulation and maturation of brain regions mediating functions other than those related to sexual behaviors. Furthermore, the differential cortical distribution of androgen receptors in fetal male versus female monkeys supports the view that prenatal androgens from the fetal testes may affect the differentiation of sexually dimorphic, site-specific, cortical activity and functions.

4.31.3 Sex Differences in Cognitive Functions

Although sex differences in cognitive functions in nonhuman primates have not been extensively studied, there exists growing evidence to suggest significant differences between males and females in several cognitive processes that have been observed throughout the life span.

4.31.3.1 Juvenile Social Behaviors

Early social experience in monkeys emerges from playful interactions between peers. Several sex differences have been noted in the behavior of juvenile monkeys and appear to result from the prenatal androgen environment of the developing fetus (for review, see Meaney, 1988; Wallen, 1996). During play behaviors, juvenile females tend to use social vocalizations more than males, whereas juvenile males engage in mounting behaviors and rough-and-tumble play more than females. Manipulation of steroid gonadal environment during gestation can in fact alter these sexual traits. For example, experimentally increasing the prenatal androgen environment of female fetuses increases the expression of the male-typical sexually dimorphic behaviors (mounting and rough-and-tumble plays) when these female monkeys are juvenile, and these effects varied depending on when in gestation the androgen manipulation occurred (Goy *et al.*, 1988; Eaton *et al.*, 1990; Wallen, 1996). Thus, androgen exposure started at day 40 of gestation and maintained for 25 days results in extensive masculinized female genitalia and increases mounting behaviors, but not play behaviors. By contrast, androgen treatment started at 110 days of gestation yields no apparent genital masculinization, but masculinizes both mounting and play behaviors. These results indicate that the effects of prenatal androgen exposure on behavior were independent of its effects on genital masculinization, and that the developing nervous system in nonhuman primates is sensitive to the organizational actions of androgens throughout a large portion of gestation. Finally, they demonstrate that the critical periods for the development of specific sexually dimorphic behavior can occur at different gestational times, whereas a single critical period seems to be present for genital masculinization. Although the possible neural locus for these hormonal effects in monkeys remains unknown, studies in rodents have indicated that the amygdala could be a potential neural substrate (Meaney, 1988). Amygdaloid lesions made on days 21 or 22 in rats reduce the levels of play-fighting in male pups to those of normal females. The same lesions have no effect on the play-fighting of females. This finding suggests that a sex difference in some portions of the amygdaloid complex might, at least in part, mediate the sex difference observed in the play-fighting of juvenile rats. Because the amygdala is a principal target of gonadal hormones in rodents as well as in monkeys (Pfaff, 1976; Pfaff *et al.*, 1976; Clark *et al.*, 1988), it is possible that androgens might act directly on this limbic structure to masculinize play-fighting in monkeys as it does in rodents (Meaney, 1988).

4.31.3.2 Sex-Typed Toy Preference

Recent findings have also documented that nonhuman primates show preferences for sex-typed toys

similar to those seen in human children (Alexander and Hines, 2002). In this particular study, colonies of vervet monkeys were provided with toys typically preferred by boys (a toy police car and a ball), toys typically preferred by girls (a rag doll and a cooking pot), and toys preferred equally by boys and girls (a picture book and a stuffed dog). Videotapes were made of the animals interacting with the toys, and researchers unaware of the sex of the animals recorded the amount of time that male and female vervet monkeys spent in contact with each type of toys. Male monkeys spent more time than females in contact with toys usually preferred by boys, female monkeys spent more time than males in contact with toys usually preferred by girls, and male and female monkeys spent similar amount of time in contact with toys that both girls and boys enjoy. Since vervet monkeys had no prior experience with any of the toys and had no cultural expectations about which toys were for males or females, it was concluded that the differences between male and female vervets in toy preferences cannot be attributed simply to social/cognitive mechanisms. The authors suggested that a differential influence of androgens on the development of cortical visual areas related to either object features (ventral visual pathway) or object movements (dorsal visual pathway) (Ungerleider and Mishkin, 1982) may influence perception of object characteristics that have different adaptive significance for males and females, and promote the formation of children's sex-typed object categories.

4.31.3.3 Learning and Memory Abilities in Infancy

Two separate lines of evidence strongly support the role of androgens in the development of sex differences in learning and memory abilities in nonhuman primate infants.

Acquisition of an object discrimination reversal task, known to depend on the integrity of the orbital frontal cortex in the adult monkey, is significantly more rapid in male infant monkeys than in females (Clark and Goldman-Rakic, 1989). Postnatal injections of testosterone propionate (TP) in the females enhance their performance to the level of normal infant males. When orbital prefrontal cortex is removed in infancy, intact male monkeys and androgenized female monkeys are as impaired as adult monkeys with the same lesions, whereas treated infant females do not differ from untreated age-matched females. The data suggest that the orbital frontal cortex matures earlier in male than in female monkeys.

Conversely, acquisition of a concurrent visual discrimination learning task (Figure 1), known to depend on the integrity of the inferior temporal cortex, is significantly more rapid in female infant monkeys than in males (Bachevalier and Hagger, 1991), and this sex difference is positively correlated in 3-month-old male animals with circulating levels of testosterone (the higher the level of testosterone, the poorer the score on the task), but not with estradiol levels. Neonatal orchiectomy, which reduced plasma testosterone levels, hastens performance on visual discrimination learning in male infant monkeys, whereas treatment of androgens (DHT) in neonatally ovariectomized female infant monkeys delays their performance. Finally, early postnatal inferior temporal cortex lesions affect performance of female but not of male infant monkeys, though male and female adults with the same lesions are equally impaired. The data suggest that this temporal cortical area is functionally more mature in female infant monkeys than in males.

The two sets of findings lead to intriguing conclusions. On the one hand, they suggest that sex differences in the development of learning and memory abilities are due to the influence of gonadal steroid hormones on the maturation of the

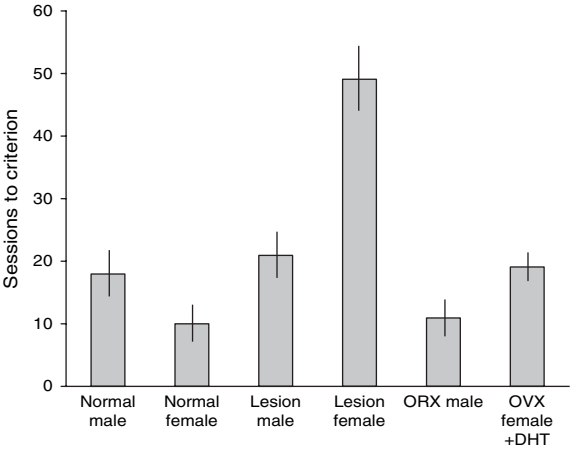

Figure 1 Summary of rate of acquisition of 20 concurrent discrimination problems in 3-month-old monkeys (*Macaca mulatta*). Normal females learned at a faster rate, and required fewer trials than normal males to reach the learning criterion. Young female monkeys were impaired after lesions to the temporal cortical area TE (lesion female), requiring more trials than did females that did not have surgery (normal female). By contrast, males with the same lesions (lesion male) and normal males learned at the same rate. Newborn males that were orchiectomized (ORX male) learned faster than normal males and at the same rate as normal females. By contrast, newborn females (OVX female + DHT) that were ovariectomized and treated with DHT (2 mg kg^{-1} three times a week) learned at a slower rate than normal females.

specific cortical regions underlying these abilities. On the other hand, they also indicate that the directionality of such hormonal influences on structure and function varies from one cortical area to the other. Specifically, whereas the orbital frontal cortex appears to mature earlier in males than in females, inferior temporal cortical area TE appears to mature earlier in females than in males. Although the specific biological mechanisms for such cortical effects of androgens are still unknown, it has been hypothesized (Bachevalier and Hagger, 1991) that the actions of TP and DHT might be mediated by different receptors in the cortex. It has been shown in nonhuman primates that TP, which is aromatized into estrogens at a neural level, has a high affinity for estrogen receptors, whereas DHT, an androgen not significantly aromatized in the primate brain, has higher affinity than TP for the androgen receptors (Michael *et al.*, 1986). Since both estrogen- and androgen-binding systems have been located in all cortical areas of the primate brain, there might be local cerebral metabolic differences (estrogenic vs. androgenic action) that underlie some of the different behavioral effects of gonadal steroids in primates. Such regional metabolic differences were underscored by the work of Michael *et al.* (1989).

4.31.3.4 Learning and Memory Abilities during Adulthood and Aging

In adult rhesus monkeys, females outperform males in spatial working memory as measured by the delayed-response task, and, in adult chimpanzees, females exhibit superior short-term stimulus memory, as measured by the delayed matching-to-sample task (for review, see Mitchell, 1977). A more recent investigation showed that, although male and female rhesus monkeys perform similarly on tasks of object memory and executive function, young males outperform young females on a spatial memory task (Lacreuse *et al.*, 1999). This superior level of spatial ability in young males declined sharply with age, and, at older ages, performance on this task is similar for both sexes. These findings have now been replicated with a large group of 90 rhesus monkeys (Lacreuse *et al.*, 2005a) and thus suggest that biological rather than sociocultural factors underlie the sex differences in cognition.

Further studies have also shown that gonadal hormone levels in male and female rhesus monkeys are associated with differential levels of spatial memory performance. In a recent study (Lacreuse *et al.*, 2001), young adult female rhesus monkeys were tested in an object recognition memory task (with two different delays) and spatial span memory task during the entire menstrual cycle. Spatial memory scores varied consistently during the menstrual cycle, with better performance during the follicular and lutheal phases (when estradiol levels are low) than during the periovulatory phase of the cycle, indicating that spatial memory performance in female rhesus monkeys is affected by cyclic estradiol variations. Furthermore, estrogen replacement therapy in ovariectomized female rhesus monkeys decreased performance on the recognition of conspecifics' faces but had no effects on spatial memory or object recognition memory (Lacreuse and Herndon, 2003). Nevertheless, another study demonstrated substantial improvement in spatial working memory performance mediated by the prefrontal cortex and modest improvement in an object recognition memory task mediated by the medial temporal lobe following estrogen replacement therapy (Rapp *et al.*, 2003). These findings clearly need to be replicated and extended to other memory tasks to further our understanding of the role of estrogen in cognitive functioning.

Finally, cognitive sex differences were also found with aging in monkeys. Thus, a progressive deterioration of motor functions was demonstrated in aged male rhesus monkeys, but not in aged females (Lacreuse *et al.*, 2005b). The authors also showed no volumetric differences in the size of the caudate nucleus and putamen in aged male and female monkeys, even though dysfunction of the nigrostriatal dopaminergic system has been associated with the decline of motor abilities in male monkeys. Together the results indicate that ovarian hormone status seems to broadly modulate neural systems and influence normal cognitive functions in monkeys across the life span.

4.31.4 Sex Differences in Brain Areas Related to Cognitive Abilities

Although no direct correlation has been established between sex differences in cognitive abilities and morphology of brain areas, there exist sex differences in numerous neural structures related to cognitive abilities. The majority of this work has been carried out in rodents (for reviews, see Beatty, 1979; Juraska, 1991; Kawata, 1995). In the limbic system (bed nucleus of the stria terminalis and hippocampus), the number and volume of neurons differ in male and female rats. These morphological differences are reversed after postnatal treatment with gonadal steroid hormones. In the cortex, the rate of cell proliferation has been shown to be

slower in male than in female rats, indicating a delayed maturation of the neocortex in males compared with that of females. Finally, recent findings indicated that the rat's corpus callosum is sexually dimorphic; the males' corpus callosum and more specifically the splenium of the corpus callosum are larger than the females (Fitch *et al.*, 1990; Nunez and Juraska, 1998). Manipulations of testosterone levels in males (castration) and females (injection of TP) had a significant impact on females but not males, in that injection of testosterone in female rats masculinized the corpus callosum.

Sex differences in brain structures have not been well documented in nonhuman primates. However, the advent of magnetic resonance imaging (MRI), which provides excellent anatomical resolution, gives a compelling new tool to investigate sex differences in the size and volume of different brain regions, not only in the adults but also across the life span and across different nonhuman primate species. Earlier anatomical studies in monkey postmortem brains (Cupp and Uemura 1981), showed that males' brains (90.57 g) weighed 11% more than females' brains (81.50 g). A similar sexual dimorphism in total brain volume has also been found using MR images in rhesus monkeys (Franklin *et al.*, 2000). However, sexual dimorphism in corpus callosum measurements in nonhuman primates is still controversial. Holloway and Heilbroner (1992) measured the volume of the corpus callosum in four different species of primate (*Macaca mulatta, M. fascicularis, Callithrix jacchus,* and *Saguinus oedipus*) and found no evidence for a sexual dimorphism of this brain structure, although they noted that in *M. mulatta* the splenium of the corpus callosum in the males was larger than in the females. More recently, Franklin *et al.* (2000) reported that, while the total brain volume and total callosal volume are smaller in young female rhesus monkeys than in the males, the area of the splenium of the corpus callosum was larger in the female monkeys than in the males. No sex difference was found in the volume of the amygdala. Using MRI volumetric measures to follow the maturation of the total brain, hippocampus, and amygdala volumes in male and female rhesus monkeys (*M. mulatta*) ranging from 1 week to 2 years of age, Machado *et al.* (2002) indicated that male macaques have slightly larger total brain volume (10%) than females in infancy, but this difference was absent in adulthood. In addition, while the initial volume of the hippocampal formation did not differ between the left and right hemispheres for males and females, the right amygdala displayed a significantly larger initial volume than the left in males but not in females. These data suggest the presence of sexually dimorphic differences in specific limbic regions in the macaque brains that could vary during maturation. Furthermore, a recent report in chimpanzees (*Pan troglodytes*) indicated a right hemispheric asymmetry for the hippocampus but not the amygdala, with a tendency for males to be more lateralized than females (Freeman *et al.*, 2004). While interesting sex differences in brain structures are starting to emerge in the literature, additional data in this area will greatly inform on the neural bases of sex differences in cognitive functions across the life span.

4.31.5 Sex Differences in Cognitive Abilities in Humans

There is also accumulating evidence that gonadal steroid hormones in humans do influence brain and behavior in a manner similar to that documented in other species (for reviews, see Witelson, 1991; Collaer and Hines, 1995; Wisniewski, 1998). During the developmental period, both behavioral and cognitive development is protracted postnatally in boys compared to girls (Maccoby and Jacklin, 1974). Girls generally learn to sit, crawl, and walk earlier than boys. This precocious behavioral ability in girls is accompanied by precocious abilities in stimulus processing (Tighe and Powlison, 1978; Clifton *et al.*, 1984; Creighton, 1984; Held *et al.*, 1984; Bauer *et al.*, 1986) and in certain learning and memory functions (Diamond, 1985). Girls also acquire language earlier than boys (McGuiness, 1976), and are superior in most forms of visual and verbal recall (Duggan, 1950; Mittler and Ward, 1970), although the superiority of boys in spatial tasks is well documented (Tyler, 1965; Guilford, 1967; Garai and Scheinfeld, 1968; Hutt, 1972; Kerns and Berenbaum, 1991). Childhood play behaviors, such as toy choices and activity and playmate preferences, also show differences between boys and girls. These sex differences can be reversed by modulation of androgen levels in the case of genetic disorders, such as congenital adrenal hyperplasia, as well as in the case of normal variability of androgen levels in the prenatal period (for review, see Hines, 2003).

Interestingly, the double dissociation found in infant monkeys with the object discrimination reversal and concurrent discrimination tasks (see above) has been replicated in very young children using almost identical cognitive tasks and nonverbal procedures (Overman, 2004; Overman *et al.*, 1997). Boys under the age of 29 months significantly outperform girls on the object reversal task, but girls

outperform boys on the concurrent discrimination task. Given the close parallel in learning behavior in human infants and infant monkeys, it is reasonable to propose that the gender differences are mediated by similar biological mechanisms in both species. Therefore, in children, as in infant monkeys, there may be a more rapid maturation of orbital prefrontal circuits in boys and a more rapid maturation of inferior temporal circuits in girls. The sex differences found on tasks measuring orbital frontal functioning appear to persist in adolescents and adults (Overman, 2004).

Sex differences in cognitive functions have also been reported during adulthood. Many studies showed that women excel in verbal abilities, perceptual speed, articulation, and fine motor skills, whereas men generally excel in tasks measuring visuospatial abilities, particularly those requiring mental rotation of objects and imagining what an object would look like from a different vantage point (Hyde and Linn, 1988). Numerous studies also report sex differences in perceiving, interpreting, and reacting to social cues that signify the potential presence of threat. Thus, females tend to perform more accurately than males when labeling others' feelings, such as anger and hostility, based on facial expressions (Hall, 1978; McClure, 2000), but they also show greater increases in skin conductance and a tendency toward stronger startle potentiation to threatening scenes (Bradley *et al.*, 1999; McManis *et al.*, 2001). These sex differences appear to be largest after the transition from adolescence to adulthood (Hall, 1978; McClure, 2000; Nelson *et al.*, 2002).

While direct manipulation of gonadal hormones is not possible in humans, the organizational and activational effects of the hormones on cognitive functions have usually been inferred from studies of different populations of individuals (for review, see Sanders *et al.*, 2002). They include boys and girls suffering from long-standing prenatal hormonal anomalies or who have been exposed to exogenous hormones *in utero*, women during regular fluctuations of estrogen and progesterone throughout the menstrual cycle or postmenopausal women receiving hormone replacement therapy, and elderly individuals showing a decline in cognitive functions. Thus, girls with congenital adrenal hyperplasia, who are genitally masculinized and prenatally exposed to excessive androgens, show significant enhancement of visuospatial ability as compared to unaffected females. Also, boys exposed with diethylstilbestrol (a synthetic estrogen) in the perinatal period show poorer performance on several spatial tasks than males who suffer from low testosterone due to postpuberty pathology (for review, see Reinisch *et al.*, 1991). In addition, when performance on several visuospatial tasks is compared at different phases of the menstrual cycle, women perform significantly more poorly during the estrogen surge just prior to ovulation than at other points in the cycle. Conversely, higher levels of estrogen during the menstrual cycle are associated with better performance on many tasks in which women typically excel. When postmenopausal women are tested either when receiving estrogen therapy or when they are off medication for at least 4 days, performance on fine motor and spatial tasks tends to be faster and more accurate during the treatment compared with the off-treatment phase (for review, see Hampson and Kimura, 1992). Finally, estrogen deficiency in menopausal women is associated with memory impairments that are reversible by estrogen replacement therapy, whereas testosterone replacement therapy in elderly men enhances spatial performance (Janowsky *et al.*, 1994).

The advances of neuroimaging techniques in the last 10 years have tremendously increased the number of studies in humans devoted to sexual dimorphism in brain structures. Both structural and functional MRI techniques are providing mounting evidence of small but robust sex differences in brain morphology, metabolism, weight, volume, and neocortical neuron number (Gur *et al.*, 1995; Pakkenberg and Gundersen, 1997; de Courten-Myers, 1999; for review, see Hampson and Kimura, 1992). These differences seem to be evenly distributed among the four lobes, although regional tissue composition show sex differences only within the parietal lobes, with females having proportionally more gray matter on the right side (Nopoulos *et al.*, 2000). Finally, sex differences are found in hemispheric and cortical asymmetry (for review, see Voyer, 1996; Witelson, 1991; Wisniewski, 1998). In general, males tend to exhibit more accentuated asymmetries and stronger right-hemisphere dominance compared with females, while females typically exhibit more diffuse lateralization patterns and greater left-hemisphere bias compared with males.

Sex dimorphism in human brain anatomy appears also to be present during development (Giedd *et al.*, 1997; De Bellis *et al.*, 2001). Males show a 9% larger cerebral volume. When corrected for cerebral volume, the caudate nucleus is relatively larger in females and the globus pallidus larger in males. Furthermore, the amygdala and hippocampal volumes increase for both sexes, but with the amygdala volume increasing significantly more in males than females, and hippocampal volume increasing

more in females. Similarly, women with gonadal hypoplasia have smaller hippocampi than controls (Murphy *et al.*, 1993).

More recently, the use of functional MRI has provided a way perhaps to understand better the relationship between sex differences in brain structures and functions. Thus, Killgore *et al.* (2001) reported a sex difference in the functional maturation of the affect-related prefrontal-amygdala circuits during adolescents. Females show a progressive increase in prefrontal relative to amygdala activation in the left hemisphere, whereas males fail to show a significant age-related difference. Interestingly, as shown with the sex dimorphism found in the orbital frontal cortex in monkeys (see above), a recent study indicated larger orbital frontal cortex in women than in men (Gur *et al.*, 2002), supporting mounting behavioral evidence for sex differences in emotion processing (Canli *et al.*, 2002; McClure *et al.*, 2004; Schirmer *et al.*, 2004).

4.31.6 Conclusions

This article provides a brief survey of sex dimorphism in behaviors and cognitive abilities not related to sexual behaviors, its expression across primate species and across the life span, and its relation to brain anatomy and function. Although sex differences in cognitive and learning abilities are presently widely acknowledged, the basis for these differences remains controversial. The data reviewed here suggest that androgens organize the brain pre- and perinatally for all sexually dimorphic behaviors, including problem-solving behaviors, and this appears to be true in mammals, nonhuman primates, and humans. Moreover, the pattern of variation in learning abilities documented recently over the menstrual cycle and during aging raises the possibility that sex differences in cognitive abilities in nonhumans and humans may also be due at least partly to an activational influence of gonadal hormones, both androgens and estrogens, on the brain throughout life. Thus, it is becoming clear that sex differences in structure and function are likely to be a pervasive characteristic of brain organization mediated by gonadal steroid hormones. Nevertheless, the challenge is to specify precisely the biological mechanisms by which these differences occur and to take into consideration the circular interactions between biological factors and socioenvironmental factors. Ultimately, the understanding of cognitive sex differences will necessarily depend upon converging evidence from many different disciplines, including anthropology, evolution, endocrinology, animal and human behavior, neurobiology, electrophysiology, and brain imaging.

Although the causes and functions of sex differences in cognitive processes are largely unknown and remain puzzling, sex differences in cognitive functions are likely to result from selective pressures on both male and female traits, as already proposed for sex differences in reproductive behaviors (Plavcan, 2001). In addition, sex differences result from different developmental pathways, all of which can be affected by different selective pressures throughout an animal's entire life history. This article provided few examples that seem to support the idea that sex-related differences in cognitive functions appeared early in human evolution, prior to the emergence of a distinct hominid lineage. Many have proposed that the diverse requirements based on tasks conducted more by males or females may have promoted differential selection pressures on diverse cognitive functions.

For example, sex differences in preferences for sex-typed toys have been demonstrated in both children and nonhuman primates (for review, see Hines, 2003). Alexander and Hines (2002) have proposed that these sex differences by males and females may be associated to sex differences in visual perceptual categories (color, shape, and movement) that are in turn mediated by sex differences in cortical visual areas, such as the ventral visual pathway involved in the processing of object features and the dorsal visual pathway involved in the processing of object movements (Ungerleider and Mishkin, 1982). They argued that, like chromatic color vision in primates, which appears to have evolved to facilitate foraging for fruit and edible leaves, sex-typed toy preferences may have evolved from the formation of perceptual categories of objects with differential adaptive significance for males and females. Similarly, the sex differences in spatial abilities observed in nonhuman species and humans may be related to sex differences in hippocampal volume, a brain structure critical for the processing of spatial information. While the significance of sex dimorphism in brain structure and function related to spatial abilities is unknown, it could also be based on different task requirements for males and females. Greater spatial abilities in males could have evolved for a role in males in gathering food and hunting, both abilities requiring good spatial skills. Conversely, greater use of communication and language in females could have evolved from a task requirement for females in regulating cohesion in the social group. Although we can offer only broad speculations at this time due to the large gaps in our understanding of the evolution of sexual dimorphism in general and, more

particularly, of the evolution of sex differences in cognitive functions, the growing interest in this field of research together with the great advances in technology to investigate genetic and molecular factors as well as brain/behavior relationships are likely to augment our understanding of this controversial topic.

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4.32 Evolutionary Psychology

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Glossary

altruism A social interaction in which the actor derives a net fitness cost and the recipient derives a net fitness benefit.

cognitive module A psychological mechanism, instantiated as neural architecture, that accomplishes a specific function by handling a certain class of inputs and generating a certain class of outputs, for example, a face-recognition module.

Natural selection (Darwin, 1859) is the inexorable engine of adaptive evolution. It results from the differential success of alternative genetic variants in creating phenotypes that usefully address the challenges of surviving and reproducing in actual environments (Williams, 1966). In each generation new genotypes assemble new phenotypes. Because they better meet the demands of the local environment, some of those phenotypes reproduce more than others. In the course of doing so, they differentially transmit the genes that built them to the next generation. Seen in this way, the process is inevitable, ubiquitous, and unending.

As selection sorts variation, it produces what can logically be regarded as designs for reproduction (Dawkins, 1976). Designs for reproduction are not limited to the sex organs. The heart, lungs, and liver are designs for reproduction to the extent that the sex organs perish without them. The same is true for countless other aspects of anatomy and physiology. Organisms are richly integrated bundles of such designs. Each design tends to do one job. The heart, lungs, and liver are functionally integrated, but each is specialized for a specific task. The reason for this is that hearts that both pumped and aerated blood were worse pumps. An improvement in a system with respect to one function will usually decrease its ability to perform others. Because selection favors improvements, it tends to build specialized systems.

Evolutionary psychology, like the rest of academic psychology, is materialistic. In other words, it assumes that, at base, psychology reduces to anatomy and physiology. If this approach is accepted, it means that natural selection will have designed psychological systems and mechanisms in precisely the same way it builds other bodily systems – by the spread of genetic variants that favorably influence the growth, organization, and functioning of the underlying architectures. Selection refines any function (be it circulation of the blood or parental care) by improving the various organs that accomplish it. By the same logic developed above, the output of such selection is expected to be an integrated bundle of specialized psychological mechanisms.

There are two related conclusions that follow from this line of thinking: the brain is not one organ but many; and the brain is not a general-purpose computer (Tooby and Cosmides, 1990, 1992). These concepts are related because they both follow from the principle of specialization. Psychological systems should have been designed by natural selection to produce adaptive (i.e., reproduction enhancing) behavior. That ultimate function would be served by many different and specific kinds of behaviors – effective communication, distinguishing reliable from unreliable associates, recognizing other's emotional states, and predicting their behavior, parsing speech, avoiding dangerous situations, recognizing nutritious foods, assessing the needs of offspring; the list is long. There is no evidence, nor theoretical reason, suggesting that the psychological mechanisms involved in, say, phoneme discrimination might serve other functions, such as the ability to read facial expressions or know when your child is in danger. The brain is many specialized organs precisely because there are many distinct tasks that must be accomplished along the road to reproduction.

The opposite side of the same coin is that the brain is not a generalized computational device primarily because it was not shaped by the demands of any 'general-purpose problem'. Selection sorts genes with reference to real-world situations. Since the barriers to reproductive success were specific, our psychologies comprise many specific mechanisms. The computer provides a useful analogy. Imagine an immensely powerful general-purpose computer with lots of computational resources in the form of vast amounts of hardware, but without any

task-specific software. It will be useless, just like a general-purpose brain would be.

A lot of controversy has swirled around the concept of psychological modularity (Fodor, 1983; Pinker, 1997). An evolutionary view of the mind predicts functional modularity in the sense that there will be different mental 'organs' specialized for different tasks. This stance is agnostic as regards anatomical modularity; functions may or may not be spatially localized. This localization debate is not relevant to the argument for cognitive modularity.

What kind of modules should we expect the mind to comprise? From the foregoing, the answer is obvious: modules that solved the problems recurrently faced by our ancestors (Tooby and DeVore, 1987). Understanding the full import of this conclusion goes a long way towards clarifying the approach of evolutionary psychology. The assumption is not, for example, that humans go about calculating the relative impact of fixed versus variable-rate mortgages on the expected number of their surviving grandchildren. For 99.9% of the 7 million years since our lineage diverged from the one leading to chimpanzees, fixed abodes, never mind compound interest rates, did not exist. Why should our minds be in any way prepared for this problem?

Consider two pictures: a sleek coiled snake and a shiny new Porsche. Which is more frightening; which more attractive? Fine, but how about a reality check? How many people die or are seriously injured each year by snakes versus cars? Obviously, the statistics tell us that cars are presently more much dangerous than snakes. Perhaps we are, as some economists might assert, irrational; but that conclusion has no temporal perspective. We are not irrational in relation to the world where our minds were designed, a world without motorized transport of any kind, but a world brimming with many natural dangers such as poisonous snakes (Gaulin and McBurney, 2004).

These examples raise two issues. The first is the one just discussed. Selection cannot design for the future, so our psychological adaptations will be the ones that functioned over the long haul of human evolution, not necessarily the ones that would be useful in the novel world of the twenty-first century (see Human Cognitive Specializations). The second is that psychological adaptations do not necessarily and automatically put fitness maximization in the spotlight of consciousness. In fact, they are often cognitively impenetrable (Pylyshyn, 1999). Let us return to the snake. The rapid, 'gut-level' emotional and behavioral response to the snake does not involve a conscious chain of reasoning: snakelike objects are often poisonous; poison may harm or kill me, thus probabilistically reducing my genetic contribution to future generations. The response is much quicker, more automatic, 'unreasoned' and inaccessible in the sense that no amount of introspection will allow us to say what happened between the glimpse of the snake and our response. The benefits of automaticity are obvious: no opportunities to make reasoning or inference errors, quick and reliable output.

In large part, evolutionary psychology consists in trying to map the environmental pressures that would have operated over the big sweep of human evolution, and testing whether the psychological mechanisms necessary to meet these challenges are present (Tooby and DeVore, 1987; Tooby and Cosmides, 1992). Critics will argue that they could be present by chance, or as incidental side effects of other mechanisms. Thus, the best way to proceed is to examine whether structurally (cognitively, emotionally) similar mechanisms that would not have served the same function are absent. This approach is applicable to all of psychology. A great deal of attention has been focused on psychological adaptations to the challenges of mating and parenting (e.g., Buss and Schmitt, 1993), and this focus is reasonable because these mechanisms are closely linked to evolutionary fitness. But any of the traditional domains of psychology – cognition, emotion, development, perception, and learning to name a few – are amenable to evolutionary analysis (e.g., Gigerenzer and Todd, 1999; Damasio, 1994; Bugental, 2000; McBurney *et al*., 1997; Gallistel, 1990).

This short list may raise a flag for some. What about learning? Aren't learned behaviors complementary to evolved ones? Absolutely not; and this insight is one of the most powerful that evolutionary psychology can offer. To say that a behavior is learned sweeps away all the interesting questions with a mere label. When learning occurs, behavior is modified by experience: but which behaviors and which experiences? The older Pavlovian and Skinnerian (e.g., Skinner, 1956) views would assert that it does not matter; any combination is possible. But this formulation was being undermined even as it was being articulated.

Consider food-deprived versus water-deprived rats, each rewarded with the withheld substance in a T-maze. Which group will learn the task more quickly? The answer depends on reward placement. In the 'fixed' design, each rat is always rewarded in the same arm. In this case thirsty rats learn where water is more quickly than hungry rats learn where food is. Perhaps thirst is more 'motivating' than hunger. Possible, but consider the next experiment. In the 'reversal' design, reward placement alternates – right,

left, right, left – in successive trials. This is a harder task, to be sure, but rats can learn it, if the reward is food. However, they never learn the task if the reward is water (e.g., Petrinovich and Bolles, 1954). So much for open-ended learning, but can we make sense of these results?

Remember that all adaptations are formed by natural selection operating in real-world environments. In the real world, food often moves around but water typically does not. Rat brains are apparently set up to handle information about food and water locations differently, such that the former is easily updated while the latter is not. This is a nice example of a fine-grained cognitive specialization. I am not aware that anyone has tested this hypothesis in humans but, because the selection pressures in these domains would have been similar in the two species (stable water sources, mobile food sources), the result could be expected to generalize.

The topical focus of evolutionary psychology is – like all of psychology – mind, brain, and behavior. But its explanatory stance unites it methodologically with evolutionary biology. Thus, one studies psychological adaptations with the same two tools used to analyze any adaptation: cross-species comparison and reverse engineering. I will explain each method and then, for each, give two examples of their use by evolutionary psychologists.

Cross-species comparison can be used to work forward from a given selection pressure to ask what kind of adaptation it would cause, or to work backwards asking what adaptive function a given trait might have been designed to serve. This method assumes that closely related species will tend to be similar because of their shared inheritance from a recent common ancestor. But if closely related species are subject to different selection pressures, their adaptations should diverge. And if distantly related species are subject to similar selection pressures, their adaptations should converge. By examining such cases of divergence and convergence, one can evaluate whether particular adaptations are systematically associated with particular environments. For example, the notion that eyes are an adaptation for forming images from reflected light gains support from the finding that, in a variety of taxa (fish, shrimp, crayfish), closely related species differ in the possession of eyes: those that live in environments where light penetrates have eyes, whereas those that live in deep caves lack them.

An early example of this approach in evolutionary psychology involves questions about the possible adaptive basis of sex differences in spatial ability. Men – and male laboratory rodents – make fewer errors on spatial tests such as navigating a maze or visualizing objects from multiple perspectives. Such a pattern should only occur where male reproductive success is more heavily dependent on such abilities than is female reproductive success. This would be the case under some types of polygynous mating systems, where individual females forage for food in a limited area, but individual males travel much more widely attempting to maximize their exposure to sexually receptive females. Such mating systems are not universal however; for example, in monogamous systems opposite-sex pairs travel together and thus exploit quite similar ranges. From these ecological facts one can predict that sex differences in spatial ability should be limited to the polygynous species. Based on experiments and observations with wild microtine rodents (voles), this prediction is supported both for spatial performance and for the relative size of the hippocampus, the brain structure most heavily involved in spatial processing (Gaulin and FitzGerald, 1986, 1989; Jacobs *et al*., 1990). Independent confirmation comes from comparisons among and wild icterids ('blackbirds'; Sherry *et al*., 1993).

Quite a different example concerns the function (and malfunction) of parental love. Here again an initial hypothesis is easy: parental love primes solicitous behavior and thus promotes the survival of the parental genes. Obvious test cases would be situations where parents and nonparents find their reproductive interests in conflict. More specifically, in some species male tenure in breeding groups is relatively brief (as a result of strong competition among males) and this turnover regularly brings adult males into close proximity with dependent young they could not have sired. Lactating females generally do not ovulate in wild populations, thus producing a conflict of interest: females are better off nursing their current offspring to independence, but new males can profit by terminating investment in the offspring of their predecessors. This pattern of infanticide has evolved repeatedly in species where new males frequently displace fathers from breeding units and not in species without such displacements. In their studies of humans, Daly and Wilson (1988) have shown that the replacement of the father by a new breeding male is a common correlate of infanticide in the ethnographic record. Likewise, living with a step-parent is the single most predisposing factor for both child abuse and infanticide in industrialized societies (Daly and Wilson, 1988, 1996, 1999). Those investing in bearers of their own genes are decidedly more solicitous than those who are not.

Reverse engineering provides another method for testing adaptive hypotheses. If a trait is to serve function x, it must have the components that allow

it to accomplish this function. Returning to the eye, we can predict that an organ designed to form sharp images from reflected light would have to possess an appropriately adjustable aperture, a lens, a focal plane at an appropriate distance from the lens, light-sensitive elements that capture the image on the focal plane, etc. If eyes do not have these features, they are probably not adaptations for forming images from reflected light.

To illustrate the reverse-engineering method, let us choose the problem of altruism. Why is altruism a problem? It seems unlikely to evolve by the differential replication of altruistic (as opposed to selfish) alleles because the former would be at a competitive disadvantage. There are (at least) two ways around this problem only one of which – reciprocity – will be treated in this brief article. If: (1) actors were in a position to provide relatively large benefits to others at small costs to themselves and (2) actor and recipient regularly exchanged roles, then both would derive net long-term benefits from such reciprocity (Trivers, 1971). This works as long as actors are not drawn into too many relationships with cheaters (=nonreciprocators); if they fall prey to such exploitation, they do not get enough benefits to offset their costs and selection will eliminate altruism. This means that selection for reciprocity can only evolve in the context of the simultaneous evolution of adaptations for withholding benefits to cheaters.

Reverse engineering requires us to ask the question: what features would an evolved system like this have to have? One basic element is the ability to recognize when cheating has occurred. Leda Cosmides and John Tooby have used the study of logical abilities as a platform for investigating cheater detection. In general, people are not very good at solving logical problems and require significant training to perform well. What Cosmides and Tooby have done is to construct a wide array of logical problems, all of which have the same logical structure. Each problem describes some social situation and each requires the same logical response, but some describe a situation where an actor may have cheated and some contain no such possibility. The striking result is that performance on these two classes of problems is very different: some 70–90% of subjects solve the problems where cheating may have occurred but only 20–40% do so where there is no such possibility (Cosmides, 1989; Cosmides and Tooby, 1992; Gigerenzer and Hug, 1992). The conclusion: there is no general logic-solving module (the brain is not a general-purpose computer), but there is a specialized cheater-detection module.

Holding to the example of reciprocity, one can suggest other plausible engineering features of system. It would be adaptive, for example, to remember who had cheated in the past. Linda Mealey and her associates (Mealey *et al*., 1996) tested the idea that facial memory is primed by the assignment of a 'cheater' label. They first obtained a sample of facial photographs and created a sample of positive, neutral, and negative statements about individuals; the negative statements emphasized cheating. Then they combined these statements and photographs (using an appropriately counterbalanced design) and showed these captioned photographs to people without explaining their purpose. One week later, the participants returned and were shown another set of faces without statements; one-half of this second set was new and one-half had been paired with statements the week before. The participants' task was simply to say which faces were new, a task that obviously required them to remember which faces they had seen. In this experiment faces previously assigned a 'cheater' label were more likely to be recalled (see Evolutionary Specializations for Processing Faces and Objects).

The power of this approach is that it usefully guides our search for psychological mechanisms. It should be clear from these examples that one can generate explicit and testable predictions about specific psychological features from a consideration of evolutionary theory and the context of human evolution. George Williams wrote nearly 40 years ago: "Is it not reasonable to anticipate that our understanding of the human mind would be greatly aided by knowing the purpose for which it was designed?" (Williams, 1966, p. 16). Evolutionary psychology is beginning to answer that question in the affirmative.

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4.33 The Interpreter in Human Psychology

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Glossary

corpus callosum	A large myelinated axonal tract that connects the two cerebral hemispheres, allowing interhemispheric interaction at the cortical level. Interhemispheric tracts are known as commissures.
split brain	Patients in whom the corpus callosum has been severed, for the relief of intractable epilepsy, in an operation known as a callosotomy. Occasionally other, smaller, commissures are also severed in a procedure known as a commissurotomy.
left-hemisphere interpreter	The term used to describe the tendency for the isolated left hemisphere in split-brain patients to generate hypotheses about information with which it is presented, including explanations of the patient's actions that were initiated by the right hemisphere.
confabulation	The production of fictitious explanations for past or present events and behavior occurring as a result of brain damage. Confabulations, which can diverge sharply from reality, are usually accompanied by an appearance of conviction on the part of the patient.

4.33.1 The Interpretive Nature of Consciousness

Although writers may disagree on the degree to which the brain exhibits modularity of function (Pinker, 1997; Fodor, 2000), there is growing evidence that the brain is not a generalized computing device exhibiting equipotentiality between regions (Lashley, 1950). Rather, the brain is a collection of functionally specialized areas that carry out domain-specific processing, such as for object or face identity, spatial location, or emotional content. This evidence has motivated the view that consciousness, although experienced as a unitary and coherent whole, is instead a composite of fractionated and disparate processes that are drawn together in a dynamic network or workspace (Baars, 1989; Dehane and Naccache, 2001). According to this hypothesis, consciousness, at any one time, comprises only those distributed processes that have been integrated into a large-scale system of cortical activity, and which receive attentional amplification. Consequently, much processing occurs outside of awareness (Zeki, 2003).

The most striking demonstration of the disunity of consciousness comes from studies of patients who deny that they have a severe cognitive deficit. For example, patients who exhibit hemispatial neglect, commonly as a result of right parietal damage, are often aware that they have been diagnosed with a deficit, but sometimes refuse to accept the diagnosis. To the patient, the notion that they neglect a side of space can seem nonsensical since their conscious experience has been reduced to encompass only a subset of perceptual and memory processes. As one patient put it "I knew the word 'neglect' was a sort of medical term for whatever was wrong but the word bothered me because you only neglect something that is actually there, don't you? If it's not there, how can you neglect it?" (Halligan and Marshall 1998).

Similarly, in anosognosia for hemiplegia, the paralysis of a limb is denied, even when the patient is confronted with their paralyzed hand (Ramachandran and Rogers-Ramachandran, 1996). This usually results from damage to the right hemisphere (Bisiach *et al.*, 1986; Gainotti, 1989). According to the workspace view of consciousness, this happens because any remaining processing in the damaged domain is unavailable for integration into a widespread cortical system and cannot therefore contribute to awareness. Thus, a lesion in a specific location may wipe out not only processing of an attribute, such as input from a side of space or

the body, but also the awareness that the attribute exists. For the patient, therefore, denying a deficit is congruent with their conscious experience.

These patients often exhibit a tendency to interpret experiences in a way that rationalizes conflicting information and involves considerable confabulation, or storytelling, that may diverge markedly from reality (Gazzaniga, 2000; Cooney and Gazzaniga, 2003). These confabulations are attempts to produce logical explanations for evidence of the patient's impairments, which fit with their conscious experience. Bizarre confabulations that seem untenable to most people, because of conscious access to information that contradicts them, probably seem completely normal to patients to whom only a subset of the elements of consciousness are available for integration.

Confabulations reveal the piecemeal and interpretive nature of conscious experience. To construct a coherent narrative from the various elements of experience, a system for resolving ambiguities is needed. This system must take the information available from other brain processes – perceptual, motoric, mnemonic, and emotive – and integrate them into a stable description of reality that can be used for ongoing behavioral maintenance.

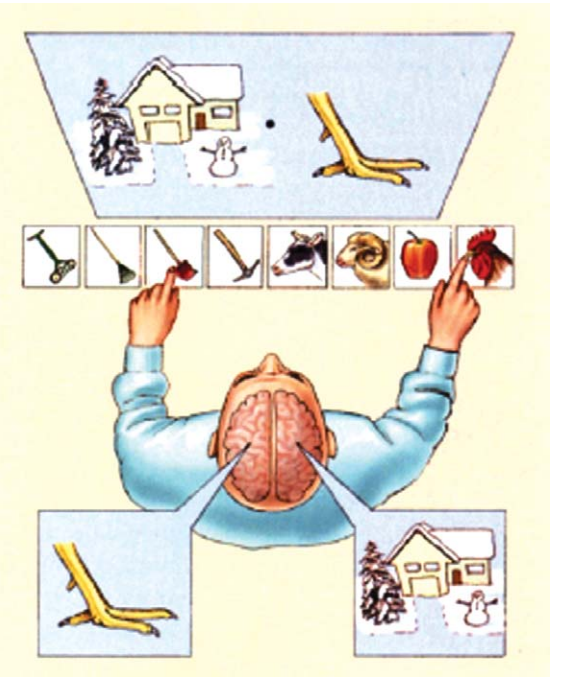

Figure 1 The method for testing each hemisphere of a split-brain patient. Each hemisphere receives only the visual input presented contralateral to fixation. Each hemisphere then chooses an item congruent with the scene presented. Reproduced from Cooney J. W. and Gazzaniga, M. 2003. Neurological disorders and the structure of human consciousness. *Trends Cogn. Sci.* 7, 161–165, with permission from Elsevier.

4.33.2 Interpretive Processes in the Two Hemispheres

A number of experimental findings suggest that the left hemisphere is particularly involved in incorporating available information into a stable representation of reality. Cutting the corpus callosum, the large band of axons that joins the two hemispheres, divides consciousness between the two hemispheres (Sperry, 1969). Lateralized presentation of visual stimuli can allow one hemisphere to be conscious of some event while the other hemisphere remains unaware of the exact nature of the stimulus (see Cortical Commissural Connections in Primates, The Evolution of Hemispheric Specializations of the Human Brain). These split-brain patients typically profess to feel completely normal.

Dividing the hemispheres can, however, reveal the interpretive nature of consciousness in the left hemisphere. In fact, the left hemisphere seems driven to generate hypotheses about information with which it is presented. Gazzaniga (2000) has termed this the left-hemisphere interpreter. In one experiment (Gazzaniga, 2000), the patient is presented with two different scenes simultaneously, one to each hemisphere (Figure 1). The patient is then asked to use his or her left hand (controlled primarily by the right hemisphere) to choose an appropriate item from an array of pictures of objects that may or may not be typically found within the presented scenes. The left hemisphere, which has no knowledge of what was presented to the right hemisphere, can observe the actions of the right hemisphere. When the patient is asked to describe why they chose a particular item, the left hemisphere, which houses the main centers essential for language production in most people, will often reply with an explanation for the action that is congruent with the scene presented to the left hemisphere. These rationalizations can be elaborate, including experiences from outside the experimental setting, and resemble the confabulatory explanations made by patients. Similarly, if a verb such as 'laugh' is presented to the isolated right hemisphere, the represented action may be carried out. When the patient is asked what he is doing, the left hemisphere usually replies with a reasonable explanation for his action, while the patient remains unperturbed by the discord between the explanation and the true stimulus for the action (Figure 2).

Memory tasks also reveal the predilection of the left hemisphere for generating hypotheses about recent experiences, even when this is detrimental to performance. Studies in which each hemisphere of a split-brain patient is required to determine whether a

Figure 2 Lateralized presentation of a command to the right hemisphere can lead to left-hemispheric interpretation of the actions initiated by the isolated right hemisphere. Reproduced from Cooney J. W. and Gazzaniga, M. 2003. Neurological disorders and the structure of human consciousness. *Trends Cogn. Sci.* 7, 161–165, with permission from Elsevier.

picture was drawn from a previously viewed set of scenes suggest that recognition–memory performance by the left hemisphere is more affected by expectations based on the meaning of the scenes than is performance by the right hemisphere (Phelps and Gazzaniga, 1992; Metcalf *et al.*, 1995). For instance, the left hemisphere often falsely recognizes pictures as having been presented in an earlier session if they are congruent with one of the previously viewed scenes. It is thought that this is because the left hemisphere elaborates on the scenario presented and consequently confuses these internal elaborations with novel semantically related distractor stimuli. The isolated right hemisphere does not exhibit this tendency, but instead seems to maintain a more veridical representation in memory of recent events. Thus, a left-hemispheric interpretive system that elaborates on the information it receives may be primarily responsible for the distortions of memory that have been well documented in normal subjects and for the apparent lack of memory deficit in some patients who have suffered insult to right-frontal cortex (Miller and Gazzaniga, 2000).

Another finding that hinted at the tendency for the left hemisphere to generate hypotheses about input it receives involved a task in which subjects had to predict the location (high or low on a screen) of a target stimulus. Neurologically normal subjects usually distribute their responses according to the probability that each stimulus will appear, a strategy known as frequency matching, even though this is suboptimal. This tendency reflects a search for sequences in the stream of stimuli (Yellott, 1969, cited in Wolford *et al.*, 2004). Wolford *et al.* (2000) found that this response strategy was also displayed by the isolated left hemisphere of two split-brain patients, but that the right hemisphere maximized the number of correct responses by consistently choosing the location at which the target was most often displayed. The mode of responding adopted by the right hemisphere is also exhibited by animals. Essentially the same finding was obtained by testing patients who had sustained damage to either the left or the right frontal lobe. Thus the left hemisphere based its responses on a hypothesized pattern in the stimuli, but the right hemisphere did not. More recent tests have revealed that the right hemisphere does, in fact, frequency-match when presented with stimuli for which it is specialized for processing, such as faces, while the left hemisphere responds randomly (Miller and Valsangkar-Smyth, 2005). These results were interpreted as suggesting that one hemisphere cedes control of the task to the other hemisphere, essentially giving up on the task, if the processing required is outside of its domain of expertise (Wolford *et al.*, 2004).

Another area of relative expertise in which the right hemisphere seems to interpret available evidence is visuospatial perception. Split-brain testing suggests that both hemispheres are capable of a range of visual perceptual tasks, such as determining whether objects are of the same size or luminance. The right hemisphere, however, outperforms the left in tests of spatial ability, such as determining alignment and orientation (Corballis *et al.*, 1999b, 2002), and some, more sophisticated visuospatial processes are only able to be performed by the right hemisphere in split-brain patients. Amodal completion, for example, in which occluded contours are perceived, and which requires the visual system to group stimuli and infer the contours, cannot be performed by the left hemisphere (Corballis *et al.*, 1999a). Similarly, only the right hemisphere is able to extract the causal structure from the spatial and temporal properties of the movements of colliding stimuli (Roser *et al.*, 2005), and functional imaging of normal subjects has also suggested right-hemispheric involvement in this task (Fugelsang *et al.*, 2005). Thus the right hemisphere

might also be said to engage in interpretation of a kind that extracts structure from a fragmented sensory representation. Corballis (2003) has referred to this construction of higher-level representations of the visual environment as a right-hemispheric visual interpreter and speculated that the poor performance of the left hemisphere on tasks requiring complex visual analysis may be due to its having lost visuospatial abilities it once possessed as lateralization of function evolved (Corballis *et al.*, 2000).

The interpretive abilities of the isolated right hemisphere are, however, limited. The extraction of causal structure from collision events undertaken by the right hemisphere was evident in the absence of the ability to perform another task that required logical reasoning about causal contingencies at a level that is achieved by 2-year-old children (Roser *et al.*, 2005). For this task, the left hemisphere was necessary. Earlier testing of two split-brain patients also found that only the left hemisphere could perform above chance on inferential tasks, such as choosing the picture representing the correct consequence of combining objects represented by two other pictures and carrying out simple mathematical operations, despite these two patients possessing some language comprehension in the right hemisphere (Gazzaniga *et al.*, 1984).

Recent functional imaging studies suggest that logical reasoning, such as deducing a conclusion from a set of premises, seems to involve mostly left-hemispheric regions, including the left prefrontal cortex (PFC) (Goel and Dolan, 2003; Noveck *et al.*, 2004). Reasoning about abstract problems with no semantic content, the so-called content-independent reasoning, has been found to elicit activity in a left-hemispheric frontal-parietal system. Content-dependent reasoning, in which tasks involve stimuli relevant to an individual's beliefs, values, and goals, involves a left-hemispheric frontal-temporal system. These left-hemispheric regions overlap with, but extend beyond, areas involved in language.

Right-hemispheric activity is also apparent in some imaging studies of reasoning (Houdé *et al.*, 2000, 2001), although sometimes at levels of significance much lower than those seen in the left hemisphere (Goel and Dolan, 2003; Noveck *et al.*, 2004). Heterogeneity of experimental tasks may account for some differences in observed areas of activation, but results suggest that emotional factors, and conflicts between logical conclusions and beliefs, engage right ventral-medial PFC. This area was activated by the inclusion of an emotive component in a task in which subjects had to learn to inhibit a commonly made logical error (Houdé *et al.*, 2000, 2001). This activation was in the same location as the damage sustained by Phineas Gage and by patients assessed by Damasio (1999), who exhibit logical reasoning problems thought to be due to the destruction of a crucial emotional component in reasoning (Houdé and Tzourio-Mazoyer, 2003). This same region was also found to be active when a syllogism's logically valid conclusion was not believable and is thought to be involved in inhibiting the response associated with a belief bias (Goel and Dolan, 2003). The involvement of additional regions outside the left hemisphere may underlie the behavioral observation that content-dependent (nonabstract) reasoning is performed better than abstract reasoning even if the logical structure is the same. The developing picture, however, is that processing of logical rules is primarily undertaken by networks of regions within the left hemisphere.

That the left hemisphere solves syllogisms by abstracted reasoning is also suggested by the observation that the suppression of the right hemisphere by electroconvulsive therapy (ECT) leaves patients inclined to accept conclusions that are absurd but based on strictly true logic. After left-hemispheric ECT, the same absurd conclusions are indignantly rejected (Deglin and Kinsbourne, 1996). Thus, if the right hemisphere is suppressed by ECT or damaged, as in hemispatial neglect, the left hemisphere seems to persist in applying logical rules to its reduced subset of consciously experienced inputs, with little heed paid to their common sense likelihood. The resulting confabulatory explanations for life events may serve to resolve conflicts between potentially contrary information and may make perfect sense within the limited sphere of the left hemisphere's consciousness, but they can often diverge sharply from reality. Disconnection of the right hemisphere from the left by callosotomy also results in interpretation from a reduced range of available input although, in split-brain patients, the intact right hemisphere may prevent confabulations from becoming too outlandish.

4.33.3 An Evolutionary Perspective

Maintaining a stable representation of reality over time must involve the incorporation of input from multiple distributed brain processes, but for the resulting construct to bear a resemblance to reality this process must occur in an orderly, logical fashion. Evidence suggests that interpretation of perceptual and cognitive processes, and the construction of a personal narrative, depends on the left hemisphere, as, to a large degree, do language and logical reasoning. The common functional requirement between

these domains is the need to manipulate units of information in a syntactic manner, that is, according to formal rules sometimes involving several levels of recursion. For instance, in constructing an explanation for one's response to a stimulus, such as the actions of another, the interpretive system must take account of the rule that causes must always precede effects. Taking multiple perceptual, mnemonic, and cognitive processes and combining them in a rule-based manner in order to generate a novel description of current experience is likely subserved by brain systems in the left hemisphere, including, but not limited to, the areas classically associated with language. Although verbally expressing a confabulatory story to explain one's experiences obviously depends on language, it is probably not the case that nothing more than language mechanisms are involved in interpretation. The observation that a number of extralinguistic areas are recruited in logical inference, and the placid acceptance of circumscribed conscious experience as representative of reality by many brain-injured patients, suggest that interpretation is not merely storytelling, but is instead an essential component of consciousness involving many brain areas.

The close association between language, logic, and interpretation suggests a similar evolutionary history (see Evolutionary Psychology). PFC in humans does not seem particularly large when compared to other primates (Roth, 2001), suggesting that human interpretive abilities cannot be accounted for simply by appealing to absolute or relative PFC size. Instead, changes to structural and functional organization and the relative size of regions within the PFC may be more promising avenues for investigation (Fuster, 1997).

Important differences between human and nonhuman brains do, however, exist in this area. For instance, Broca's area in the left frontal lobe, which is involved in syntactic processing in language, is asymmetric in humans. Modern apes do not exhibit the same hemispheric asymmetry in the homologue of this area (Sherwood *et al.*, 2003). The earliest date for the emergence of Broca's area is around 2 Mya, with the genus *Homo*, as suggested by its appearance in skull endocasts (Falk, 1983; Tobias, 1987). If syntactic combinatorial abilities in tool production (Ambrose, 2001), and in either manual or vocal communication (Corballis, 2002), first emerged with this genus, then interpretive abilities, drawing on the same mechanisms for rule-based operation, may have emerged alongside.

In fact, interpretation of one's own behavior, and the behavior of others, in mental terms may only have become necessary once behavioral complexity and flexibility evolved to a point at which it became impossible to represent the range of possible behaviors as simple abstractions based on statistical co-occurrence. The uniqueness to humans of a mentalistic representation of the self and of others is suggested by research that delineates the limitations of chimpanzee cognition (Premack, 2004), and which points out that behavioral abstraction can account for many findings that have been taken as evidence for mentalistic representation in chimpanzees (Povinelli and Giambrone, 2000; Povinelli and Vonk, 2003).

Interpreting one's conscious experience in a coherent manner underlies a conception of oneself as a mental entity, with continuity through time, and with control over one's actions. This capacity stems from the systems that make humans unique, the left hemisphere's ability for generative, recursive thought.

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4.34 Human Cognitive Specializations

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Glossary

emulation	A type of social learning characterized by copying a rule pertaining to environmental effects (causes), results, or goals using idiosyncratic movements. Emulation is often contrasted with imitation, which is typically defined as copying specific actions and their respective goals.
episodic memory	Memories about one's personal past; autobiographical recollections.
percept	A representation of something perceived by the senses, regarded as the basic component in the formation of concepts.
proprioception	The perception of bodily movement and the spatial position of limbs relative to the rest of the body arising from internal (bodily) stimuli.
retroduction	A general process of logical inference that generates possible causes (theories) for available facts. Competing (causal) theories are evaluated based on their relative predictive abilities. Also known as hypotheticodeduction.
theory of mind	The ability to reason about unobservable psychological states such as seeing and knowing.

4.34.1 Introduction

What makes the human mind 'human'? Arguably, Charles Darwin articulated the most influential answer to this question. In *The Descent of Man*, Darwin (1871) challenged orthodoxy and many of his champions, including the co-discoverer of the theory of natural selection Alfred Russell Wallace, and argued in favor of the view that the likeness between humans and other primates was not simply skin deep:

> . . .man and the higher animals, especially the primates, have some few instincts in common. All have the same senses, intuitions, and sensations. . .they practice deceit and. . .possess the same faculties of imitation, attention, deliberation, choice, memory, imagination, the association of ideas and reason, though in very different degrees. . .Nevertheless, the difference in mind between man and the higher animals, great as it is, certainly is one of degree and not of kind (Darwin, 1871, p. 82).

This theory, known as the theory of the continuity of mind, made two radical assertions: (1) the mind is like every other morphological feature – subject to selection and change over time and (2) having directly descended from other living organisms, human and nonhuman animal minds evidenced only quantitative but not qualitative differences.

However, from the outset, such an idea was fraught with problems. Principally, the second point articulated by the theory of continuity of mind was more consistent with pre-Darwinian ideas that espoused a Great Chain of Being – the notion that organisms are ranked from the lowest forms, such as bacteria, to the highest forms, such as humans, angels, and God (Mayr, 1985). According to Hodos and Campbell (1969, 1991), the notion of the Great Chain of Being exists today in the form of the phylogenetic scale. That is, the notion that species may be ranked on a single ladder of ascending complexity. In spite of the obvious limitations and

contradictions with neo-Darwinian theory, the idea of psychological continuity continues to influence how scientists think about the evolution of mind and the broader question of human cognitive specialization. As a result, whereas the modern biologist has thrived on understanding the genetic and morphological diversity that exists both within and among populations of species, those interested in the evolution of mind and behavior have largely shunned an exploration of diversity. Perhaps, when compared to the evolution of physiological features, the evolution of mind has significant social and political ramifications. This was true in Darwin's time (Mayr, 1985) and it is certainly true today, as, for example, the academic resistance to sociobiology (Rose *et al.*, 1984; Wilson, 1975/2000; Lewontin *et al.*, 1985; Alcock, 2001). Therefore, we should not be surprised that those scientists who study the minds of both humans and animals – comparative psychologists – have been the most resistant to elucidating phylogenetic psychological differences. Indeed, the resistance to the idea of a significant and qualitative difference between the minds of human and nonhuman animals has been noted by many (e.g., Hodos and Campbell, 1969; Lockard, 1971; Wasserman, 1981; Boakes, 1984; Kamil, 1984; Macphail, 1987).

But why are so many scientists inclined to believe that the mind has escaped evolution? One possibility is that the domains in which comparative psychologists have traditionally searched for qualitative phyletic differences are precisely those in which we should least expect to find them. In this sense, the statement of Macphail (1987) that "causality is a constraint common to all ecological niches" exposes a more general claim that there are no differences in intelligence among vertebrates. Given that causality is a universal feature of biological environments, the types of general-purpose learning mechanisms that early behaviorists championed should be expected to be present in all animals. This approach has come to be known as general process learning theory (Seligman, 1970). This learning theory attempted to account for all learning with the same set of principles (Shettleworth, 1997).

The general process learning theory turned out to be too simplistic and, eventually, untenable. In a series of now classic papers that were adamantly resisted by establishment psychologists, Garcia and Kimeldorf (1957), Garcia *et al.* (1968, 1976) reported that, when rats are made ill from X-rays at the time they ate food pellets, they form associations about the flavor but not the size of the pellets. However, if, while eating, they are treated with a painful electric shock (rather than X-rays), they form an association with the size but not with the flavor of the pellet. In subsequent tests, rats were systematically treated to electric shock whenever they drank flavored juice. In this condition, rats never learned to avoid the flavored drink. This result perplexed behaviorists but delighted evolutionary thinkers. From an evolutionary perspective it made perfect sense that the consumption of liquids and food does not result in pain in your skin; however, ingesting toxic substances can have damaging internal effects. It follows that animals capable of detecting internal damage and linking these sensory cues with foods or fluids that were recently consumed would have been able to modify their diet adaptively. No such benefit comes from circuitry that enables rats to associate specific foods or fluids with skin pain since ingested substances have no way of acting on external sensors (Alcock, 2001). Refocusing research efforts on ecologically relevant domains in this way might lead to the detection of psychological differences.

Although psychological innovations are rare, rarity should not imply a lack of importance. For instance, in the case of morphological evolution, there have been very few radical transformations in basic animal body plans, yet these core innovations constitute the basis for the classification of distinct phyla (Mayr, 1985, 2001). So, too, radical alterations in psychological forms might occur relatively infrequently. But this is not to state that they never occur. Indeed, comparative psychologists might have already detected the evolution of several such innovations (see Bitterman, 1960, 1975; Gallup, 1982; Rumbaugh, 1990; Itakura, 1996). Thus, in addition to the detection of differing finely scaled psychological dispositions among species, large-scale transformations might also be detectable.

4.34.2 The Reinterpretation Hypothesis

Evidence that has accumulated over the past years suggests that one possible discontinuity between human and nonhuman minds is the ability to interpret observable phenomena, such as an individual's gaze or the propensity for unsupported objects to fall, in terms of unobservable psychological concepts such as desires or physical concepts such as gravity (Povinelli and Preuss, 1995; Povinelli, 2000; Povinelli and Vonk, 2003). For example, when reasoning about behaviors prior to the evolution of a theory of mind system (TOM) in the genus *Homo*, social animals possessed complex nervous systems equipped to detect the various statistical regularities

in the behaviors of others (Heyes, 1997; Povinelli, 2000). The very first social systems were probably quite simple and the information that individual organisms needed to keep track of was relatively limited. However, as some lineages evolved increasingly complicated social interactions, brain systems dedicated to processing information about the regularities of the behaviors of others became increasingly sophisticated as well. The general point is that, for hundreds of millions of years, vertebrates and other taxa have been under steady and unending selection pressures to detect, filter, and process information about the regularities in both their social and physical environments. The hypothesis presented here makes one simple claim: about 3 Mya, one peculiar lineage – the human one – began to evolve the additional ability to interpret these statistical regularities in terms of unobservable causal states. Naturally, this reinterpretation of physical and behavioral events in terms of unseen causal forces was integrated with a pre-existing mechanism for interpreting the observable features of these events.

If this hypothesis (or something like it) is correct, what causal role does the representation of unobservable states play in generating behavior? After all, if complex social behaviors such as self-awareness, gaze-following, social learning, and so forth evolved prior to a TOM and complex technological behaviors such as tool selection, construction, and use evolved prior to an understanding of physical forces, this implies that other psychological systems are independently capable of controlling their execution. Does this mean that the representation of causal forces plays no role in one's actions? We do not think so. Rather, the initial evolutionary advantages of this new psychological system that reinterprets observable phenomenon in terms of imperceptible concepts was that it allowed already existing behaviors (such as social learning or tool use) to be employed in more flexible and proactive ways, without discarding the ancestral psychological systems. As a result, we contend that, for any given behavior, humans will have multiple causal pathways of executing it.

So what evidence exists that phylogenetically ancient behaviors coexist with the uniquely derived ability to interpret these ancient behaviors in terms of invisible causes such as belief and force? A number of laboratories, including our own, have pursued various lines of research in various domains in an effort to answer this and related questions.

Below, we review the results from three general domains: (1) self-awareness, (2) social cognition, and (3) physical cognition. We conclude with an overarching view of human cognitive uniqueness.

4.34.3 The Self

4.34.3.1 Mirror Self-Recognition

In 1970, Gordon Gallup reported that chimpanzees used their mirror reflections to explore body parts difficult to see without the aid of a mirror such as their under arms, teeth, and anogenital region (Gallup, 1970; Figure 1). Gallup also reported that, after lengthy exposures to mirrors, monkeys continued to display social behaviors toward their mirror image, which suggested that they failed to see their reflections as representations of their selves (Gallup, 1970). Following this study, additional research has reported mirror self-recognition in bonobos (Hyatt and Hopkins, 1994; Walraven *et al.*, 1995) and orangutans (Lethmate and Dücker, 1973; Suarez and Gallup, 1981). Gorillas, however, have failed to recognize their mirror image (Suarez and Gallup, 1981; Ledbetter and Basen, 1982; Shillito *et al.*, 1999) with one exception (Patterson and Cohn, 1994; Patterson and Linden, 1981). Subsequent studies with monkeys confirmed Gallup's initial negative findings (e.g., Suarez and Gallup, 1981; Hauser *et al.*, 2001).

Among human infants, evidence of mirror self-recognition first appears around 18 months of age (Amsterdam, 1972; Bertenthal and Fischer, 1978; Johnson, 1982; Anderson, 1994). At this age, infants begin to use their reflection to investigate marks on body parts such as their nose and head much as nonhuman apes do (Figure 1a). The distribution and development of mirror self-recognition within the primate order suggests that the ability to recognize one's self-image represents an example of a phylogenetic cognitive specialization (see Evolutionary Specializations for Processing Faces and Objects).

The pattern of performance reported for apes in front of mirrors raises an important question: do apes and young human children equally depend upon representing psychological and temporal dimensions of the self? One view of self-recognition has emphasized the role of the kinesthetic dimension

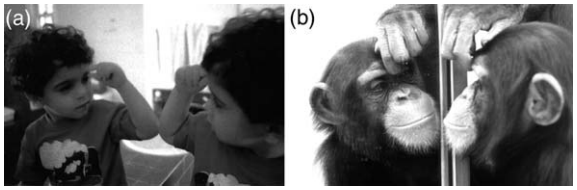

Figure 1 Mirror self-recognition. Examples of a child (a) and of a chimpanzee (b) using a mirror to explore marks on their faces.

of the self (e.g., Povinelli, 1995; Povinelli and Cant, 1995; Barth *et al.*, 2004). In this view, once an organism can hold in mind a kinesthetic representation of the current state of its body, it is able to match this information with the one seen in the mirror. Accordingly, self-exploratory behaviors arise from an association between proprioception and contingent visual cues provided by the mirror's reflection. This kinesthetic–visual matching can be contrasted with a psychological interpretation of the mirror's reflection (e.g., That's me!). In this case, subjects must reinterpret the association between proprioception and visual perception as an abstract self that guides actions independently of both proprioception and visual perception.

To address important aspects of this question, Povinelli and colleagues used live video feeds to explore the role of temporal contingency in supporting mirror self-recognition in 2- to 5-year-old children (Povinelli *et al.*, 1996; Povinelli and Simon, 1997; Povinelli *et al.*, 1999a, 1999b). In those studies, an experimenter played a game with the children in which the subjects were regularly praised. On some occasions the experimenters used this opportunity to secretly put a sticker on the child's head. One group of children saw a live video feed (i.e., they saw the experimenter placing a sticker on their head), the other group saw a 3 min delayed video showing the placement of the sticker. Most of the children that saw the live images retrieved the sticker, whereas few of the younger children who saw the delayed video retrieved the sticker. However, it is important to note that the younger children did not fail to retrieve the sticker on their head because they failed to recognize themselves in the delayed images. In fact, they would accurately state that they saw themselves in the video, but would refer to him/her (i.e., speaking in the third person) as having a sticker on their head. This suggests that for children younger than 4 years of age it is difficult to link the present self to a past self. This is a remarkable fact when one considers how early in development mirror self-recognition appears (see Amsterdam, 1972).

As has been noted by various scientists, the ability to recognize one's image has a number of implications. Gallup (1977) repeatedly proposed that the evidence of mirror self-recognition may be used as an index of self-consciousness or, as he phrased it, the ability to become the object of one's own attention. This interpretation of the results was premised on the notion that, to recognize an image in a mirror as one's own, one had to have an abstract (unobservable) concept of self. Later, Gallup (1982) speculated further, arguing that, if chimpanzees, bonobos, and orangutans (and by extension, 18-month-olds) were self-aware in this sense, they might also have the capacity to reflect upon their own experiences and, by inference, the experiences of others; this topic we discuss below at length.

4.34.3.2 Episodic Memory: The Self in Time

Tulving (1983; Tulving and Markowitsch, 1998) named the ability to reflect upon one's experiences as episodic memory. Tulving and Markowitsch (1998, p. 202) defined episodic memory as having to do with "the conscious recollection of previous experiences of events, happenings, and situations." In short, episodic memory concerns events experienced in one's personal past. Such autobiographical memories are, presumably, defined by a concept of self that is not anchored to facts about our lives in the here-and-now, but is free to move seamlessly backward and forward in time while reflecting on its history.

Schwartz and Evans (1994, 2001) have argued that episodic memory is characterized by three critical features: (1) it refers to a specific event in one's personal past; (2) retrieval involves re-experiencing a past event; and (3) it is accompanied by a strong sense of confidence in the veracity of the memory. Clayton and Dickinson (1998) developed criteria to examine features of episodic memory in nonlinguistic animals. In their view, the critical components of episodic memory is the binding of information about the what, where, and when of a given event. Others have included who, as well (Schwartz *et al.*, 2002). These researchers have resorted to the term episodic-like in recognition of the fact that with nonlinguistic animals it is impossible to ascertain whether they are reflecting on or re-experiencing their past.

To date, the strongest evidence of episodic-like memory has been reported in food-storing birds (scrub jays) and in apes (Clayton and Dickinson, 1998; Schwartz and Evans, 2001; Schwartz, 2005). Scrub jays are particularly interesting because in the wild these birds cache extra food. When food is in short supply, they return to the cache sites. In a series of laboratory studies, Clayton and Dickinson measured whether scrub jays remembered the location of cached food on a single and unique trial of learning. In these studies, jays had to encode information about the type of food (what), it's freshness (when) and its location (where). In a typical experiment, crickets were stored on one side of an ice tray and peanuts were stored on the other side. Jays naturally prefer to eat crickets, but, whereas peanuts remain edible for long periods of time, crickets do

not. To respond adaptively, jays had to encode when a given food was cached, switching from crickets to peanuts after long delays. This is, in fact, how jays responded. Clayton *et al.* (2001) argued that this is evidence that jays bind information about the what, the where, and the when of events.

There is only a single published account of a nonhuman primate encoding multiple types of information in a single event. Schwartz and his colleagues have reported that a gorilla named King made what and who judgments in some cases after a 24h delay (Schwartz *et al.*, 2002, 2004, 2005). In one study, King had to select different cards that contained information about a type of food (e.g., banana) or an individual trainer. During training, King learned to respond appropriately to the commands "what did you eat?" and "who gave you the food?" During testing, King was asked both what and who questions. King responded correctly to what and who questions on 43% of the trials (chance was 10%).

While intriguing, the presence of this type of memory binding in species that do not typically evidence spontaneous mirror self-recognition, such as birds and gorillas, suggests that encoding multiple components of an event as described by Clayton and Dickinson and Schwartz and colleagues is independent of a concept of self (kinesthetic or otherwise) free to move forward and backward in time (Tulving, 1983). In this regard, we expect that future studies will show that many animal species are able to bind different facts about an event. Yet, we are doubtful that this paradigm, by itself, will answer whether nonhuman animals are able to re-experience their past in the same way humans do.

4.34.4 Social Cognition

4.34.4.1 Gaze-Following

One of the features that characterize the primate order is its gregariousness. For example, our closest living relative, the chimpanzee, resides in medium-sized groups that consist of males and females (Goodall, 1986). Males patrol the borders of their territories and cooperate when hunting small monkeys (Mitani, 2006). They also engage in complex social struggles for control over valuable resources such as food, mates, and allies. De Waal (1982) has aptly referred to this feature of chimpanzee societies as chimpanzee politics. In order to navigate their social worlds, chimpanzees, like humans, probably form representations of the behavior of others, predict future actions and adjust their own conduct accordingly (see Relevance of Understanding Brain Evolution). For example, when a chimpanzee sees a conspecific pursing his lips with hair bristling, he need not represent each of these behaviors separately. Rather, a concept of threat display can be formed. In like fashion, primates are likely to form all sorts of concepts based on observable behaviors.

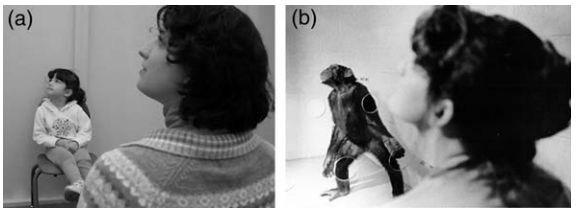

Figure 2 Gaze-following. Examples of a child (a) and of a chimpanzee (b) following the gaze of an experimenter.

Consider the behavior of gaze-following and joint-attention. Primates in general and apes in particular are acutely sensitive to the direction of gaze (Figure 2). Determining the precise direction of another's attention is an important ability because it provides salient information about the location of objects such as food and predators. In social settings, a great deal of information is communicated by means of following other individuals' gaze to specific individuals or to call attention to specific events.

Several field studies suggest that primates can follow the gaze of conspecifics (e.g., Chance, 1967; Menzel and Halperin, 1975; Whiten and Byrne, 1988). However, in field studies, it is difficult to identify which object, individual, or event is the focus of two individuals' attention and whether they arrived at the focal point by following one another's gaze. For instance, individuals may come to fixate on the same object because the object is inherently interesting even if they do not follow gaze. Such interpretational confounds can be effectively excluded in laboratory studies. In fact, various studies have demonstrated that many primate species follow the gaze of others to objects (e.g., chimpanzees, mangabeys, and macaques) (Call *et al.*, 2000; Emery *et al.*, 1997; Tomasello *et al.*, 1998; Tomonaga, 1999). They do this even when the target is located above and/or behind them (Itakura, 1996; Povinelli and Eddy, 1996b, 1997). Itakura (1996) studied the ability of various species of prosimians, monkeys, and apes to follow a human experimenter's gaze. Only chimpanzees and one orangutan responded above chance levels. Neither Old nor New World monkeys (i.e., brown lemur, black lemur, squirrel monkey, brown capuchin, whiteface capuchin, stump-tailed macaque, rhesus macaque, pig-tailed macaque, and Tonkean macaque) responded above chance levels.

The clearest evidence for the ability to follow gaze in nonhuman primates comes from laboratory work

on great apes, in particular chimpanzees (Figure 2). For instance, Povinelli and Eddy (1996a), in order to investigate how chimpanzees follow another individual's gaze, installed an opaque barrier in a testing room, obstructing subjects' line of sight. In cases where the experimenter looked to an object next to the barrier (outside the immediate line of sight of the subject), chimpanzees followed the experimenter's line of sight around the barrier to the unseen object. These results have been replicated and extended to all four great ape species (Bräuer *et al.*, 2005) and human children (Moll and Tomasello, 2004). This ability might be important when trying to extrapolate information from other's attention, specifically when the focus of attention is out of sight (rhesus monkeys: Emery *et al.*, 1997; chimpanzees: Tomasello *et al.*, 1999). These findings suggest that primates do not reflexively follow gaze to the first available object within their view, but actively track the gaze of others geometrically to locations or objects that are the focus of others' attention.

One method commonly used to investigate nonhuman primates' ability to use gaze cues, is the object-choice task. In this task, subjects must choose one of two containers, only one of which is baited (Figures 3 and 4). In a series of studies, Anderson and his colleagues used this task to investigate whether capuchin monkeys (Anderson *et al.*, 1995) and rhesus macaques (Anderson *et al.*, 1996) use human gaze to locate hidden food rewards. Subjects were tested in various conditions: pointing only, gaze only (head orientation and eyes cues), and gaze and pointing. None of the capuchin monkeys or rhesus macaques could be trained to use the gaze-only cue to retrieve a concealed reward. However, some subjects eventually learned to use either the pointing only or the gaze and pointing cue. However, it is likely that local enhancement (Thorpe, 1956) may explain these subjects' success in the gaze and pointing situation (e.g., responses may be guided by the hand's proximity to the container).

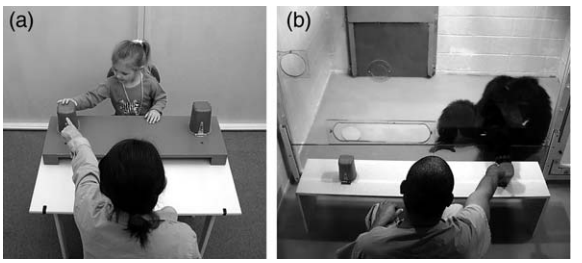

Figure 3 Using proximate pointing cues. A child (a) and a chimpanzee (b) using an experimenter's proximate pointing cue to locate hidden rewards.

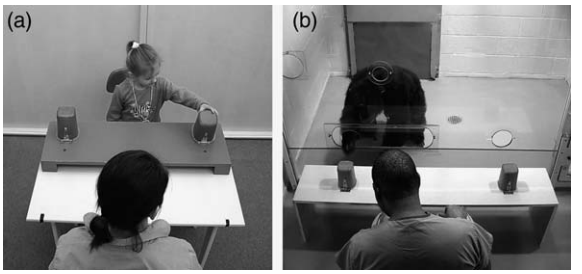

Figure 4 Using distant gaze cues. A child (a) and a chimpanzee (b) using an experimenter's distant gaze cue to locate hidden rewards.

Using a similar paradigm, Itakura and Tanaka (1998) found that chimpanzees, an enculturated orangutan, and human infants (18–27 months old) used an experimenter's gaze, including pointing and glancing (without head turning), to choose a baited container. These responses appeared to be spontaneous and independent of training. Povinelli *et al.* (1999), however, found that chimpanzees failed to use the eyes only (glancing) cue when responding in a similar task. These differences may be due to the age, experimental experience, testing design, and developmental history of the different groups of chimpanzees. Nevertheless, the available research suggests that there is a qualitative difference between monkeys' and apes' understanding of gaze cues in the object-choice task (see also Itakura and Anderson, 1996).

Povinelli and Eddy (1996a, 1996b) have offered an explanation for the differences between monkeys and apes in this task. They theorized that following another individual's gaze might be an automatic response and form part of a primitive orienting reflex triggered by a reward. This reflex does not require the attribution of a mental state. The use of an operant task to test gaze-following would fail to test for the presence of a primitive orienting reflex compared to a more complex social cognition mechanism (e.g., a theory of mind). Monkeys, for example, might follow the gaze of conspecifics yet fail to use the same cue in operant tasks.

The development of this ability in chimpanzees and humans closely parallel one another. For instance, Okamoto *et al.* (2002) demonstrated that, starting at 9 months of age, a chimpanzee infant began using various social cues such as tapping or pointing and head turning to direct the attention to an object. By 13 months of age the infant reliably followed eye gaze. Starting at 21 months of age, the infant looked back to targets located behind him, even when there was a distracter in front of him (Okamoto *et al.*, 2004). Research

with human infants has produced similar results. From 3 months of age, human infants are able to discriminate changes in an adult's eye direction (Hains and Muir, 1996). The development of gaze-following in human infants has been widely studied (e.g., Scaife and Bruner, 1975; Butterworth and Cochran, 1980; Butterworth and Jarrett, 1991; Corkum and Moore, 1995; D'Entremont *et al.*, 1997). By 12 months of age, human infants begin to follow their mother's gaze toward particular objects in their visual field, and at around 18 months they can direct their attention to objects outside of their visual field. Although there are some developmental differences in the onset of gaze-following, on the surface the development of gaze-following in human and chimpanzee infants appears to be remarkably conserved.

But alongside these similarities in the gaze-following behavior of humans and nonhuman primates important differences exist. For example, Okamoto *et al.* (2002, 2004) reported that an infant chimpanzee failed to look back at the experimenter after following her gaze to an object located behind him. This triadic interaction between mother, child, and object of interest has been widely reported in the human developmental literature but is largely absent in the animal literature. Researchers have offered various explanations for these differences. Among humans, a number of changes in social communication occur at around 6 to 9 months of age (Carpenter *et al.*, 1998a). By 6 months, human infants interact dyadically with objects or with a person in a turn-taking (or reciprocally exchanging) sequence. However, they do not interact with the person who is manipulating objects (Tomasello, 1999). From 9 months on, infants start to engage in triadic exchanges with others. Their interactions involve both objects and persons, resulting in the formation of a referential triangle of infant, adult, and object to which they share attention (Rochat, 2001; Tomasello, 1999). That is to say, shared attention is an important component of social cognitive skills in human infants 12 months of age and older. These theories suggest that the chimpanzee infant described in Okamoto *et al.* (2004) and the human experimenter jointly attended to the object behind the infant without engaging in shared attention.

Nevertheless, results from our own laboratory (Povinelli and Eddy, 1996c; Povinelli, 2000) have revealed that chimpanzees and humans share many aspects of gaze-following behavior exhibited by 18-month-olds, including: (1) the ability to extract specific information about the direction of gaze from others; (2) the ability to display the gaze-following response whether it is instantiated by movements of the hand and eyes in concert or the eyes alone; (3) the ability to use another's gaze to visually search into spaces outside their immediate visual field in response to eye plus head/upper torso movement, eye plus head movement, or eye movement alone; (4) no requirement to witness the shifts in another's gaze direction in order to follow it into a space outside their immediate visual field; and (5) the possession of at least a tacit understanding of how another's gaze is interrupted by solid, opaque surfaces.

4.34.4.2 Understanding Seeing

There are two broadly different ways of interpreting the level of social understanding associated with chimpanzees' gaze-following abilities. First, chimpanzees and other nonhuman primate species (and even human infants) may understand gaze not as a projection of attention, but as a direction cue. It is possible that the ancestors of the modern primates evolved an ability to use the head/eye orientation of others to direct their own visual system along a particular trajectory. Once their visual system encountered something novel, the orientation reflex would ensure that two chimpanzees, for example, would end up attending to the same object or event, without attributing an internal (psychological) state to each other. This kind of gaze-following system may have evolved because it provided useful information about predators or social exchanges at little or no cost to individuals involved. A second account is that apes follow gaze because they appreciate its connection to internal attentional states. We will refer to these two accounts as the low-level and the high-level account of gaze-following.

In an effort to distinguish between the low- and the high-level account of gaze-following, Povinelli and colleagues executed a series of studies that measured chimpanzees' and human children's understanding of 'seeing' as a psychological (unobservable) function of eyes. To address this question, they used the chimpanzee's natural begging gesture (Figure 5), a gesture that this species uses in a number of different communicative contexts, including soliciting allies, requesting food, or reconciliation with others after hostile encounters. The apes were trained to use this gesture in a standardized routine: the apes entered a test unit in which they were separated from human experimenters by a Plexiglas partition, and they quickly learned to gesture through a hole directly in front of a single, familiar experimenter who was either standing or sitting to their left or to their right. On each trial that

they gestured through the hole to the experimenter, this person praised them and handed them a food reward. This training set the stage for examining the animals' reactions to two experimenters, one whose eyes were visible and therefore could respond to their gestures, and another whose eyes were covered or closed and therefore could not respond to their gestures. Several treatments recreated this problem (Figure 6).

When first confronted with two experimenters during one of these treatments, the animals' first reaction was to pause. But after noticing the novelty of the conditions, the apes in these studies were as likely to gesture to the person whose eyes were covered/closed as to the person whose eyes were visible/open. In other words, the chimpanzees displayed no preference for gesturing toward the experimenter who could see them. Yet, on trials when subjects were presented with a single experimenter, the apes gestured through the hole directly in front of them on virtually every trial. Thus, despite their general interest and motivation in the test, when it came to the seeing/not seeing treatments, the animals responded indiscriminately seemingly, oblivious to the psychological state of seeing. These same chimpanzees were tested in a number of other experiments, which further manipulated the presence of eyes and/or the orientation of the experimenter's posture (Figure 6). Nevertheless, in all instances, chimpanzees ignored the eyes as cues and relied almost exclusively on global cues such as the back/front posture of the experimenter. These results have now been independently replicated by other comparative psychologists working with captive chimpanzees (Kamisky *et al.*, 2004).

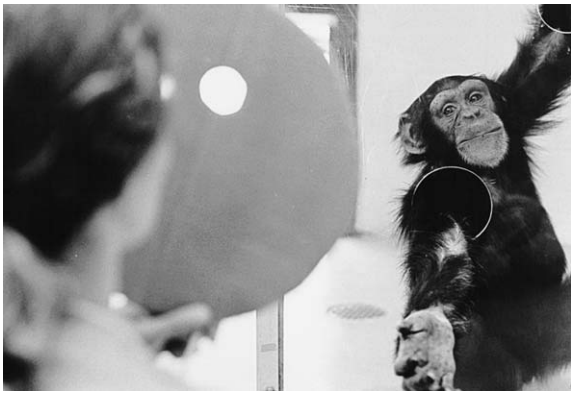

Figure 5 Chimpanzee begging gesture. Example of a captive chimpanzee gesturing to an experimenter.

This pattern of performance contrasted sharply with the performance of human children. Children, like the chimpanzees, were trained to gesture to an experimenter for brightly colored stickers. They were tested on several of the conditions used with the apes and it was found that the youngest children (2-year-olds) were correct in most or all of the conditions from their very first trial forward (Povinelli and Eddy, 1996a).

Hare and associates have challenged these results (Hare, 2001; Hare *et al.*, 2000, 2001, in press). They used a competitive paradigm (individuals must compete with conspecifics or human experimenters for food) because they argue that this paradigm is more ecologically valid than the cooperative paradigm (in which subjects gesture to an experimenter) used by Povinelli and Eddy (Hare, 2001; Hare and Tomasello, 2004). In the paradigm of Hare *et al.*, a dominant and a subordinate chimpanzee were

Figure 6 See/not see paradigm. Different experimental manipulations used by Povinelli and Eddy (1996c).

placed in opposite sides of a large enclosure. In certain trials, both the subordinate and the dominant animal were in view of one another and food was placed in a position that was visible to both the subordinate and the dominant animal. In other trials, food was strategically placed in a position that was only visible to the subordinate. Hare and colleagues reported that subordinate animals avoided the food that was visible to the dominant animal but not the food that had been strategically positioned so that only the subordinate animal could see. These results were interpreted as evidence that chimpanzees infer some aspects of mental states such as seeing (Hare, 2001; Hare *et al.*, 2000, 2001, in press). Povinelli and colleagues have offered alternative interpretations for these results (see Karin-D'Arcy and Povinelli, 2002; Povinelli and Vonk, 2003, 2004).

However, the see/not-see paradigm (whether competitive or cooperative) poses at least three distinct problems. The first problem involves whether or not the cooperative paradigm of Povinelli and Eddy (1996b, 1996c) or the competitive paradigm of Hare *et al.* (2000, 2001, in press) can adequately isolate nonhuman primates' understanding of unobservable psychological states such as seeing from their understanding and/or use of nonpsychological, observable cues associated with the psychological interpretation of seeing, such as the visibility of the face and the eyes. The main concern is that neither of these particular competitive or cooperative paradigms is adequate to answer the question of whether or not chimpanzees understand seeing as a psychological state. In either paradigm, subjects can develop behavioral rules based on observable cues such as the visibility of the competitor's face, or they may develop rules premised on psychological interpretations of these observable (nonpsychological) cues. However, because the psychological inference depends on the availability of observable cues, and the use of either rule would lead to the same behavioral consequence, it is impossible to discern which rule – psychological or behavioral – subjects are using.

The second problem concerns whether competitive paradigms are better than cooperative paradigms in terms of eliciting psychological interpretations of others' behavior(s). If, in fact, the performance of subjects in Hare and colleagues' studies is dependent upon a specific setting or paradigm, it further suggests that observable cues (unique to the setting), rather than unobservable (psychological) inferences, are guiding the subjects' behavior. This possibility is reinforced by the assertions of the senior authors who have stressed that competitive paradigms mimic the type of situations that might elicit such psychological inferences in the wild (Hare and Tomasello, 2004). But rather than eliciting psychological inferences, such settings can activate arousal/motivational mechanisms that make subjects more sensitive to a competitor's behavior. Regardless, as noted above, because reasoning about what competitors can and cannot see necessarily involves the ability to reason about observable (nonpsychological) variables such as the visibility of the face and eyes, the argument that competitive paradigms are more ecologically valid does not resolve the problem that chimpanzees can use either a behavioristic or mentalistic rule when making a response.

The third problem involves the interpretation of the results and its implication for chimpanzee and human cognition. Despite our skepticism of the studies described above, we do not believe that chimpanzees are mindless automatons. The results reported here and elsewhere speak to the contrary. Chimpanzees use information in a flexible and adaptive manner. In particular, chimpanzees' performance on social (e.g., Hare *et al.*, in press) and physical tasks (e.g., Visalberghi *et al.*, 1995; Povinelli, 2000) speaks volumes about this species' problem-solving abilities as well as their unique perception of the world. We should be neither discouraged nor insulted by the suggestion that chimpanzees may reason about the world in a way that's unique and different from our own. Rather, we should celebrate it.

4.34.4.3 Intentional Communication

In the middle of the twentieth century, a number of studies sought to inculcate into nonhuman primates a uniquely human behavior: language (e.g., Hayes, 1951; Kellogg and Kellogg, 1967; Gardner and Gardner, 1969; Terrace, 1979; Terrace *et al.*, 1979). At best, this tradition highlighted what apes might be capable of learning were they trained under ideal circumstances; at worst, it demonstrated that language is a uniquely human trait and of little use to nonhuman primates (Chomsky, 1964; Terrace, 1979; Pinker, 1994). A different tradition has sought to explore how apes naturally communicate with each other. This vein of research explores parallels in the intentional desire to express goals, desires, and intentions through a means other than language.

But what separates intentional communication from other forms of communication? Tomasello and Call (1997) argue that, in order for a signal (or gesture) to be an intentional form of communication, it must involve a goal and some flexibility for attaining it. This entails using the behavior in

different contexts and with different communicative functions, or, conversely, using different signals in the same communicative context. For these authors, this entails learning. But the learning is not of the signal itself – rather, learning the appropriate social contexts in which to use such signals. Another important feature of identifying intentional communication is that the intentional cue has to be directed to a specific individual rather than to a general (i.e., nonspecific) audience. This appears to be the case with the vervet alarm call system. Vervet monkeys have three general calls for three different predators: eagles, leopards, and snakes. Each call is associated with a specific behavioral response: eagles – run to the center of trees and look up; leopards – run to the limbs of trees; snakes – stand up and look at surroundings (Cheney and Seyfarth, 1990).

Tomasello *et al.* (1985, 1989) recorded a number of gestures used by juveniles in a group to solicit food, play, grooming, nursing, etc. Although they collected no systematic data, these investigators reported that the behaviors were flexibly used in different contexts. Tomasello and Call (1997, p. 244) cite two examples of gestures being used to initiate play:

> . . . the initiation of play often takes place in chimpanzees by one juvenile raising its arm above its head and then descending on another, play-hitting in the process. This then becomes ritualized ontogenetically into an 'arm-raise' gesture in which the initiator simply raises its arm and, rather than actually following through with the hitting, stays back and waits for the other to initiate the play . . . In other situations a juvenile was observed to actually alternate its gaze between the recipient of the gestural signal and one of its own body parts . . . (an invitation to grab it and so initiate a game of chase) . . .

This view of chimpanzee communication has found support among a number of field researchers. For example, Whiten and a number of other renowned primatologists reported 39 behavioral patterns, including a number of behavioral patterns that the authors described as "patterns customary or habitual at some sites yet absent at others, with no ecological explanation" (Whiten *et al.*, 1999, p. 683). Of those, five are described as having communicative functions: rain dance (display), branch slap (attention-getting), branch din (warn/threat), knuckle-knock (attract attention), leaf-strip (threat). There were two other actions with possible communicative/affiliative functions: stem pull-through (which makes a loud sound like leaf-strip and might be used as a threat), and handclasp (where two individuals clap hands above their heads while grooming as a specific affiliative gesture).

A number of controlled studies, however, suggest that apes have difficulty reasoning about (and hence communicating) beliefs and desires (Premack and Premack, 1994; Tomasello and Call, 1997). This apparent inability to reason about the beliefs of others may handicap nonhuman primates' ability to use communicative signals in a meaningful and intentional fashion. Although some studies suggest that chimpanzees might be able to use pointing gestures to located occluded rewards (Menzel, 1971, 1974; Povinelli *et al.*, 1992; Call and Tomasello, 1994; Itakura and Tanaka, 1998), other work has demonstrated that, when humans use pointing gestures to inform chimpanzees about the location of hidden food, chimpanzees appear to rely more on the proximity of the finger or pointing hand than on the referential aspect of the pointing hand/finger (Povinelli *et al.*, 1997; Barth *et al.*, 2005; but see Itakura and Tanaka, 1998).

Chimpanzees may have a more difficult time understanding the referential cues of humans than a conspecific. While no long-term field study on chimpanzee social behavior has ever documented an instance in which a member of this species pointed to something in a referential manner (Nishida, 1970; Goodall, 1986), chimpanzees do use a gesture that topographically resembles pointing: holding out a hand (Bygott, 1979; Figure 5). This gesture does not appear to be used in a referential fashion, rather it appears to be used to solicit food, bodily contact, or as a means to recruit allies during conflicts (De Waal, 1982; Goodall, 1986). In captivity, however, chimpanzees exhibit a number of gestures that look like pointing, but these seem to be restricted to their interactions with humans (Woodruff and Premack, 1979; Savage-Rumbaugh, 1986; Gomez, 1991; Call and Tomasello, 1994; Leavens *et al.*, 1996; Krause and Fouts, 1997). How might we explain such gestures in captivity? One possible explanation is that chimpanzees construct pointing-like gestures from their existing behavioral repertoire because humans consistently respond to their actions (such as reaching) in a manner that the chimpanzees themselves do not understand or intend (Povinelli *et al.*, 2003). A number of people have argued that this is also the case in infancy (Vygotsky, 1962). But whereas human infants begin to redescribe their gestures in an intentional manner between the ages of 18 and 24 months (Karmiloff-Smith, 1992), a similar redescription process might never occur in the development of nonhuman primates.

4.34.4.4 Imitation Learning

As with the attribution of mental states, there has been a long-standing controversy over whether or

not humans are unique in the ability to learn from others. In fact, Aristotle argued in the *Poetics* that humans are "the most imitative creatures in the world and learn first by imitation." In the past 30 years, interest in imitation has experienced a renaissance, particularly as scientists have found that, from birth, neonates copy the facial expressions of adults (Meltzoff and Moore, 1977), and primatologists have documented various instances of tool traditions in populations of wild chimpanzees (McGrew, 1992, 1994, 2001; Whiten *et al.*, 1999) and orangutans (van Schaik *et al.*, 2003).

To date, seven studies have directly compared imitation learning in human and nonhuman (adult) apes using analogous procedures (Nagell *et al.*, 1993; Tomasello *et al.*, 1993; Call and Tomasello, 1995; Whiten *et al.*, 1996; Horner and Whiten, 2005; Horowitz, 2003; Call *et al.*, 2005). Four of these studies reported that, on an operational task for which a tool had to be manipulated in a certain manner to retrieve a reward, humans reproduce the demonstrator's actions with greater fidelity (i.e., imitation) than mother-reared apes (Nagel *et al.*, 1993; Tomasello *et al.*, 1993; Call and Tomasello, 1995; Call *et al.*, 2005). The other two studies reported both similarities and differences between humans and peer-reared chimpanzees when executing specific actions on an object following a demonstration (Whiten *et al.*, 1996; Horner and Whiten, 2005); and one found no differences between the performance of adult humans and chimpanzees (Horowitz, 2003).

However, the notion that humans are unique when learning by imitation has been challanged by Subiaul and colleagues. Subiaul *et al.* (2004) have distinguished between motor imitation (the imitation of a motor rule) and cognitive imitation (the imitation of a cognitive rule). In a series of studies, they reported that rhesus macaques – primates that typically do poorly in motor imitation tasks (Chamove, 1974; Thorndike, 1898; Whiten and Ham, 1992; Tomasello and Call, 1997) – excelled in a cognitive imitation task in which the execution of specific motor rules was independent of the execution of specific serial (cognitive) rules. These researchers suggested that human and nonhuman primates may differ fundamentally in the manner in which they plan, coordinate, and represent the actions of others. This conclusion is buttressed by a number of studies showing a dissociation between action and perception (monkeys: Hauser, 2003; Fitch and Hauser, 2004; human infants: Diamond, 1990; Spelke, 1994, 1997; apes: Myowa-Yamakoshi and Matsuzawa, 1999).

Researchers from a number of disciplines have reported that human and nonhuman primates share

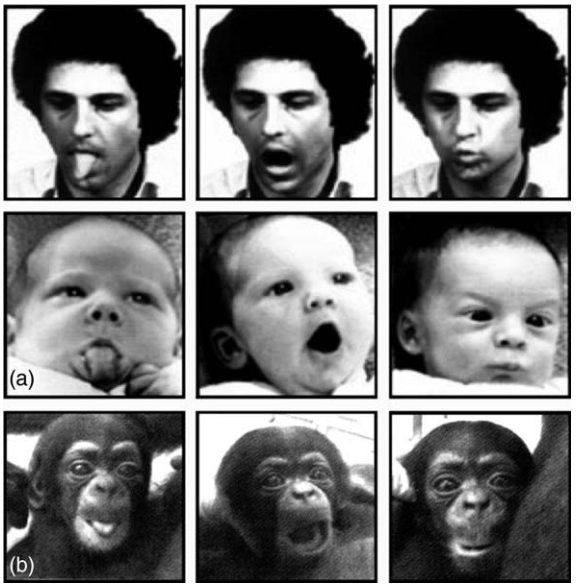

Figure 7 Oral facial imitation. a, Human infants (Meltzoff and Moore, 1977) and b, neonatal chimpanzees (Myowa-Yamakoshi *et al.*, 2004) copying three distinct orofacial movements. Reprinted from Meltzoff, A. N. and Moore, K. W. 1977. Imitation of manual and facial gestures by human neonates. *Science* 198, 75–78. Copyright 1977 AAAS.

a number of homologous mechanisms mediating behavior-matching. For example, Iacoboni *et al.* (1999) and Rizzolatti *et al.* (1988) reported that neurons in the inferior frontal lobe of humans (BA44) and macaques (area F5) are active both when subjects execute a specific action and when they observe a demonstrator execute the same action. Investigators have concluded that BA44 and F5 are evolutionarily homologous (Rizzolatti *et al.*, 2002).

Behavioral research by comparative developmental psychologists has found no significant differences between a human and a chimpanzee infant's ability to copy the orofacial expressions of a model. Chimpanzees, like human infants (e.g., Meltzoff and Moore, 1977), reproduce tongue protrusions, lip protrusions, and mouth openings in response to a model displaying the same expression (Myowa-Yamakoshi *et al.*, 2004). Figure 7 illustrates the similarities of responses between human infants (e.g., Meltzoff and Moore, 1977) and those of neonatal chimpanzee (Myowa-Yamakoshi *et al.*, 2004).

There are also parallels in the developmental trajectory of orofacial imitation in both of these species. Myowa-Yamakoshi *et al.* (2004) report that, after 9 weeks of age, the incidence of orofacial imitation in chimpanzees slowly disappears. A similar phenomenon has been reported for human infants (Abravanel and Sigafoos, 1984). In short, this study found no qualitative differences between humans infants and infant chimpanzees in orofacial imitation.

Nevertheless, there is considerable evidence suggesting that, when learning from others, humans differ from other primates in significant ways. This has become evident in various imitation experiments with young children who evidence reasoning about unobservable mental concepts such as a model's goals and intentions. For example, in one study, Carpenter *et al.* (1995, 1998a, 1998b) exposed children to a model which, while executing a target action, made superfluous movements that were not necessary to achieve the goal. Children only copied the actions that were necessary to achieve the objective, omitting movements that were unnecessary. Gergeley *et al.* (2002) has reported a similar phenomenon. No comparable results have been reported for nonhuman primates.

The performance of human subjects also differs from that of nonhuman primates in a ghost control; that is, a treatment in social learning experiments in which target actions are executed in the absence of a demonstrator. A number of studies have employed this control to isolate imitation from emulation learning (Heyes *et al.*, 1992; Fawcett *et al.*, 2002; Klein and Zentall, 2003; Subiaul, 2004; Subiaul *et al.*, 2004; Thompson and Russell, 2004; Huang and Charman, 2005). However, whereas a number of investigators have reported that human subjects benefit from the standard social learning condition as well as the ghost condition (Subiaul, 2004; Thompson and Russell, 2004; Huang and Charman, 2005), comparative psychologists have reported that animals that copy a rule executed by a conspecific do not copy a similar rule in the ghost control (Heyes *et al.*, 1992; Atkins *et al.*, 2002; Subiaul *et al.*, 2004). This difference between the performance of humans and animals suggests that the ghost treatment is a measure of something other than emulation because, at least among primates, emulation appears to be the default social learning strategy (Horner and Whiten, 2005; Call *et al.*, 2005). Although increasing the salience of the target actions in this control treatment might be sufficient for learning in certain paradigms (Klein and Zentall, 2003), we suspect that learning novel rules in the ghost condition might involve grappling with unobservable concepts. Depending on the experimental context and the task employed, learning in this control condition may require inferring (implicitly or explicitly) actions, intentions, or agency.

The research we have summarized above leads to a number of interesting questions and, potentially, new avenues of research. Some possible questions for future research in social cognition include:

1. Do human and nonhuman primates differ in their sensitivity to behavioral cues and/or the statistical regularities of behaviors?
2. Does the propensity of a human to reinterpret behavioral regularities in terms of unobservable concepts lead to predictable errors that nonhuman primates do not make?
3. Is there a nonverbal experimental paradigm that can distinguish between the use of a behavioral rule and a psychological rule without confounding the two?

4.34.5 Physical Cognition

We live in a world governed by invisible forces such as gravity, strength, weight, and temperature. Although they are invisible, we reason about these forces constantly. A long-lasting question in the comparative sciences has been: do nonhuman primates similarly reason about these forces that cannot be directly perceived but must be inferred?

From a very young age, humans are predisposed to make these kinds of inferences about the physical world. So, when young children see a ball, hit a stationary ball, and then see this second ball darting away, they insist that the first ball caused the second ball to move. Indeed, as the classic experiments of Michotte (1962) revealed, this seems to be an automatic mental process in humans. But what is it, exactly, that humans believe causes the movement of the second ball? As Hume (1739–1740/1911) noted long ago, this belief goes beyond the mere observation that the balls touched. Rather, humans redescribe this observation in terms of the first ball transmitting something to the second ball. That 'something' is, of course, a theoretical force that is ubiquitous, yet unseen.

At the very least, the earliest comparative studies on physical cognition date back to Köhler (1925). In the past decade, there has been a resurgence of interest in nonhuman primates' folk physics. Empirical attention has focused both on tools and on the conceptual systems that govern their use (Köhler, 1925; Boesch and Boesch, 1990; Matsuzawa, 1996, 2001; Hauser, 1997; Visalberghi and Tomasello, 1998; Santos *et al.*, 1999, 2003; Munakata *et al.*, 2001; Santos and Hauser, 2002; Fujita *et al.*, 2003). A significant number of studies have investigated how monkeys understand the relationships between means and ends (e.g., Hauser, 1997; Hauser *et al.*, 1999, 2002b). Of these, some have focused on the

question of whether or not the ability to reason about invisible causal forces mediating the behavior and properties of objects represents a human cognitive specialization (see Visalberghi and Trinca, 1989; Visalberghi and Limongelli, 1994, 1996; Visalberghi, 1997; Limongelli *et al.*, 1995; Visalberghi and Tomasello, 1998; Povinelli, 2000; Kralik and Hauser, 2002; Santos and Hauser, 2002).

In a series of studies, Hauser and his colleagues repeatedly demonstrated that a New World monkey – the cotton-top tamarin – once trained how to use a tool, will readily transfer what it has learned to novel tools that differ in terms of shape and color (Hauser, 1997; Hauser *et al.*, 1999, 2002a, 2002b). A more recent study with capuchin monkeys replicated this result, but, in addition, showed that these monkeys, while not being distracted by the irrelevant features of the tools, nevertheless failed to attend to relevant variables of the task. For instance, they did not learn to pull in the appropriate tool to procure a reward when obstacles or traps impeded performance (Fujita *et al.*, 2003).

In another study, Hood *et al.* (1999) adapted a paradigm used to test gravity rules in human children (Hood, 1995) for use by cotton-top tamarins. The task involved dropping a food reward down a chimney which was at times clear and at other times opaque. The chimney was connected to various solid containers. Whereas children eventually learned to search in the container connected to the chimney, tamarins always searched in the container where the food was dropped on the first trial, ignoring whether the chimney was connected to that container or not. This result suggests that tamarins do not understand general principles of gravity (or connectedness).

However, some authors have suggested that, whereas the same representational abilities characterize the tool-using capacity of monkeys and apes (Westergaard and Fragaszy, 1987), others have implied that chimpanzees use tools in a more complex and sophisticated fashion than monkeys (Westergaard, 1999). In particular, these researchers have hypothesized that the apes succeed where the capuchin monkeys fail because of apes' ability to represent the abstract causal forces underlying tool use (Visalberghi, 1990; Limongelli *et al.*, 1995; Visalberghi *et al.*, 1995).

In an effort to test this and other hypotheses, Povinelli and colleagues in the mid-1990s systematically explored what they termed chimpanzee folk physics (Figure 8). Specifically, they focused the apes' attention on simple tool-using problems such as those used by Köhler, Hauser, and Visalberghi (Povinelli, 2000). Given chimpanzees' natural proclivity with tools (e.g., Whiten *et al.*, 1999), the goal was to teach them how to solve simple problems. All the tasks involved pulling, pushing, poking, etc. Carefully designed transfer tests assessed chimpanzees' understanding of why the tools produced the observed effects. In this way, Povinelli and his associates attempted to determine if their subjects reasoned about things such as gravity, transfer of force, weight, and physical connection, or whether they only reasoned about spatiotemporal regularities. Throughout, these researchers contrasted such concepts with their perceptual properties (see Table 1).

For instance, a series of experiments explored in detail the chimpanzees' understanding of physical connection – the idea that two objects are bound together through some unseen interaction such as the force transmitted by the mass of one object resting on another, or the frictional forces of one object against another. Or, conversely, the idea that

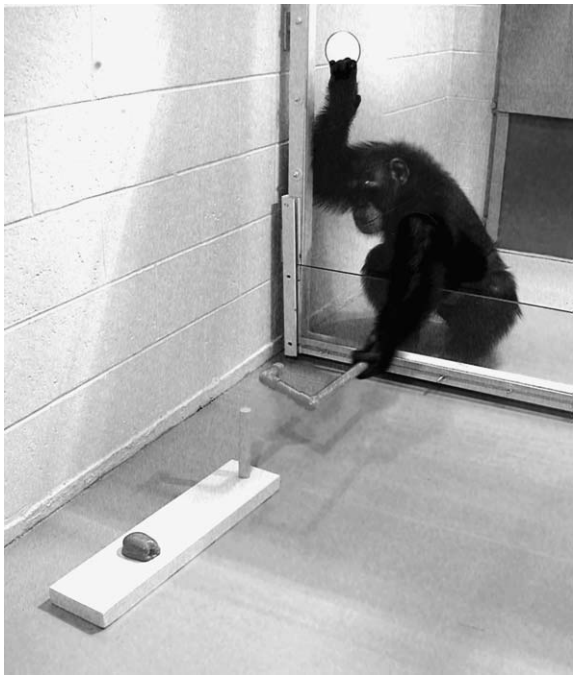

Figure 8 Chimpanzee tool use. One example of a tool task employed by Povinelli (2000) to assess captive chimpanzees' understanding of connectedness, shown here.

Table 1 Theoretical concepts and their observable properties

Theoretical concept	*Paired observable properties*
Gravity	Downward object trajectories
Transfer of force	Motion–contact–motion sequences
Strength	Propensity for deformation
Shape	Perceptual form
Physical connection	Degree of contact
Weight	Muscle/tendon stretch sensations

simply because two objects are touching each other does not mean there is any real form of connection. To answer this question, Povinelli and colleagues presented the chimpanzees with numerous problems. In one set of studies chimpanzees were first taught to use a hooked tool to pull a food tray within reach. Chimpanzees quickly mastered this task. In order to address exactly what the chimpanzees had learned, they were presented with two choices: one was consistent with a theory of intrinsic connection (transfer of force); the other choice was consistent with a theory of superficial contact. In all cases, perceptual and/or superficial contact seemed to be chimpanzees' operating concept. In fact, any type of contact was generally sufficient for chimpanzees to think that a tool could move another object.

Bates and colleagues presented 10-month-old children with a battery of tests similar to those Povinelli (2000) presented to chimpanzees. In each case, a fuzzy toy could be attained only with the aid of a tool. The conditions varied in the amount of contact between the tool and the toy, from a toy resting on a cloth, to a toy positioned next to a stick. Children as young as 10 months old successfully retrieved the toy when it was making contact with the tool, but not in instances where the toy did not make direct contact with the tool or in cases where the contact was implied (Bates *et al.*, 1980).

In another group of studies, Brown (1990) trained 1.5-, 2-, and 3-year-old human subjects to use a tool to retrieve a reward. Once they had mastered the task using a training tool, she presented these same subjects with a choice between two tools differing in their functional properties. One of these tools retained the correct functional characteristics; for example, the tool was sufficiently long, rigid, or it had an effective pulling end. The second tool was perceptually more similar to the training tool; that is, it was the same color or shape, but it was functionally ineffective, being too short, made of a flimsy material, or did not have an effective end. Brown reported that children as young as 24 months virtually ignored surface features such as color or the shape of the effective end of the tool. Instead, young children's choices, unlike the choices of chimpanzees, were guided by abstract physical properties such as rigidity, length, and an effective end; that is, the tool properties that were related to the causal structure of the task.

In spite of the fact that chimpanzees attend to statistical regularities associated with objects and events – using these regularities to execute behaviors that are coherent and rule-governed – they fail to reason about these same regularities in terms of invisible causal forces. Indeed, we have speculated that, for every unseen causal concept that humans may form, chimpanzees will rely exclusively on an analogous concept, constructed from the perceptual invariants that are readily detectable by the sensory systems (see Table 1). Of course, like chimpanzees, humans rely on these same spatiotemporal regularities most of the time, perhaps relying on systems that are homologues of those found in chimpanzees and other primates. But, unlike apes, we believe that humans evolved the unique capacity to form additional, far more abstract concepts that reinterpret observable phenomenon in unobservable terms (such as force, belief, etc.). If this interpretation of the data is correct, future research should address the following:

1. Can animals ever be taught to explicitly reason about unobservable physical forces such as gravity or connectedness?
2. For any given unobservable learned through explicit training, is it stable and generalizable across tasks and domains or restricted to a limited set of problems?
3. Do human and nonhuman primates form different percepts when confronted with identical sensory stimuli? If so, how might these differences affect nonhuman primates' conceptualization of physical unobservables?

4.34.6 Conclusions

The evidence reviewed above demonstrates that various features of the human and nonhuman mind are remarkably conserved. As a result, human and nonhuman primates are remarkably similar in each of the cognitive domains reviewed (see Figures 1–7). However, this same evidence also suggests that the ability to wield abstract theoretical concepts is the basis for much of what is deemed higher-order cognition in humans. We speculate that primate minds come in two forms: minds that are capable of generating predictions about regularities (physical and/or behavioral) alone and minds that are capable of generating predictions about regularities in addition to generating predictions about abstract (theoretical) concepts. For instance, the ability to interpret a given behavior, such as reaching for an object, as intentional depends on the ability to infer from observable behavior an unobservable intervening variable, and to use this intervening variable to describe the behavior in psychological terms. But note that describing a behavior as reaching (for an object) need not be additionally redescribed as wanting (an object). In fact, the same observable behavior – reaching – may lead to predictions

understood in behavioral terms alone (reaching=consumption or possession) or in terms of mental states (reaching=wanting or needing). Note that both types of minds describe the behavior and may respond to an individual reaching for a desirable object such as food in the same way.

Importantly, the system that describes observable phenomena in terms of mental states or physical forces did not replace the older system that only analyzed observable features. Instead, this newer integrated system co-evolved with the existing psychological systems of primates. Because the ability to reason about unobservable concepts such as minds co-evolved with a phylogenetically older behavioral system, we found ourselves in the position of being able to represent ancient behavioral patterns in explicitly psychological terms, and of using these new representations to modulate an existing behavioral repertoire in order to cope with the newly uncovered mental world in addition to the directly observable aspects of the social and physical world with which our ancestors had been coping for millions of years. If this view of human cognitive specializations is correct, the most crucial differences between humans and apes are defined by cognitive, not behavioral, innovations. This view contrasts with a number of hypotheses about the evolution of primate intelligence. First, unlike the social intelligence hypothesis, our theory does not assume that the ability to predict behaviors based on unobservable psychological states produced an entirely new class of behaviors. To the contrary, we believe that the nonlinguistic behaviors of organisms with minds that can generate unobservable concepts and use these concepts to redescribe certain behaviors do not qualitatively differ from the behaviors of organisms with minds that can generate only observable concepts. Second, the ecological (e.g., Parker and Gibson, 1977, 1979) and technical intelligence hypothesis (Byrne, 1997; Parker and Gibson, 1977, 1979), which argues that challenges in the physical environment favor unique behavioral and cognitive traits, has the same limitations. As in the social domain, selection likely favored the ability to successfully and accurately interpret the observable statistical regularities that characterize objects in the environment (e.g., flowering plants or tools). We agree with the assessment of Byrne (1997, p. 293) that, "Rapid learning and efficient memory, having evolved because of social [and physical] profits, evidently also allow benefits in quite different, nonsocial tasks." But we do not agree that apes' unique technical abilities requires the evolution of an additional system that reinterpret spatiotemporal regularities in terms of unobservable forces. The sophisticated behaviors that characterize apes in general requires, "efficient learning and large memory capacity...and possession of theory of mind [or a system for representing unseen forces] is not necessary for the case" (Byrne, 1997, p. 292).

The ability to reinterpret observable phenomena in terms of unobservable concepts may depend on a specific type of inference which the philosopher Charles Sanders Pierce called retroductive inferences. For Pierce, "Retroduction comes first and is the least certain and...the most important kind of reasoning...because it is the only kind of reasoning that opens up new ground" (as cited by Kehler, 1911). Pierce viewed retroduction as fundamental to the scientific enterprise because it depended upon the development of hypotheses about observable phenomena. Elsewhere (e.g., Povinelli and Dunphy-Lelii, 2001; Povinelli and Vonk, 2003; Povinelli, 2004), it has been argued that there is a difference between a mind that predicts events and one that seeks to explain them. But, of course, there is nothing trivial about predictions. Note that predictions come in two varieties: forward (e.g., classic conditioning), and backward (e.g., descriptive). If the reinterpretation hypothesis is correct, we can imagine, on the one hand, a mind that responds in a predictive manner to events and cues, and, on the other, a mind that generates rules that makes predictions (from hypotheses) across domains. In other words, a mind that engages in retroductive reasoning.

Thus far we have focused on the aspects of the conceptual systems of humans that may be unique in the primate order. But the human conceptual system may be distinct because fundamental features of the human peripheral nervous system are unique. As noted in the introduction of this article, it has been assumed since time immemorial that the differences between humans and other primates is not only skin deep; as a result, physiologists and psychologists have assumed that basic features of the nervous system (e.g., receptors and effectors) of primates do not meaningfully differ. Yet, differences in the sensory systems of primates will result in the generation of different percepts. If two organisms form different percepts from the same sensory experience, they will develop different concepts of the same event. Imagine the different visual percepts formed by the eyes of prosimians (who are largely nocturnal) versus the eyes of catarrhines (who are diurnal). If these differences at this basic level are real, we can be certain that the percepts that develop from these differences are similarly real.

In short, we should expect that humans and other primates differ in ways large and small. These

differences may be instantiated at the conceptual level as well as in more basic levels. We should not be surprised if differences at more basic levels of information processing (i.e., sensory system) have an effect on cognition. In fact, it is entirely possible that quantitative differences in the sensory systems may result in qualitative differences in the conceptual systems of primates. Only through a systematic exploration of these various problems will we ultimately come to understand human and nonhuman cognitive specializations.

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4.35 The Evolution of Language Systems in the Human Brain

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Glossary

acheulean The stone tool technology associated with the hominid species *Homo erectus*.

akinetic mutism Immobility and nonresponsiveness due to dorsal frontal midline cortical damage involving the anterior cingulated cortex, that includes verbal nonresponsiveness.

allometry The nonisometric scaling of anatomical structures with growth and comparative size.

arcuate fasciculus The fiber tract extending from the temporal and inferior parietal lobes to the inferior frontal lobes on the human brain passing beneath the supramarginal gyrus somatic and motor areas just dorsal to the Sylvian fissure.

Baldwin effect A theoretical evolutionary mechanism proposed independently by James Mark Baldwin, Conwy Lloyd Morgan, and Henry Osborne in 1896 arguing that physiological and behavioral plasticity could shield a lineage from elimination by natural selection long enough for new variations to accumulate (e.g., by chance mutation) that could supplement or replace the plastically acquired adaptatation.

Broca's aphasia (area) The language disorder first described by Paul Broca in 1861 and elaborated in 1865 that was produced by damage involving the posterior part of the inferior third frontal convolution on the left side of the brain (commonly referred to as Broca's speech area). This aphasia syndrome is typically associated with nonfluent, telegraphic speech and often agrammatism (a difficulty constructing or assessing syntactic structures).

co-evolution An evolutionary dynamic that is a consequence of two interacting selection processes, typically occurring between species whose survival is linked or between organizational levels of the biological hierarchy. In this context, it is

	used to describe the complex interactions that have likely characterized the evolution of the human brain and the evolution-like processes of language transmission and change which may have imposed novel selection pressures on human brain evolution as an artificial niche (see 'niche construction').
diffusion tensor weighted MRI	A structural MRI technique that uses oriented diffusion processes (constrained by fiber tract orientation) to visualize three-dimensional organization of major axonal pathways.
FOXP2	A highly conserved transcription factor gene of the fork-head family of genes that is associated with an inherited disorder of speech articulation and syntactic regularization.
generativity (generative grammar)	The capacity for indefinite novelty of combinatorial uses of words provided by the grammatical and syntactic apparatus of a language. Generative grammars are theoretical rule-governed grammars that by recursive application of these rules enable generativity of sentential forms.
genetic drift	The random mixing, accumulation of genetic variants, and elimination of alleles due to the relaxation of the effects of natural selection or the greater effect of probabilistic factors in small breeding populations.
Homo erectus	A long-persisting fossil precursor species to modern humans (from approximately 1.6 Mya to 350 kya, depending on which specimens are included) with roughly modern stature and postcranial skeletal structure, and a brain size average in the range of 950 cm^3 (at the very low end of the modern range). *H. erectus* is found in Africa and also throughout Eurasia, extending into Europe, central Asia, China, and Indonesia.
Homo habilis	One of the two earliest identified fossil members of our genus *Homo* dating to approximately 1.8 Mya in northeast Africa and associated with early stone tools. This species is characterized by reduced dentition and a slightly larger brain case in comparison to earlier australopithecine hominids.
Homo rudolfensis	One of the two earliest identified fossil members of our genus *Homo* dating to approximately 1.8 Mya in northeast Africa and associated with early stone tools. This species is characterized by a significantly larger brain case but minimally reduced dendition in comparison to earlier australopithecine hominids.
hopeful monster	A hypothetical significantly deviant member of a species produced by a mutation that radically alters some phenotypic character which just happens to be better suited to the current environment and thereby manages to outreproduce and eventually replace the more typical phenotypes of the population. This theory was championed by Goldschmidt in the early 1900s and is often tacitly assumed by proponents of a saltational origin of language abilities.
index (and indexicality)	The mode of reference that works by virtue of correlational relationships, as in pointings, symptoms, samples, and simple learned associations.
Lamarckian inheritance	The mode of trait inheritance proposed by Jean Baptiste de Lamarck in the early 1800s in which physical and behavioral traits acquired by effort, exercise, or exposure to demanding conditions were presumed to be passed directly to offspring during reproduction.
Mousterian	The stone tool industry associated with Neanderthals and early modern humans prior to the upper Paleolithic period beginning somewhere between about 60 and 75 kya.
niche construction	The effect that a species has in altering its immediate environment so that the influences of natural selection are significantly affected by this modification, as in the way that beaver dam construction has played a significant role in providing selection favoring the evolution of aquatic adaptations.
symbol (symbolic)	The mode of reference that picks out objects of reference by virtue of a system of sign–sign relationships (correlational relationships, as in pointings, symptoms,

	samples, and simple learned associations).
universal grammar (UG)	The hypothetical common core of grammatical rules that all human languages share. The theory was championed by the linguist Noam Chomsky and is argued by many linguists to be the innate endowment of all humans from which the specific grammars of existing languages are derived.
upper Paleolithic	An archeologically delineated period of human prehistory beginning roughly between 60 and 75 kya (most notably in Europe, but with more ancient precursors appearing in Africa) in which stone tool technologies begin to exhibit significant regional varieties and the first unambiguous representational forms (i.e., carvings and cave paintings) appear.
Wernicke's aphasia (area)	The language disorder first described by Wernicke (1874) that is produced by damage involving the posterior part of the superior temporal lobe on the left side of the brain (commonly referred to as Wernicke's speech area). This aphasia syndrome is typically associated with fluent speech that includes inappropriate, phonologically deviant, and/or semantically deviant word choice, and typically a deficit in comprehending sentence meaning and in naming objects.

4.35.1 Introduction: Human Neural Language Adaptations

4.35.1.1 Language Uniqueness and Nonhuman Communication

The comparative uniqueness of language is probably its most important and troubling feature. Besides being vastly more complex, language is substantially different in referential function, behavioral organization, and neural control than any other known animal communication system. Although a number of features are shared in common with the communication systems of some species, for example, vocal–auditory medium, social transmission, its most distinguishing characteristics – symbolic reference, grammar, open-ended generativity, and combinatorial patterning – are unprecedented. The lack of clear behavioral homologies in other species renders the comparative method problematic. There are no other species with various grades of language to provide clues about the contexts that support language evolution (though there are species that exhibit the ability to acquire aspects of language; see below), or the range of brain systems that can be involved. There is only one exemplar, *Homo sapiens*, and if there were intermediate levels of these abilities in our ancestry they have all been eliminated. This noncomparability is made all the more enigmatic when we consider the apparent absence of neurological dishomologies with respect to major neuroanatomical structures that might be expected to correlate with such a significant cognitive-behavioral discontinuity. Generations of comparative neuroanatomists have failed to identify even one major novel brain structure in humans. This suggests that our special adaptations for language are the result of using previously evolved primate brain structures in new ways and in new combinations.

4.35.1.2 Linguistic Context of Language Adaptation

Because of this unusual status of language, it has long been regarded as one of the defining features of human distinctiveness. Historical efforts to explain its origins have consequently been confounded with efforts to define the essence of humanness. This tendency is well exemplified by linguistic debates about the origin and basis for language. Under the influence of persuasive arguments by the linguist Chomsky (1972) and the psychologist Lenneberg (1967), it became popular to argue that language depended on elaborate innate capabilities unique to humans. Chomsky has been particularly influential in articulating what this might entail. At the center of this theory is the claim that all humans have inherited a common innate universal grammar (UG). This innate faculty is presumed to make the acquisition of language possible even at a stage in the life when other forms of learning are undeveloped, and makes effortless the unconscious deployment of a vast set of syntactic rules. These rules are thought to underlie the real-time capacity to interpret or generate a nearly infinite number of grammatical sentences (generativity). Despite the fact that Chomsky, and other colleagues, locate this language capacity in the brain, he maintains the view that it cannot be explained as an adaptation, whereas other linguists (e.g., Jackendoff, 1994; Pinker, 1994) argue that it is an evolved adaptation (see Section 4.35.5). The formal tools developed by generative linguists over the past four decades have

provided unparalleled rigor for the analysis of morphology and sentence structure; however, despite their theoretical commitment to an inherited biological substrate for linguistic capacities, these methods have yielded relatively little in the way of verified neurological predictions, and it is also not clear that they could be substantiated or falsified by brain research. More than a generation has elapsed since this view achieved ascendancy; neither a discrete neural locus for grammatical processes or a neurological lesion that selectively disrupts core features of UG nor a genetic defect that produces systematically divergent forms of grammar have been identified (though neural and genetic impairments of certain features of morphological or syntactic processing have been identified; see below). One major reason for this may be the difficulty of translating highly abstract linguistic formalisms into concrete anatomical predictions. At least superficially, language appears to be generated according to symbolic principles that are very different from the phylogenetic and epigenetic principles that determine functional organization within brains. Nevertheless, linguistic lists of the necessary and sufficient capacities for language remain highly influential, and linguists have been the staunchest proponents of a radical discontinuity between humans and other animals with respect to language.

4.35.1.3 Animal Exceptions and the Significance of Animal Language Experiments

Efforts to identify analogues to human language features in nonhuman species' naturalistic communication have demonstrated only limited behavioral and functional overlap. The most influential examples include the vocal learning of parrots and songbirds, the socially transmitted songs of humpback whales, and the referential alarm calls of numerous species, but most notably vervet monkeys (see Section 4.35.3). Vocal learning is deemed significant because the vast majority of terrestrial mammals to not exhibit any significant capability to learn or mimic noninnate species-typical vocalizations. The examples of complex socially transmitted vocalizations in many bird lineages and in humpback whale pods thus exhibit a deviation from the norm that parallels a key characteristic of language. The referential function of alarm calls to pick out distinctive classes of predators has been demonstrated in primates, birds, and even rodents. Classic theories of animal calls had caricatured them as merely extrinsic symptoms of emotional states, so demonstrations of specific extrinsic reference linked to specific innate calls also suggested parallels with the ubiquitous referential function of words and sentences. However, these superficial similarities are to be contrasted with many unprecedented language features.

Despite a failure to demonstrate any naturally occurring language-like systems outside of humans, partially successful efforts to train nonhuman species to perform certain limited language tasks have helped focus attention on the specific cognitive differences that separate them from humans. Studies of ape, dolphin, and parrot abilities to acquire language-like systems tailored for their different propensities and sensory-motor capacities have variously demonstrated the simple use of symbolic reference and a very basic understanding of syntactic operations, even if not anywhere near the level of interpretive and generative competence observed in a 3-year-old human child. Significantly, these three animal groups represent considerably different brain structures, since dolphin and especially bird brains (see below; and see Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?, The Evolution of Vocal Learning Systems in Birds, The Evolution of Vocal Learning Systems in Birds and Humans, Forebrain Size and Social Intelligence in Birds, The Hippocampal Formation in Food-Storing Birds, Cetacean Brain Evolution) are organized quite differently than are human brains. One possible implication is that at least rudimentary language-like capacities are not dependent on primate (or even mammalian) brain architecture, and so may be achievable in diverse ways.

4.35.1.4 Gestural Language: Neural Correlates and Evolutionary Scenarios

A counterpart to this diversity of potential neural substrates for language-like behaviors is demonstrated by the modality independence of language in humans. The manual languages that have developed in numerous deaf communities throughout the world show that fully complex languages can be acquired independent of the aural–vocal modality. However, despite this significant modality difference, there is also considerable overlap in neural representation with spoken language (Neville *et al.*, 1997). This may indicate that the critical neural adaptations supporting language are not, as is often suggested, merely specializations in motor or auditory processing, though these are also likely. Many scenarios for language origins have suggested that manual or gestural language preceded spoken

language in evolution (see, e.g., Hewes, 1973; Corballis, 2002). Neurological support for this view comes from the absence of voluntary articulate control of laryngeal musculature in most terrestrial mammals that have been studied, including all great apes (anatomy discussed below). Humans are the sole exception. It is therefore likely that the common ancestor of humans and chimpanzees lacked this control and that articulate vocal control is a derived trait that arose at some point in hominid evolution, possibly after the emergence of the genus *Homo*. Although most face-to-face speech is accompanied by gesture for emphasis and indexicality, language develops almost exclusively in the vocal channel, not manually, if speech and hearing are possible. Also, the locations of the major cortical systems critical for language processing are similar in both speakers and deaf signers. So support for a separate prior specialization of the brain for gestural language is weak. More likely, spoken and gestured symbolic communication were employed in linked fashion for a significant part of the evolution of language, with vocal capacities lagging behind but eventually becoming the more prominent modality.

4.35.2 Human Neuronatomical Features Associated with Language

4.35.2.1 Gross Neuroanatomical Homologies

Generations of comparative neuroanatomists have explored the possibility that human brains contain species-unique large-scale structures (e.g., distinct nuclei, cortical areas, fiber tracts) that might correlate with our species-unique form of communication. However, since the famous nineteenth century debate between Thomas Huxley and Richard Owen in which Huxley disproved the existence of a uniquely human hippocampal structure, there has been widespread confirmation of the extensive homologies linking human and great ape brains and no verified claims of any phyletically unprecedented macroscopic structure in the human brain. Nevertheless, claims of the evolutional functional divergence of brain structures are widespread in the literature. Two cortical regions have been consistently implicated in these claims: Broca's region in the inferior frontal lobes and the angular gyrus region at the temporal-parietal–occipital junction. The uniqueness of speech has led to hypotheses that Broca's region is uniquely developed in human brains, and the supramodal nature of semantic associations has led to hypotheses that a cross-modal association area is uniquely developed in the region of the angular gyrus. The hypothesis that Broca's region was uniquely developed in the hominid lineage led to investigations of fossil skull endocasts (see critical discussion below) and reports that the distinguishing sulci of this region could first be detected on an endocast of a *Homo rudolfensis* specimen, KMNER 1470 (then identified as *Homo habilis*) (Falk, 1983; Tobias, 1987). Phyletic novelty of this structure has since been cast in doubt by the evidence of both cytoarchitectonic and connectional homologies of this region with corresponding regions in ape and even monkey brains (Deacon, 1992b; see Section 4.35.2.3). The hypothesis that the human angular gyrus region is an unprecedented cross-modal association area critical for language was first articulated by the neurologist Geschwind (1964). Early claims of poor cross-modal transfer of information in monkeys were subsequently disproven (Wegener, 1965; Blakeslee and Gunter, 1966), and subsequent studies have since identified polymodal function in homologous cortical regions as well as other inferior parietal and middle temporal areas (e.g., Ettlinger and Wilson, 1990). In response to this failure to find unprecedented brain structures relevant to human language facility, most attention has turned to quantitative, connectional, and peripheral dishomologies that may be relevant.

4.35.2.2 Allometric Deviations Potentially Associated with Language Adaptation

That the human brain has been subject to quantitative deviation from ape brain proportions is indisputable. Human brains are both absolutely and comparatively larger than expected for an anthropoid primate or even a great ape. For this reason, much attention has been focused on the plausible link between this deviation in brain proportions and the deviant features of human language. Both brain/body proportions and the internal scaling of brain structures with respect to each other and the gross brain size are highly correlated (Sacher, 1970; Gould, 1975; Finlay and Darlington, 1995), but most brain structures do not scale up or down isometrically with respect to total brain size across species. Allometric scaling patterns are also exhibited at every level of brain structure. For example, larger brains tend to have higher proportions of telencephalon to diencephalon, more neocortex to limbic cortex, more eulaminate cortex to specialized agranular and sensory koniocortex, more white matter to gray matter, more glia per neurons, and so on.

Generally, it is argued (on theoretical, not empirical grounds) that structural proportions that are predictable from allometric scaling (e.g., with respect

to the trend exhibited by large interspecific sample as a background) indicate nondeviant function as well. Consequently, interest has mostly focused on quantitative findings of allometric deviation of human brain structures with respect to apes or to anthropoid primates in general (e.g., Stephan, 1969; Stephan *et al.*, 1981). These investigations are complicated by disparities of results obtained using different statistical approaches, different methods of structural measurement, and disagreements about the significance of deviations that these analyses suggest. At present, there is no agreed upon theoretical basis (and limited empirical data) for predicting the functional correlates of either the allometric or deviant changes in relative proportions of brain regions. Comparative studies showing quantitative structural correlations with peripheral specialization offer the most useful comparisons. Examples of regional enlargements with respect to manipulative forelimbs (e.g., large forelimb tactile representation in primates and raccoons), elaborated or degenerate sensory organs (e.g., specialized tactile representation in the star-nose mole or elimination of visual cortical responses in the blind mole rat, respectively), or highly modified and hypertrophied organs (e.g., cerebellum in electric fish) offer support to the phrenological null hypothesis that increase in relative size equals functional augmentation as well. Perhaps those most relevant to the cognitive-behavioral specialization of language are the size correlations between song complexity in songbirds and the relative sizes of forebrain nuclei involved in singing (e.g., DeVoogd *et al.*, 1993). It is likely, however, that there are other possible correlates of allometric deviation that have yet to be explored (other alternative possibilities are explored in Deacon, 1990b).

Despite this uncertainty about the significance of allometric scaling and deviations in brain structure proportion, there has long been an interest in searching for possible correlations between allometric deviations of human brain structure and language. Different studies have, for example, provided analyses that suggest that human brains have divergent enlargement of cerebral and cerebellar cortices, prefrontal cortex, and certain thalamic nuclei, and divergent reductions of primary visual cortex, primary motor cortex, and olfactory bulbs. Similarly, quantitative studies have found hemispheric asymmetries in language-related areas of cortex, though such asymmetries have also been reported for nonhuman apes. Many of these findings must be considered preliminary, however, since most have been contradicted by studies using different methods that have come to different conclusions. There is also a considerable variation in the size of cytoarchitectonically identified language areas to contend with (e.g., Amunts *et al.*, 1999). One illustrative example of a quantitative dispute with implications for language concerns the allometric predicatability or deviation of the human prefrontal cortex. Studies based on histological analyses of the cytoarchitectonic distinction between granular prefrontal and agranular premotor cortex have reported that human prefrontal cortex is allometrically larger than predicted with respect to other anthropoids (e.g., Deacon, 1997). In contrast, MRI-based studies using major sulci and fissures as morphological markers to discern frontal from parietal and temporal cortex suggest no deviation (Semendeferi *et al.*, 1997). Claims of disconfirmation of one or the other result are, however, clouded by the use of these different anatomical methods, different definitions of frontal and prefrontal cortex, comparison of nonhomologous structures, including different primate species as a comparison set, and employing different statistical tests for deviance (Deacon, 1997, 2004).

Despite these unresolved methodological issues, most studies have concluded that human brains deviate from allometric predictions in a number of internal relationships that might be relevant for language. Probably the most consistently reported finding is that human cerebral cortex is larger than allometrically expected with respect to the two major forebrain nuclear complexes that are most intimately related to it: the thalamus and the basal ganglia (Deacon, 1988, 1990a; Dunbar, 1993; Rilling and Insel, 1999). Additionally, there is evidence for allometric deviation of kiniocortex and agranular cortex to eulaminate cortex (Deacon, 1990a), visual cortex (e.g., Holloway, 1979), temporal lobe morphology (Rilling and Seligman, 2002), and prefrontal cortex (Deacon, 1988, 1997; Rilling and Insel, 1999). It is hard to believe that significant deviations in these major forebrain relationships would not have an impact on language. For example, Deacon (1997) argues that this disproportion may have aided invasion of cortical efferents into brainstem vocalization nuclei (see below), as well as biasing developmental competition among cortical afferents affecting parcelation of functional cortical areas. Finally, difficulties of discerning comparable boundaries of cortical areas across species of widely differing sizes have made more fine-grained allometric studies of the scaling of individual cortical areas even more problematic.

Efforts to link allometric deviations of some of these structures with language adaptation have mostly focused on two findings, the expansion of

prefrontal cortex (e.g., Aboitiz and Garcia, 1997; Deacon, 1997) and quantitative deviations and asymmetries of Broca's and Wernicke's language areas compared to their homologues in chimpazees (e.g., Gannon *et al*., 1998). For example, prefrontal expansion may provide working memory support for symbol learning, visual cortex reduction may reflect parietal cortex expansion and an augmentation of cross-modal cognition, and asymmetries of language cortex may provide the substrate for hemispheric specialization for language.

4.35.2.3 Connectional Homologies and Dishomologies Relevant to Language

Neither the lack of novel human brain structures nor the uncertainties about the existence or relevance of allometric deviations precludes the possibility of evolutionary changes in neural circuitry. Unfortunately, methods used to accurately trace axonal connections between brain structures require lethal experiments and so are only available for study of nonhuman species, and indeed are not even applicable to apes because of their endangered status and the ethical issues involved. Thus, information concerning human neural connections is mostly lacking and must be extrapolated from nonhuman data. However, indirect evidence from human functional differences, clinical studies, and *in vivo* imaging can be compared to connectional data derived from nonhuman primates to support a handful of fairly robust connectional claims.

Probably the most robust behavioral distinction between the vocal abilities of humans and other primates (and in general with respect to all terrestrial mammals) is the ability for humans to produce a wide range of vocal sounds that can be freely organized into diverse combinations. In addition, we also have an unprecedented ability (for a terrestrial mammal) to mimic vocal sound combinations that we hear others produce. In contrast, the vast majority of terrestrial mammals, including primates, has relatively fixed vocal repertoires, for which sound mimicry learning plays almost no role. Associated with our lack of constraint on productive sound combination, we experience a relative freedom from specific correlations between vocalizations, emotional states, and stereotypic referential contexts, unlike what is characteristic of the other primates (Deacon, 1997). This is a critical requirement for language, as it allows socially transmitted patterns of sound production (e.g., words) to be learned in association with any given reference. Though humans still exhibit a small repertoire of innate stereotypic species-specific vocal 'calls' such as laughter and sobbing, which do have fixed structure and are associated with highly constrained emotional contexts, this call repertoire is both small compared to that in chimpanzees and atypical in form and context (Deacon, 1997; Provine, 2000).

These differences are probably in part attributable to a change in the central innervation of the laryngeal control nucleus of the brainstem, the nucleus ambiguus. Tracer studies in nonhuman primates have demonstrated that this nucleus is almost entirely innervated by subcortical structures from midbrain and adjacent brainstem regions (Jürgens *et al*., 1982). This is an expected pattern given that the nucleus ambiguus is a visceral motor nucleus that is segregated from significant influence from volitional systems in order to provide reliable automatic responses for a system that is associated with life-and-death consequences. Though electrical stimulation of ventral motor cortex regions in the macaque monkey brain can result in vocal muscle movement, there is little evidence that this is mediated by a direct projection. In addition, bilateral ventral frontal motor cortex damage in monkeys does not appear to block their ability to vocalize. In contrast, unilateral damage to left inferior motor cortex in humans (even just in the left hemisphere) can produce significantly impaired vocal ability, and even mutism. This clinical evidence is supported by experimental studies that also suggest that the human nucleus ambiguus is directly innervated from motor cortex (Jürgens *et al*., 1982). Taken together, this makes it likely that this connection constitutes a uniquely human feature, by virtue of which precise control of pitch and vocal timing is achieved in speech and song. This would also explain the remarkable coordination of vocalization with the other cortically controlled tongue, jaw, and facial muscles that are necessary for articulate speech. In this regard, humans have dual control of vocalization, as is exhibited in the tendency for speech to be interrupted with impulses to laugh or sob in response to intense emotional states (Provine 2000; see Figure 1).

The left inferior frontal cortical region that likely includes Broca's speech area has been subject to conflicting claims concerning (1) which components of this region are responsible for the deficits associated with Broca's aphasia, (2) how they are functionally connected with other cortical and subcortical regions also associated with language processing, (3) whether the cortical area itself or its connections with other areas are more important for its language role, and (4) whether or not its

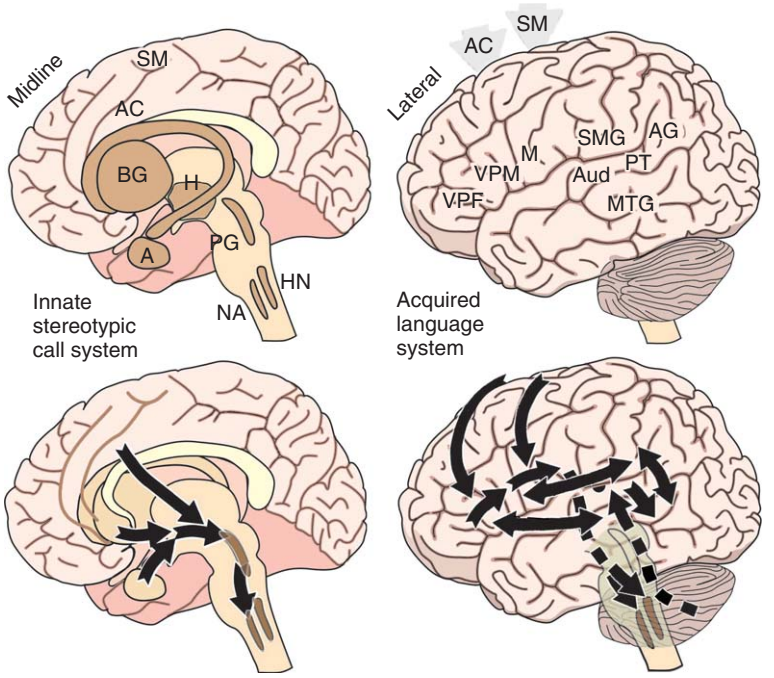

Figure 1 Highly simplified schematic comparison of brain structures (above) and major connections (below) comprising the mammalian innate call production system and the human spoken language system. Many connections and relevant structures are not shown, including notably the involvement of a cortical–basal ganglia–thalamic–cortical loop and the cerebellum in language production. The forebrain structures of the innate call system are almost exclusively associated with arousal control, whereas those supporting language are almost exclusively sensorimotor and 'association' cortical structures. So these two vocal communication systems of the brain are largely nonoverlapping in terms of structures and connections, with the exception of final brainstem output systems controlling oral, vocal, and respiratory muscles, and the anterior cingulate cortex. Projections from motor cortex extend directly to brainstem vocal motor nuclei, whereas forebrain output controlling innate calls is mediated by the periaqueductal gray area of the midbrain. In humans, both systems operate in parallel, and may compete for the control of vocal output. The differential involvement of numerous interconnected forebrain systems in language as compared to the few involved in innate vocalizations is superficially similar to the neural differences between birds that learn complex variable songs and those with innate stereotypic songs (see Figure 2). A, amygdala; AC, anterior cingulate cortex; AG, angular gyrus; Aud, auditory area; BG, basal ganglia; H, hypothalamus; HN, hypoglossal nucleus; M, motor cortex; MTG, middle temporal gyrus; NA, nucleus ambiguus; PG, periaqueductal gray; PT, planum temporale; SM, supplementary motor area; SMG, supramarginal gyrus; VPM, ventral premotor; VPF, ventral prefrontal.

connectivity with other structures is typical of other primate brains. The clinical literature is even still split on what Brodmann's areas (Brodmann, 1909) are the substrates for Broca's area language functions (Dronkers *et al*., 1992), whether multiple frontal cortical areas subsume component language functions (Paulesu *et al*., 1997; Deacon, 2004), and whether these cortical areas are the primary locus, rather than the underlying white matter and striatal structures (D'Esposito and Alexander, 1995; Lieberman, 2002). Nevertheless, claims that this area is in some way uniquely organized in humans are reinforced by the common incidence of agrammatism in Broca's aphasics and by the belief that it is grammar that sets humans apart from the other species.

One of the long-standing assumptions about Broca's area is that it is a convergence zone where auditory input contributes to the formulation of speech. Could this connection pattern be a unique feature of human brains supporting speech? Combining connection data from primates with new *in vivo* functional image data on language processing, it is possible to settle some of these long-standing questions. Tracer studies of connections of the macaque inferior frontal cortex demonstrate linkages with other cortical and subcortical sites that include both parietal and temporal cortical areas. But these tracer results delineate at least three quite different connection patterns associated with different subregions of the monkey ventral frontal cortex (Deacon, 1992b). Motor and premotor cortical areas are primarily interconnected with inferior parietal and superior insular regions of cortex, and premotor cortex is connected with dorsal midline supplementary motor cortex. In comparison, the rostrally adjacent ventral prefrontal area is primarily interconnected with superior temporal and middle temporal gyrus areas, as well as with

dorsal prefrontal areas and anterior cingulate cortex, but lacks connections with motor areas. So primate connection data do not support a simple convergence of auditory and tactile motor functions in their anatomical homologue to Broca's region, and instead exhibit a tier-like organization with caudal–rostral segregation of parietal from temporal input zones. Physiological confirmation of an auditory projection zone in this macaque ventral prefrontal region has been provided by single cell recording (Romanski *et al*., 1999). But is this segregation of auditory and motor functions in the primate homologue to Broca's area evidence that monkeys and humans differ in this respect? The corresponding connection patterns in the human brain have recently been traced using diffusion tensor weighted MRI techniques, which enable the visualization of fiber tracks (Catani *et al*., 2005). This study mapped the course of the components of the fiber bundle known as the arcuate fasciculus, which in humans carries fibers presumed to interconnect Wernicke's area with Broca's area. The findings are consistent with the monkey brain connection pattern, not with a simple convergence zone logic. Inferior parietal projections terminate in ventral motor and premotor areas and superior and middle temporal gyrus projections terminate more rostrally in ventral prefrontal areas. Inferior parietal areas and superior temporal areas are also interconnected, but not superior temporal areas and ventral motor or premotor areas. As in the macaque brain, auditory information is relayed to the frontal areas by way of a prefrontal cortical area in front of and separate from the premotor–motor areas involved in speech production.

This evidence for fractionation of the contributions to language processing in ventral frontal cortex is also consistent with the accumulation of *in vivo* imaging data that show slightly different localizations in this region for heightened activity during language tasks that differentially involve auditory and motor processing. For example, word association and linguistically mediated mnemonic tasks preferentially activate the ventral prefrontal component, while tasks involving motor analysis preferentially activate premotor and motor areas located more caudally. The implications are first that Broca's area is not a single functional unit, but comprises two or more adjacent regions, second that only the prefrontal component utilizes temporal auditory input, and third that the language specialization of this region did not depend on any major restructuring of connectivity. In addition, if as in macaques this same auditory recipient ventral prefrontal area is linked to the anterior cingulate cortex, it would also represent a bridge between a language-specialized area and the one cortical area known to be involved in primate call production.

4.35.3 Comparative Functional Analyses

4.35.3.1 Functional Dissociation of Call and Speech Motor Control

The discovery of predator-specific alarm calls in vervet monkeys (Seyfarth *et al*., 1980) suggested that the functional dichotomy between language and primate call systems might not be so great as once believed. The existence of distinct calls given to leopards, eagles, and snakes suggested that the origins of language might be envisioned as a gradual elaboration of a larger and larger specific repertoire eventually requiring more complex production and combination mechanisms. However, evolutionary continuity is difficult to support when the difference in neural substrates between calls and language is considered. Electrical stimulation and lesion experiments established that primate calls could be elicited by stimulation of midbrain and limbic forebrain structures, including basal forebrain, ventral striatum, amygdala, hypothalamic, and anterior cingulate cortex, but not by cerebral cortical areas (e.g., Jürgens, 1979). Correspondingly, damage to monkey's cerebral cortical areas homologous to those involved in language processing in humans do not interfere with call production. Conversely, damage to limbic and telencephalic structures homologous to structures supporting primate calls do not produce language deficits in humans. There are some exceptions that prove this rule. One is the anterior cingulate cortex, which if bilaterally damaged in humans, may result in akinetic mutism (immobility that includes vocalization). But this is arguably an impairment of the arousal to speak and move rather than a disturbance of language processing. There have also been reports that stimulation of the amygdala in human subjects can sometimes produce spontaneous curses as well as emotional cries. And in patients with global aphasia, cursing is sometimes spared or even facilitated. Expletives are, however, an interesting intermediate; an acquired vocalization that has become relatively automatic and stereotypically associated with specific intense emotional experience. Taken together, these data demonstrate that the neural substrates for language functions and

innate calls derive from almost completely dissociated brain systems. Along with evidence that speech depends on direct cortical projection to oral–vocal motor nuclei, independent of the older limbic-midbrain–brainstem pathway, and the fact that speech and human innate calls exist side by side in our vocal repertoires, we can confidently conclude that the language system is not an elaboration of the call production system. The only significant overlap of these two systems is a final common output pathway.

4.35.3.2 Songbird Comparisons

Despite the fact that telencephalic organization in birds and mammals is radically different, there are useful analogies that can be drawn from comparison to birdsong control and its differences in different species (Jarvis, 2004). Research into the organization of song acquisition and control in different bird species demonstrates a consistent pattern that distinguishes song learners able to produce complex songs from nonlearners with simple songs. Comparisons between songbirds, parrots, and hummingbirds also demonstrate that complex singing abilities have evolved independently at least three times in the course of bird evolution and that in each of these lineages motor control of song output is mediated by slightly different forebrain systems. Species with highly stereotypical innate songs utilize only one or two forebrain motor nuclei for song production. In both songbirds and birds with stereotypic songs, a primary motor output nucleus in the caudal telencephalon (RA in songbirds) projects to the common vocal output pathway in central midbrain, and from there to brainstem motor nuclei. In addition, species that learn significant aspects of their songs and produce complex variable songs may require the coordinated contributions from as many as a dozen forebrain structures. In this regard, the difference between birds that sing complex sounds and those that sing stereotypic songs is crudely analogous to the human/nonhuman primate difference. So understanding the differences between the alternative complex song control strategies in different bird lineages and the difference between complex singers and stereotypic singers may provide useful comparisons (see Figure 2).

Two major classes of forebrain systems are integrated with the forebrain motor output nuclei to enable song learning and song complexity: auditory and striatal motor systems. These are necessary for learning from auditory experience. In addition, a higher-order premotor nucleus (high vocal center (HVC) in songbirds) is also critical for song complexity and flexibility. The differences between different lineages where vocal learning evolved are also interesting as a comparison. Some of the most sophisticated learners, such as parrots, have a different motor output pathway from the forebrain than do oscine songbirds. Although it is not clear from current research whether these differences are more than variations on a theme, differences in the midbrain/brainstem output targets of these nuclei are suggestive. For example, in songbirds, a midbrain region (probably homologous to the mammalian periaqueductal gray area which mediates call production) mediates between forebrain and brainstem motor control nuclei, but in parrots and possibly hummingbirds there is also a direct projection from forebrain motor nuclei (not homologous to RA) to brainstem motor systems. It is tempting to speculate that this latter pattern is analogous to the direct forebrain (cortical) projections to the vocal motor centers that distinguishes humans. Whether these differences account for the remarkable vocal mimicry of parrots and their kin is unknown.

So, although a good deal is still to be learned about the evolution of vocal learning and vocal skill in birds, exploring this more experimentally accessible parallel to the human case may provide important clues about how vocal flexibility evolves. An intriguing example is discussed below.

4.35.3.3 Lateralization of Language Functions

One of the more enigmatic features of language representation in the brain is the fact that the two hemispheres play very different and unequal roles in controlling speech and comprehension. This is not because there are different cortical areas on the two sides. Ever since the French surgeon Paul Broca first catalogued cases of speech impairment associated with localized brain lesions (Broca, 1865), it has been known that the left-hemisphere damage is far more debilitating for language functions than the right-hemisphere damage. The brain regions in the inferior frontal gyrus and superior temporal gyrus that are the loci most likely damaged in Broca's and Wernicke's aphasia, respectively, are identified for the left hemisphere, but their right-hemisphere counterparts can often suffer damage with no obvious speech impairment. This left 'dominance' for language, as it is often described, is not universal, with just a few percent of people exhibiting complete right-sided language bias. The exceptions also correlate strongly with left handedness, suggesting a link between the asymmetrical biases. Beginning in the 1960s, a series of surgical

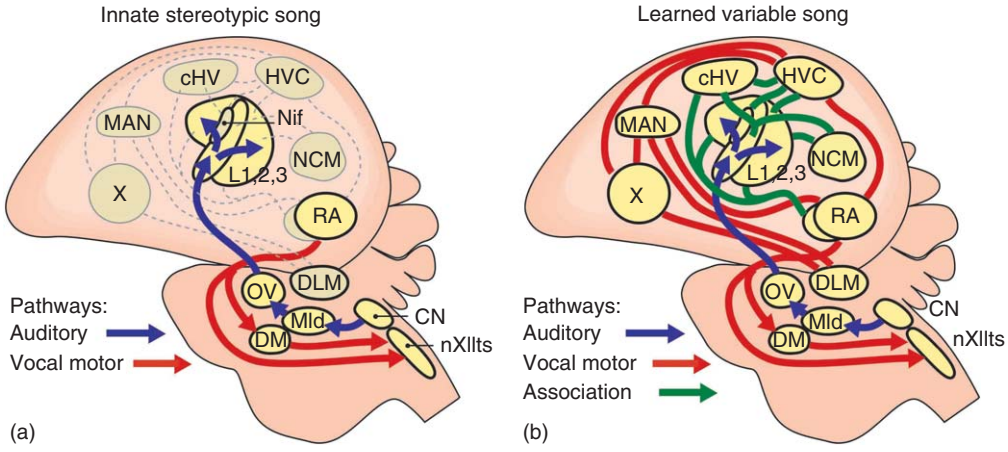

Figure 2 Comparison of major structures and connections in brains of (a) songbirds that do not learn their song and (b) those that do. Dashed connections and structures not connected by heavy lines are minimally involved in song (many structures and connections are not shown for simplicity). Innate stereotypic birdsong is relatively insensitive to damage to most forebrain structures (a) excepting a primary motor nucleus (RA) which projects to the central midbrain and to vocal motor nuclei, such as the hypoglossal nucleus (nXIIts). In contrast, striatal structures (e.g., areas X and MAN), auditory centers (e.g., L1, L2, and L3), and numerous pathways linking these with premotor (e.g., HVC) and motor (RA) nuclei are all involved in various aspects of learning a song 'dialect' from adult singers in early life. In these birds damage to any of these structures or their interconnections can disturb learning, complexity, or flexibility of song. Other bird orders exhibiting learned vocalizations, such as parrots and hummingbirds, are distinguished by differences in final motor output pathways (not shown). The distribution of song control to a diverse system of forebrain structures in song learners is loosely analogous to the shift in control from limbic structures to the diverse system of interconnected sensory, motor, and association cortical areas and striatal nuclei that evolved to control language (see Figure 1). In both systems the involvement of a diverse constellation of interconnected forebrain structures appears to be correlated with complex, flexible, context-sensitive, socially transmitted, learned vocal skills. For a more detailed account of the comparative neurology of bird vocalizations and human language see Jarvis (2004). cHV, caudal region of the hyperstriatum ventrale; CN, cochlear nucleus; DM, dorsal medial nucleus of the midbrain; DLM, medial nucleus of the dorsolateral thalamus; MAN, magnocellular nucleus of the nidopallium; HVC, high vocal center; L1–3, primary auditory fields; Mld, mesencephalic lateral dorsal nucleus; NCM, caudal medial nidopallium; Nif, interfacial nucleus of the nidopallium; nXIIts, hypoglossal nucleus (nucleus XIIth cranial nerve, tracheal syringeal division); OV, nucleus ovoidalis; RA, robust nucleus of the arcopallium; X, area X of the striatum.

interventions to limit the spread of epilepsy susceptibility from one hemisphere to the other cut the corpus callosum, and other forebrain commissures, severing the two hemispheres so that they could not exchange signals. The results were startling. If information was carefully provided to only one of the isolated hemispheres, patients could use language to describe the stimulus only if presented to the left hemisphere (input from the right side). This suggested that the right hemisphere was essentially mute.

There are a few clues to how and why this functional asymmetry evolved to be so robustly associated with language functions. The first clue comes from understanding what language-related functions, if any, are contributed by the contralateral counterparts to Broca's and Wernicke's areas in the right hemisphere. Two lines of evidence suggest that, contrary to earlier views, the right hemisphere does indeed contribute to language processes. Both kinds of evidence come from cases of right-hemisphere brain damage. First, there is a higher incidence of aprosodia with right-hemisphere damage (Ross, 1981). Aprosodia is an impairment of the ability to produce or accurately comprehend the changes in tonality and rhythmicity that is used to convey emotional tone, emphasis, or differential focus in speech. This suggests that the right hemisphere is involved in regulating the nonreferential social–emotional context of spoken conversation in parallel with the left-hemisphere production and comprehension of the syntactical and semantic content of speech. Second, right-hemisphere damage appears to impair the ability to keep track of the larger semantic and pragmatic frames of speech. Right-hemisphere-damaged patients have difficulty understanding what makes one joke funny and another lame, and also have difficulty following the theme of a story, often failing to recognize the insertion of incongruous elements (Gardner *et al.*, 1983). So although the right hemisphere appears to be minimally, if at all, involved in immediate semantic and syntactic processing of

words and sentences, it appears to be carrying out important supportive background tasks in parallel.

This parallelism may help to explain another curious feature of language lateralization: its development in childhood. Studies of very young children who have had their left hemispheres surgically removed show a remarkable sparing of language abilities, with minimal obvious impairment in adulthood (see review in Kolb, 1995; and recently Boatman *et al*., 1999). So, although left lateralization of the semantic, syntactic, and phonological processing of language is highly predictable and probably reflects an innate bias, it is only a bias and not a fixed and inflexible adaptation.

Why should there be laterally asymmetric distributions of language functions in an otherwise bilaterally symmetric brain? Although there is no certain answer to this question, considering the functional characteristics that are lateralized, the fact of their progressive differentiation during maturation, and other correlates of lateralization (e.g., handedness) some plausible hypotheses can be formulated. First, there are consistent structural asymmetries in human brains (discussed already). The relative sizes of cortical areas and morphological structures associated with Broca's and Wernicke's language areas indicate that the right side counterparts are on average smaller. This could be a source of developmental bias or also a consequence of developmental differentiation, but other left–right asymmetries in neonatal brains support the possibility that anatomical bias contributes. Second, the language functions that appear to segregate to opposite hemispheres seem to divide according to both rate of processing (rapid processing on the left) and extent of conscious online monitoring required (also left). This can probably be understood in terms of segregating functions that need to run in parallel but would likely interfere with one another because of their very different processing parameters (Deacon, 1997). Third, the correlation with asymmetric manual skill suggests a possible linkage, perhaps with tool use (Kimura, 1993). Finally, the unilateral representation (also to the left) of vocal skill learning is also characteristic of songbirds (Nottebohm and Nottebohm, 1976). Since vocalization involves muscle systems that are aligned along the midline of the body and are bilaterally controlled, it may be necessary to strongly bias control to one hemisphere in order to avoid functional conflict. In summary, lateralization may not be a requirement for the evolution of language, but it is likely a bias built in to aid functional segregation of processes that are best run in parallel systems and thus avoiding mutual interference.

4.35.3.4 The Mirror System

Although the class of cells called 'mirror neurons' are discussed elsewhere in this volume (see Premotor Cortex and the Mirror Neuron Hypothesis for the Evolution of the Language), including their possible roles in language processing, this class of neural responses has also been implicated in many language origins theories (Rizzolatti and Arbib, 1998) Recording from single neurons in a ventral premotor subregion (designated F5) of the macaque monkey brain, Rizzolati and colleagues identified a subset of neurons that preferentially spiked when the subject observed himself picking up an object and also when observing an experimenter picking up the same object in the same way. This responsiveness to the general form of the action, irrespective of the role of agency and perspective, suggested the name 'mirror neuron'. The relevance to the evolution of the language capacity is twofold: first it suggests the possibility that this kind of neural responsiveness could play a role in the ability to mimic others, and second the location of these cells in the macaque brain is in a region generally considered adjacent to the monkey brain region deemed homologous to the premotor division of Broca's area (see Section 4.35.2.1), and possibly overlapping. *In vivo* imaging data have further suggested that there is a similarly responsive premotor region in the human brain. Although it can be debated whether this response characteristic is specifically found in the same premotor region in human brains as the one that plays a critical role in language, these coincidences make it reasonable to entertain the hypothesis that this might help support the vocal mimicry necessary for word learning in language. If so, what might be the implications for language evolution? First, it must be noted that the presence of mirror neurons in macaque brains is sufficient to exclude these cells or their connections from being the difference in human brains that makes language possible; however, it could be argued that their presence predisposed this premotor zone for a later role in language processing. Second, speech mimicry demands that a parallel class of auditory–vocal mirror neurons be identified (visual–manual mirror neurons might be more important for mimicry in gestural language), though some of these neurons exhibit responses to the sound of

an object being manipulated as well. In the monkey brain, mirror neurons receive input from neurons in inferior parietal cortex that are also responsive to visuomanual stimuli, but if there are corresponding auditory–vocal mirror neurons we might rather expect them to receive input from superior or middle temporal sources. Until such a parallel class of neurons is identified it is probably premature to assume that mirror neurons are critical to language functions, but looking for them is thus a relevant enterprise. One plausible scenario – assuming that mirror neurons are indeed critical for mimicry – is that they played a role in an early, more gestural phase of language evolution, and possibly paved the way for the evolution of these hypothetical auditory counterparts.

4.35.4 Genetic Correlates of Language Adaptation

4.35.4.1 Hopeful Monsters and Megamutation Scenarios

Probably the most popularly accepted scenario for language evolution is what has sometimes derisively and sometimes seriously been referred to as the 'big bang' scenario. On the analogy to the birth of the universe, this scenario suggests that language was made possible as a result of one or just a few major mutation events that resulted in the significant reorganization of brain functions. This resonates well with assumptions about an innate UG (see Section 4.35.1.2) or a 'language acquisition device' constituting the difference between human and nonhuman brains. It also resonates with paleoarcheological theories for explaining the sudden burst of cultural artifacts (such as diverse tools types, representational cave paintings, and carvings) that arose within the last 50 000 years. Since *H. sapiens* has been around for greater than 100 000 years, and hominids with comparably large brains and complex stone tools have been around for roughly half a million years, this transition appears quite recently in human evolution. But the idea that what distinguishes speaking humans from other species and from our recent ancestors can be explained by a couple of very lucky genetic accidents seems both counterintuitive in terms of what is known about the genetics of the developing brain and what is known about the complexity of language control. But more generally, it also leaves almost the entire explanation of this adaptation to an incredibly lucky accident. This kind of evolutionary scenario is often described as a hopeful monster story, because it imagines that a mutation producing a major phenotypic distortion becomes so enormously successful that it replaces all alternatives. Though one cannot argue that it is impossible, it is a claim about evolution that is little better than invoking a miracle. Nevertheless, there is at least one serious proposal for just such a critical genetic change.

4.35.4.2 Genes Affecting Language Processing

In the mid-1980s, when excitement about the plausibility of innate UG was at its peak, a surprisingly specific inherited language disorder was described. Called specific language impairment (SLI) by researchers, it was expressed in a family (identified as the KE family) in which many members exhibited specific difficulty with regularized aspects of English syntax (Gopnik, 1990; Gopnik and Crago, 1991). Most notably, this was manifested in a problem learning to use the regular past tense ending '-ed'. Subsequent study of this deficit showed it to also be accompanied by significant oral motor apraxia (pronunciation and fluency problems) and neurological reduction of motor areas and basal ganglia (Vargha-Khadem *et al.*, 1998). Chromosomal damage to a common locus was subsequently correlated with expression of this trait, and in 2002 a transcription factor gene, FOXP2, was identified as the critical damaged gene in this disorder (Enard *et al.*, 2002), expressed in structures affected in the KE family (Lai *et al.*, 2003). It is the first single gene to be correlated with a known neurological disturbance of language function. It is not a 'new' gene unique to the human lineage, since it is present and plays a critical role in development of the brain in all mammals (a homologue is also found in birds and fruit flies), and it is a highly conserved gene in terms of sequence variations across species, and the KE family variant is damaged at a site conserved in all known species (and so likely critical).

Important with respect to its plausible role in the evolution of language are two point mutations in the human variant that distinguish it from the chimpanzee version (and basically from all other mammals in which it is highly conserved). Linkage information even suggests that these human deviations are relatively recent – possibly within the last 100 000 years – and are likely universal or nearly so in living humans. This does not prove, however, that these human-specific differences contribute to a crucial change in function (though the evidence is highly suggestive), and the alterations do not correspond to the damaged locus in the KE family. At the present

time, we cannot even say for certain that having the chimpanzee gene would result in diminished language function, or whether a chimp with a human version of the gene would have improved oral motor capacity. But damage to a regulatory gene that is critical for early brain development (as it is in all mammals) will almost certainly result in significant disruption of function, since it likely controls the expression of many other genes. So although the gene did not evolve for language, the neural features it controls during development have clearly been recruited by language, and it seems likely that the mutations that occurred in it in human evolution played some role in the evolution of speech.

Assuming that the point mutations of FOXP2 that are unique to the human lineage do play a role in our language adaptation, we next need to ask what kind of effect. And this requires considering its contribution to development of specific brain structures. Comparative and clinical data suggest that it plays a role in the development of the basal telencephalon, which will become ventral basal ganglia and basal forebrain in the adult. Though these basal ganglia structures are not classically identified as language structures *per se*, there are many reasons to think that basal ganglia structures could be important contributors to language learning and use (Lieberman, 2002), particularly of those processes that become relatively automatic. This is consistent with the critical role played by basal ganglia in skill learning, the automatization of many routine behavioral functions, and the establishment of procedural memories. Since there is an extensive interdependence between the anterior cortical areas and basal ganglia, via a recurrent circuit through the pallidum and thalamus, it should be no surprise that functions associated with frontal language cortex might also be affected by basal ganglia disturbances, especially motor functions. As a comparison, disruption of fluency, pronunciation, and syntactic processing have all been shown in Parkinson's disease patients, who also have reduced basal ganglia function (Lieberman, 2002). So if recent human-specific point mutations in the gene FOXP2 do reflect an adaptation for language processing, it is most likely with respect to aiding speech automatization. This role in learned vocalization is also supported by two additional comparative findings. First, the bird homologue to FOXP2 is found to be expressed in striatal nuclei associated with song learning (particularly in area X), and is more extensively expressed in species that learn their songs (Haesler *et al.*, 2004). Second, damaging one copy of FOXP2 in mice produces an impairment of their ultrasonic vocalization, and damaging both causes severe motor impairments, elimination of ultrasonic vocalizations, and premature death (Shua *et al.*, 2005). So although it is not specifically a gene for language, nor did it evolve only in humans, it has clearly been critical for neural systems underlying vocal motor functions in terrestrial vertebrates for a very long time, and it may have been tweaked in recent human evolution.

4.35.5 Evolutionary Processes and Brain–Language Co-Evolution

4.35.5.1 Evolutionary Scenarios

Estimates of the age of language date from as little as 50 000 years to more than 2 million years. Some of this difference reflects different definitions of language, some reflects different notions about the tempo of evolution (i.e., whether the change was sudden or gradual), and some takes different views about the number of mutational changes that were necessary. In general, those who argue that the language faculty is a highly specialized modular capacity tend to favor a recent date of origin and a saltational transition, whereas those who favor a more generalized conception of the language faculty supported by a constellation of adaptations tend to favor more ancient dates.

Exactly how the processes of natural and sexual selection might have contributed to the evolution of the human language adaptation is also contentious. Darwin (1871) argued that language might have evolved from something like courtship song under the influence of sexual selection. Modern theories that appeal to sexual selection have also focused on the use of language for social manipulation. The most common scenarios, however, focus on the role of language as a tool for social coordination and maintenance of social groups.

Two extreme language selection scenarios are commonly opposed in the literature to predict what changes in brain structure might be relevant: scenarios assuming that language is a consequence (or late-stage tweak) of a more prolonged trend toward increasing general intelligence (exemplified by a 2-million-year expansion of brain size) and scenarios assuming that language is the consequence of domain-specific neural modifications and is independent of general intelligence. These are not mutually exclusive options, but they do make different predictions with respect to neural structural and functional consequences, as well as

evolutionary timing. These functional implications can be used retroductively to probe the plausibility of each. If language has an ancient origin, it would follow that it is likely supported by a significant and extended natural selection history, including the contributions of many genetic changes affecting the brain. If, on the other hand, language is of recent origin and largely without precedent, it would follow that little time has elapsed for significant effects of natural selection to accumulate. As a result, ancient origin hypotheses predict that language functions will be more thoroughly integrated into other cognitive functions, will likely have distributed representation in the brain, and should be highly plastic with respect to both minor brain disorders and genetic variation. Recent origin hypotheses, on the other hand, are more consistent with language processes being highly modular and domain specific, localized to one or a very few neural systems, fragile with respect to brain damage and genetic variation, and possibly radically altered in grammatical organization by genetic abnormalities. With the exception of claims for domain specificity, which are controversial, the neuropsychological evidence argues against a recent rapid transition to language capacity. But archeological evidence is also brought to bear on this question. The paleoarcheological record is surprisingly stable from about 1.6 Mya to roughly 350 kya, with the transition from Acheulean to Mousterian tool culture, but does not begin to show signs of regional tool styles, decorative artifacts, and representational forms (e.g., carvings and cave paintings) until roughly 60 kya, with the dawn of what is called the Upper Paleolithic culture. This recent transition to technological diversity and representational artifacts has been attributed to a major change in cognitive abilities, which many archeologists speculate reflects the appearance of language. Fossil crania, however, provide no hint of a major neuroanatomical reorganization, and the genetic diversity of modern human populations indicates that there are some modern human lineages who have been reproductively separated from one another for at least twice this period and yet all have roughly equivalent language abilities. These considerations weigh in favor of a protracted evolution of language abilities and for the convergence of many diverse neural adaptations to support language (Johansson, 2005).

An adaptive convergence logic also helps to resolve some of the mysteries concerning the absence of direct neuroanatomical or functional homologies between language and nonhuman communication adaptations. The novelty of language can be understood in terms of the combined effects of systems which individually may have served quite different functions in ancestral species but which collectively interact in novel ways to produce emergent consequences. If the human language adaptation reflects the combined contribution of many diverse systems whose parallel evolutionary paths have come together to provide an unprecedented functional synergy, we should not expect to find highly divergent local changes in brain structure, but rather global reorganization in which most structures participate in some respect or other. But considering language functions to be emergent adaptations, in this sense, poses new questions about the evolutionary process. Specifically, we must explain how such functional synergies among diverse systems can be explored and recruited by the process of natural selection. In general, this reflects a common challenge posed to evolutionary theory since the time of Darwin, and can be generally answered the way he explained the probable evolution of the eye. He argued that even quite minimal non-image-forming light-sensitive proto-eyes would, none the less, provide an adaptive advantage over the absence of any light sensitivity, and that any minor modifications to adjacent structures that improved on this in any way would likewise be advantageous and selectively retained. As more comparative anatomical and genetic information has come to light concerning the evolution of eyes, in the century and a half since Darwin's time, his speculation has found ample justification. However, language differs from this sort of complex adaptation in one important respect: much of the detail of a language's functional architecture is transmitted socially.

4.35.5.2 Co-Evolutionary Scenarios

In contemporary behavioral biology, the concept of instinct no longer comes with the connotation of learning playing no role. Many species-typical behaviors from the social learning of birdsongs to the hunting behavior of wolf packs involve the interaction of behavioral and learning biases with socially transmitted habits and variable environmental contexts. Darwin recognized the relevance of this environmental conditionality when he described the language adaptation as "an instinct to acquire an art." Language is, of course, special with regards to the relatively massive contribution of extrinsic factors, and also with respect to the likely combinatorial and emergent character of its supporting neurology. So its emergent character is unusually dependent on interactions between diverse neural

and social mechanisms producing specific outcomes. This combinatorial co-dependence provides a challenge to simple caricatures of language evolution on the analogy of other physiological adaptations.

Recognition of this co-dependency has given rise to evolutionary scenarios that incorporate this interactional logic. Most develop from an evolutionary logic that has come to be called the Baldwin effect, after Baldwin (1896) who described how behavioral plasticity enabling the production of acquired adaptations might serve as an evolutionary precursor to a more innately produced analogue of this adaptation. The general logic of this evolutionary mechanism involves two phases: (1) the production of phenotypic plasticity (e.g., learned behaviors) making it possible for acquired adaptations to be conditionally produced that enable a lineage to persist despite a suboptimal match to the environment and (2) the appearance of new variants in that lineage that are selectively retained because they take over some fraction of the load of acquisition. This, presumably, described a Darwinian mechanism that would produce the evolutionary equivalent of Lamarckian inheritance of previously acquired traits. Proponents of innate UG invoked versions of this logic to argue that language-like behavior in our ancestors could have become progressively internalized as an innate faculty that is presently only minimally dependent on learning in the standard sense (e.g., Pinker, 1994). But the same logic could equally support the evolution of biases and aids to learning, without invoking a replacement of learned with innate knowledge of language (e.g., Deacon, 1992a, 1997). More recently, these arguments have been revisited in the context of the concept of niche construction (Odling-Smee *et al*., 2003; Deacon, 2003), in which persistent socially maintained language use can be understood as a human-constructed niche that exerts significant selection pressures on the organism to adapt to its functional requirements. This approach is compatible with the claim that language function is supported by many modest distributed evolutionary modifications of brain anatomy and chemistry. It also assumes that language-like communication was present in some form for an extensive period of human prehistory.

4.35.5.3 Degenerative Processes as Possible Contributors to Language Evolution

A co-evolutionary scenario for the evolution of language still does not account for the generation of the novel functional synergy between neural systems that language processing requires. The discontinuities between call control systems and speech and language control systems of the brain suggest that a co-evolutionary logic alone is insufficient to explain the shift in substrate. Recent investigation of a parallel shift in both complexity and neural substrate in birdsong may be able to shed some light on this.

In a comparative study of a long-domesticated bird, the Bengalese finch, and its feral cousin, the white-rump munia, it was discovered that the domesticated lineage was a far more facile song learner with a much more complex and flexible song than its wild cousin. This was despite the fact that the Bengalese Finch was bred in captivity for coloration, not singing (Okanoya, 2004). The domestic/feral difference of song complexity and song learning in these close finch breeds parallels what is found on comparisons between species that are song learners and nonlearners. This difference also correlates with a much more extensive neural control of song in birds that learn a complex and variable song. The fact that this behavioral and neural complexity can arise spontaneously without specific breeding for singing is a surprising finding since it is generally assumed that song complexity evolves under the influence of intense sexual selection. This was, however, blocked by domestication. One intriguing interpretation is that the relaxation of natural and sexual selection on singing paradoxically was responsible for its elaboration in this example. In brief, with the song becoming irrelevant to territorial defense, mate attraction, predator avoidance, and so on, degrading mutations and existing deleterious alleles affecting the specification of the stereotypic song would not have been weeded out. The result appears to have been the reduction of innate biases controlling song production. The domestic song could thus be described as both less constrained and more variable because it is subject to more kinds of perturbations. But with the specification of song structure no longer strictly controlled by the primary forebrain motor center (RA) (see Section 4.35.3.2), other linked brain systems can begin to play a biasing role. With the innate motor biases weakened, auditory experience, social context, learning biases, and attentional factors could all begin to influence singing. The result is that the domestic song became more variable, more complicated, and more influenced by social experience. The usual consequence of relaxed selection is genetic drift, increasing the genetic and phenotypic variety of a population by allowing random reassortment of alleles, but neurologically, drift in the genetic control of neural functions should cause

constraints to become less specific, generating increased behavioral flexibility and greater conditional sensitivity to other neurological and contextual factors.

This is relevant to the human case, because a number of features of the human language adaptation also appear to involve a relaxation of innate constraints allowing multiple other influences besides fixed links to emotion and immediate context to affect vocalization. Probably the clearest evidence for this is infant babbling. This unprecedented tendency to freely play with vocal sound production occurs with minimal innate constraint on what sound can follow what (except for physical constraints on vocal sound generation). Babbling occurs also in contexts of comparatively low arousal state, whereas laughter, crying, or shrieking are each produced in comparatively specific high arousal states and with specific contextual associations. This reduction of innate arousal and contextual constraint on sound production opens the door for numerous other influences to begin to play a role. Like the domesticated bird, this allows many more brain systems to influence vocal behavior, including socially acquired auditory experience. In fact, this freedom from constraint is an essential precondition for being able to correlate learned vocal behaviors with the wide diversity of objects, events, properties, and relationships language is capable of referring to. It is also a plausible answer to the combinatorial synergy problem (discussed above) because it demonstrates an evolutionary mechanism that would spontaneously result in the emergence of multisystem coordination of neural control over vocal behavior.

But although an evolutionary dedifferentiation process may be a part of the story for human language adaptation, it is clearly not the whole story. This increased flexibility and conditionality likely exposed many previously irrelevant interrelationships between brain systems to selection for the new functional associations that have emerged. Most of these adaptations remain to be identified. However, if such a dedifferentiation effect has been involved in our evolution, then scenarios hypothesizing selection for increased innateness or extrapolation from innate referential calls to words become less plausible.

4.35.6 Conclusions

Despite decades of research to identify the distinctive neuroanatomical substrates that provide humans with an unprecedented faculty for language, no definitive core of uniquely human anatomical correlates has been demonstrated. Only a few distinctive anatomical differences can be directly associated with the human language adaptation. These are associated with the special motor adaptations for speech. There is an unprecedented direct projection from motor cortex to the laryngeal motor nucleus of the brainstem (nucleus ambiguus) allowing direct control of vocalization independent of arousal state or innate vocal motor pattern. There is also one known genetic correlate with language competence, the gene FOXP2. Although it is clearly not specifically a language gene, nor can we be sure that its few human sequence differences represent adaptive modifications with respect to language, it is clear that it plays a necessary supportive role in the development of brain systems involved in speech production. Damage to gene in humans results in both generalized vocal disarthria and disruption of the ability to automate certain highly regular syntactic operations, and is associated with reduction of anterior basal ganglia structures. Besides these specific effects, however, it also appears likely that the neural changes associated with language adaptations involve more generalized allometric deviations from the ape pattern. Correlated with the increase in brain size in hominid evolution, there appears to have been quantitative remodeling of relationships between brain structures that is likely to have produced quantitative connectivity changes as well. If, as now appears likely, human brain adaptations for language involve many systems' coordinated interactions, it is likely that some or all of the quantitative alterations of brain organization reflect language adaptations. Although there is still considerable controversy concerning the proper assessment of the allometry human brain structures, candidates include overall cortical expansion, disproportion between cerebral cortex and basal ganglia, disproportionate increase in eulaminate cortical areas with respect to specialized sensory and motor areas, prefrontal expansion, increases in proportions of corticocortical and corticocerebellar connections, among others. However, the relevance of any of these cannot be discerned until there is a better understanding of the contributions of these systems to language acquisition, comprehension, and production. But a definitive assessment of the significant allometric deviations of human brain structure from typical primate trends could likewise provide hints of major differences in cognitive processing relevant to language.

The highly robust and developmentally canalized nature of language acquisition suggests that this capacity does not depend on only a few subtle neurological changes from the ape pattern but instead

likely reflects a prolonged process of selection involving many systems, and perhaps extending over a million years. The nature of this selection process appears to have involved early protolanguage use as a kind of niche construction, providing selective pressure to better support the unusual demands imposed by language. If this is an accurate assessment, it means that the neurological adaptations supporting language can at least in part be understood as adaptations for language, rather than merely accidentally giving rise to language. Some aspects of this ability may also be the result of evolutionary degradation of other functional specializations, which has allowed more diverse and distributed neural systems to directly or indirectly influence vocalization.

Though human brains unquestionably include numerous species-unique innate adaptations supporting the acquisition and use of language, there is to date little evidence for a specific neuroanatomical substrate for an UG. So, progress in understanding the language-related evolutionary changes of human brain structure can mostly be marked by what we now know is not the case, and just a few clear correlates of language adaptation. But this imposes considerable constraint on the scenarios we can consistently entertain and focuses neural research on a few notable problem areas.

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4.36 Nuclear Schizophrenic Symptoms as the Key to the Evolution of the Human Brain

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Glossary

Broca's area	A region of the frontal lobe of the left cerebral hemisphere associated with articulate language.
cerebral asymmetry	The concept that the two cerebral hemispheres differ in function. This is reflected in differences in the anatomical organization of the two hemispheres.
schizophrenia	A psychopathological disorder characterized by delusions, hallucinations, disorganized thought, and a poverty of emotion, speech, and intention.
sexual selection	The process where some individuals have greater reproductive success than others because of competitive advantages in mating.

4.36.1 The Nuclear Symptoms of Schizophrenia and the Central Paradox

The core nuclear symptoms of schizophrenia according to Kurt Schneider, as defined by the glossary of the Present State Examination (Wing *et al.*, 1974), are:

1. Thought echo or commentary: the subject experiences his own thought as repeated or echoed with very little interval between the original and the echo.
2. Voices commenting: a voice or voices heard by the subject speaking about him and therefore referring to him in the third person.
3. Passivity (delusions of control): the subject experiences his will as replaced by that of some other force or agency.
4. Thought insertion: the subject experiences thoughts *which are not his own* intruding into his mind. In the most typical case, the alien thoughts are said to have been inserted into the mind from outside, by means of radar or telepathy or some other means.
5. Thought withdrawal: the subject says that his thoughts have been removed from his head so that he has no thoughts.
6. Thought broadcast: the subject experiences his thoughts actually being shared with others.
7. Primary delusions: based upon sensory experiences (delusional perceptions) the patient suddenly becomes convinced that a particular set of events has a special meaning.

Why do the core symptoms have this form? What primary function is disturbed? What neural structure is the focus of the disturbance?

Through the presence of these features the authors of the WHO Ten Country Study of the Incidence and Manifestations of Schizophrenia (Jablensky *et al.*, 1992) reached their conclusion that:

> schizophrenic illnesses are ubiquitous, appear with similar incidence in different cultures, and have clinical features that are more remarkable by their similarity across cultures than by their difference.

Thus, schizophrenia is constant across populations that differ widely in geographic, climatic, industrial, and social environment; it seems it is a characteristic of human populations. It is a disease (perhaps the disease) of humanity.

If the core syndrome is a characteristic of populations it must somehow be intrinsic, i.e., genetic in origin. This raises the central paradox: why if the disease is associated with a biological disadvantage is this genetic variation not selected out? About the existence of the fecundity deficit there is little doubt: it is of the order of 70% in males and 30% in females (Essen-Moller, 1959; MacSorley, 1964; Vogel, 1979; Haverkamp *et al.*, 1982; Penrose 1991). To balance such a disadvantage, a substantial and universal advantage must be invoked. But what could such an advantage be, and by whom might it be carried? These questions were first clearly formulated in a paper (Huxley *et al.*, 1964) notable by an authorship that includes J. S. Huxley and E. Mayr, two progenitors of the modern evolutionary synthesis of Mendelian genetics and Darwinian theory. The first paragraph identifies these two as the originators of the notion. Yet the theory they proposed – that the balance lay in resistance to wound shock and stress – was clearly mistaken, as Kuttner *et al.* (1967) pointed out soon after. It makes no sense to suppose that the advantage of a particular genetic variation lies in a field that is unrelated to the disadvantage. Kuttner *et al.* considered three advantages – intelligence, language, and complex social ability – and favored the last. But these three are clearly related and one – language – is both of more obvious adaptive significance and is more readily defined in terms of neural function than the other two.

What is striking about the nuclear symptoms is that they can hardly be conceived except within the framework of language. Auditory hallucinations (items 1 and 2 above) are self-evidently an anomaly of the perception of the spoken word. Thought insertion, withdrawal, and broadcast (items 4, 5, and 6) are disturbances of the experience of thought and of the transition from thought to speech production. Primary delusions (item 7) represent a deviation in the attachment of meaning to symbolic representations, that is to say, they are a disturbance of semantics. Only delusions of control (item 3) are not immediately recognizable as a disturbance of speech, but these cannot be described except with the use of language. They can perhaps be understood as anomalies of identification of the self in relation to the rest of the universe of symbols.

4.36.2 The Problem of Language for Evolutionary Theory

The concept of language as the defining characteristic of humanity has an ancient origin:

> In most of our abilities we differ not at all from the animals; we are in fact behind many in swiftness and strength and other resources. But because there is born in us the power to persuade each other and to show ourselves whatever we wish, we not only have escaped from living as brutes, but also by coming together have founded cities and set up laws and invented arts, and speech has helped us attain practically all of the things we have devised (Isocrates, 436–338 BC, quoted in Harris and Talbot, 1997, p. xiii).

Darwin can be quoted in agreement with this view. On p. 53 of *The Descent of Man*, he writes that language "has justly been considered as one of the chief distinctions between man and the lower animals" and he seems not to have regarded this as a particular difficulty. But in 1873, within 2 years of the publication of *The Descent of Man*, Mueller (1873), who held the chair of Philology in the University of Oxford, delivered a series of three lectures at the Royal Institution in which he drew attention to the problems that language raises for Darwin's theory:

> My object is simply to point out a strange omission, and to call attention to one kind of evidence – I mean the evidence of language – which has been most unaccountably neglected, both in studying the development of the human intellect, and determining the position which man holds in the system of the world.

In the second lecture Mueller addresses the problem:

> There is one difficulty which Mr Darwin has not sufficiently appreciated ... There is between the whole animal kingdom on the one side, and man, even in his lowest state, on the other, a barrier which no animal has ever crossed, and that barrier is – *Language* ... If anything has a right to the name of specific difference, it is language, as we find it in man, and in man only ... If we removed the name of *specific difference* from our philosophic dictionaries, I should still hold that nothing deserves the name of man except what is able to speak ... a speaking elephant or an elephantine speaker could never be called an elephant. Professor Schleicher, though an enthusiastic admirer of Darwin, observed once jokingly, but not without a deep meaning, "If a pig were ever to say to me, 'I am a pig' it would *ipso facto* cease to be a pig."

Mueller thus raised for Darwin the problem for evolutionary theory raised 86 years later by Chomsky (1959) in his critique of Skinner's claim to have an explanation of language in operant principles derived form studies in the rat. The strong implication was that there were principles underlying language that were human-specific. The concept of universal

grammar as a defining human characteristic and of its generativity have implications for speciation theory. The question raised by Chomsky, as by Mueller, is: what is the nature of a species?

In the last chapter entitled 'Recapitulation and Conclusion' of *The Origin of Species by Means of Natural Selection*, Darwin had written:

> In the distant future I see open fields for far more important researches. Psychology will be based on a new foundation, that of the necessary acquirement of each mental power and capacity by gradation. Light will be thrown on the origin of man and his history.

The form of words – "that of the necessary acquirement of each mental power and capacity by gradation" – is of note because it clearly expresses Darwin's predilection for a gradualist account of the origin of human faculties along with other evolutionary innovations. The paradoxical point has been made that an issue on which *The Origin of Species* is weak is the origin of species. One figure appears in the book (chapter 4) to show how within one genus different varieties and species emerge over eons of time. The implication of the figure is that variation within species is qualitatively the same as variation between species, hence the absence of a clear differentiation of varieties and species.

In the same chapter Darwin wrote:

> Hereafter we shall be compelled to acknowledge that the only distinction between species and well-marked varieties is, that the latter are known, or believed, to be connected by intermediate gradations, whereas species were formerly thus connected.

His aim was to establish that species have a common origin, they are not the subjects of independent creations. Species can arise, subject to favorable environmental circumstance, out of the variation that is present in the natural world at any point in time. It is a gradualist theory. That gradualism was preserved into the evolutionary synthesis of the Darwinian theory of natural selection with Mendel's laws of genetic inheritance in the 1940s (Mayr and Provine, 1998).

The test comes in the application of the theory to actual speciation events. Such events are not generally observed, but somewhere in the figure, if it were to be applied to the case of humans, we must suppose there is a separation that leads on the one hand to the chimpanzee and bonobo and on the other to *Australopithecus*, *Homo erectus*, and modern *Homo sapiens*. The branch point and its sequelae is of theoretical and specific interest.

In 1863, T. H. Huxley addressed the question in *Man's Place in Nature*. He mounted a powerful case that on a series of anatomical comparisons the distance between humans and any one of the great apes was no greater than that between any pair of the great apes compared on their own. Huxley (1863) thus fit humans into the framework of the Darwinian theory of natural selection of which he was so powerful an advocate. But on the issue of speciation, he did not see eye to eye with his mentor. After the publication of *The Origin*, he wrote to Darwin that he was ready to go to the stake for the theory but added that he thought that Darwin had loaded himself with "an unnecessary difficulty in adopting *Natura non facit saltum* so unreservedly." Huxley was thus the first in a line of evolutionists including Bateson (1894), De Vries (1901), Goldschmidt (1940), and most recently Gould (2002), who have held that species transitions were more discontinuous, and the characteristics of species more stable, than appeared to follow from Darwin's formulation (see The Evolution of Language Systems in the Human Brain, The Interpreter in Human Psychology).

4.36.3 Darwin's Intuition on Sexual Selection

When Darwin (1871) addressed himself to the problem of human origins in *The Descent of Man* – this publication linked in a single volume *The Descent of Man and Selection in Relation to Sex* – the anatomical and paleontological case with Darwin's theory of sexual selection. In his introduction, Darwin writes that:

> During many years it has seemed to me highly probable that sexual selection has played an important part in differentiating the races of man; but in my *Origin of Species* I contented myself by merely alluding to this belief. When I came to apply this belief to man, I found it indispensable to treat the whole subject in full detail (Darwin, 1871, *The Descent of Man*, pp. 4–5).

Some passages in the second part of the book indicate that Darwin considered the two arguments to be related in a more fundamental way. Thus:

> . . .Sexual selection has apparently acted on both the male and the female side, causing the two sexes of man to differ in body and mind . . . [and] has indirectly influenced the progressive development of various bodily structures and of certain mental qualities. Courage, pugnacity, perseverance, strength and size of body . . . have all been indirectly gained by one sex or the other, through the influence of love or jealousy, through the appreciation of the beautiful in sound, color and form, and through the exertion of choice. . . (Darwin, 1871, *Selection in Relation to Sex*, p. 402).

Although Darwin believed that sexual selection and the descent of man are related, he nowhere specifies exactly how this is the case and the fact remains that these are separate books. Thus, on the one hand we have Charles Darwin committed to the

view that humans are descended from the great apes by the process of natural selection with a strong intuition that the ancillary process of sexual selection also has something to do with it but unable to integrate these processes. On the other hand, we have Friedrich Max Mueller complaining that language has characteristics that are present in the communicative abilities of no animal other than humans and that Darwin has given no account of its origins. The solution to this dispute that I have offered (Crow, 1998a, 1998b, 2000a, 2002a, 2002b, 2002c) is that the Darwinian gradualist account indeed has to give ground to a saltational version, as argued strongly for example by Goldschmidt and Gould. Speciation events have a reality that is obscured in *The Origin of Species*. But that there is a relationship between speciation and sexual selection, as Kaneshiro (1980) and Carson (1997), among others, have argued; species are distinguished by characteristics that are often sexually dimorphic. The possibility is that the first change in the process of speciation occurs in one sex, generally the male, and that this change is then subject to mate choice to define what Paterson has called a specific mate recognition system. My proposal is that changes on the sex chromosomes, including chromosomal rearrangements and subsequent epigenetic modifications of gene control, play a critical role in these transitions. This concept of the nature of speciation comes from a consideration of the problem that vexed Max Mueller – the origins of language and its relationship to the origin of humans – and its relevance to the central paradox of psychosis – the universal persistence in human populations of a genetic predisposition in the face of a biological disadvantage (Crow, 2000b).

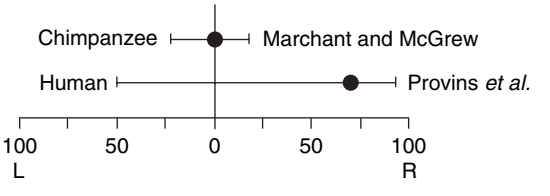
4.36.4 Paul Broca and Cerebral Asymmetry

The key to the solution lies in the asymmetry or torque that appears to be characteristic of the human brain. After he had convinced himself (Broca, 1861) of the reality of the earlier observations of Marc Dax that language in the frontal lobes is localized on the left side, Broca (1877) came to the conclusion that:

> Man is, of all the animals, the one whose brain in the normal state is the most asymmetrical. He is also the one who possesses the most acquired faculties. Among these faculties ... the faculty of articulate language holds pride of place. It is this that distinguishes us most clearly from the animals.

While it is clear that there are directional asymmetries (e.g., of the habenular nucleus) that are ancient in vertebrate phylogeny, these are presumably unrelated to those expressed in the cortex that are associated with language. Such species specificity would have been no surprise to Broca (see Harrington, 1987, pp. 49–51, for his views on the essence of human nature), but the assumption that directional asymmetry on a population level is present in other primates, perhaps based upon Darwinian gradualist principles, has been widespread in the literature. Annett introduced a discussion of the issue in her earlier volume (Annett, 1985, pp. 169–173) with reference to the work of Finch (1941), who found no evidence of directional handedness in a group of 30 chimpanzees. Subsequent studies, for example, Annett and Annett (1991), of 31 lowland gorillas in European zoos and Byrne and Byrne (1991), of 38 mountain gorillas in Rwanda, have reinforced this conclusion. Recently, Marchant and McGrew (1996) systematically studied 42 chimpanzees in the Gombe National Park and reviewed the primate literature to conclude that "non-human primate hand function has not been shown to be lateralized at the species level – it is not the norm for any species, task, or setting, and so offers no easy model for the evolution of human handedness" (McGrew and Marchant, 1997; see also Holder, 1999 for congruent conclusions from a field survey of primates in Africa). Thus, we have evidence that a putative correlate of the capacity for language that Mueller and Chomsky identify as the defining characteristic of *H. sapiens* demonstrates a discontinuity in the primate phylogenetic tree (Figure 1).

Thus, at some point in the course of hominin evolution, the dimension of asymmetry was introduced in the sequence of brain development, and this dimension, or some modification of it, is the obvious correlate of language.



	Number of Individuals	Activities
Chimpanzee	38	46
Human	1960	75

Figure 1 Hand preference for everyday activities in chimpanzees and *H. sapiens* compared. Data for chimpanzees referred to a community of 38 animals (*Pan troglodytes schweinfurthii*) observed by Marchant and McGrew (1996). Data for *H. sapiens* were collected by questionnaire by populations of undergraduates by Provins *et al.* (1982). Medians and boundary values for 95% have been extracted from graphs in the original publications.

But why should the capacity for language be variable between individuals? Perhaps only when we understand the nature of the genetic mechanism will we have a clear answer (Crow, 2002c). The important point is that Broca's hypothesis – that language is lateralized in the brain – provides an indication of the neurophysiological basis and an approach to its pathophysiology.

Buxhoeveden *et al.* (2001) have recently documented the anatomical correlate of population bias to right-handedness. Through a statistical analysis of the minicolumn structure of the cerebral cortex, they have demonstrated asymmetries, for example, of minicolumn width and separation that are present in the planum temporale of human but absent in those of the chimpanzee and rhesus monkey.

4.36.5 The Torque and Related Asymmetries in Psychosis

The form of the asymmetry – from right frontal to left occipital, described as cerebral torque – has implications for function, as the anatomy has for pathophysiology. Aspects of anatomical asymmetry are deviant in individuals who suffer from psychosis (see Crow, 1990b, 1997; Petty, 1999; Esiri and Crow, 2002) and there is evidence from a study of handedness in childhood that they are lateralizing less, or more slowly (Crow *et al.*, 1996; Leask and Crow, 2005) than the population as a whole. In postmortem studies, the anatomical changes appear to be more posterior in the brain; losses or reversals of asymmetry have been detected in fusiform, parahippocampal (McDonald *et al.*, 2000), and superior temporal (Highley *et al.*, 1999b) gyri. A curious feature of these findings is that although the loss or reversal was present in both sexes, the relationship to age of onset was different: greater anomaly related to earlier age of onset in females but to later age of onset in males.

Other sex differences have been detected in postmortem brain. Density of fibers in the corpus callosum was greater in females than in males, consistent with the generalization that connectivity is inversely related to degree of asymmetry (Highley *et al.*, 1999a). In patients with psychosis, fiber density was reduced relative to female controls, while in males it was increased relative to male controls. Consistent with these findings in an magnetic resonance imaging (MRI) study (Highley *et al.*, 2003), white matter in the occipitotemporo-occipital regions was greater in females than in males, while in female patients it was reduced and in male patients increased relative to same sex controls.

Thus, there are morphological changes in the brain in psychosis that are sex-dependent and may relate to the sex difference in age of onset: onsets are earlier in males, and earlier onsets are generally associated with a poorer outcome and a preponderance of negative symptoms (affective flattening and poverty of speech). Conversely, with increasing age of onset the proportion of females increases and the form of the illness is more likely to be paranoid, i.e., delusional. This sex difference has been identified by two evolutionary theorists (Gould and Gould, 1989) as evidence that sexual selection operates in humans. This attractive argument encounters the difficulty that on a simple developmental interpretation, the sex difference is in the wrong direction: brain size (Kretschmann *et al.*, 1979) and verbal ability (Crow *et al.*, 1998) develop faster in females than in males, yet onset of psychosis is earlier in males (Figure 2).

A solution to this problem may unravel the evolution of the human brain. Some recent data are relevant. In a postmortem study (Chance *et al.*, 2006), planum temporale asymmetry of surface area to the left was found to be greater in males than females, consistent with the sex difference in the torque. But there was also an asymmetry of Heschl's gyrus (primary auditory cortex) and this, as has been found with the asymmetry of the paracingulate sulcus in the frontal lobes (Clark *et al.*, 2006) was greater in females. A possible generalization therefore is that the brain growth is faster and the plateau is earlier in females and that asymmetries, for example of primary sensory cortex, that are formed earlier are greater in females, whereas those, for example of posterior heteromodal association cortex that have the opportunity of developing longer, are greater in males.

The principle that interhemispheric connections are inversely related to degree of asymmetry together with the hypothesis that there is a relative loss of fibers in one direction (Witelson and Nowakowski, 1991) provides a possible solution to the age of onset paradox. Myelination continues into the third and fourth decades of life in the corpus callosum and apparently continues longer in females than males (Cowell *et al.*, 1992; Pujol *et al.*, 1993). This sex difference may reflect the longer time required in females to myelinate the larger body of interhemispheric axons. If we assume that the first symptoms of psychosis emerge as a consequence of some limit imposed on interhemispheric transmission by these late myelinations (for example, that those predisposed to psychosis fail to differentiate connections in the two directions), then owing to the faster myelinization, this may be apparent earlier in males. In

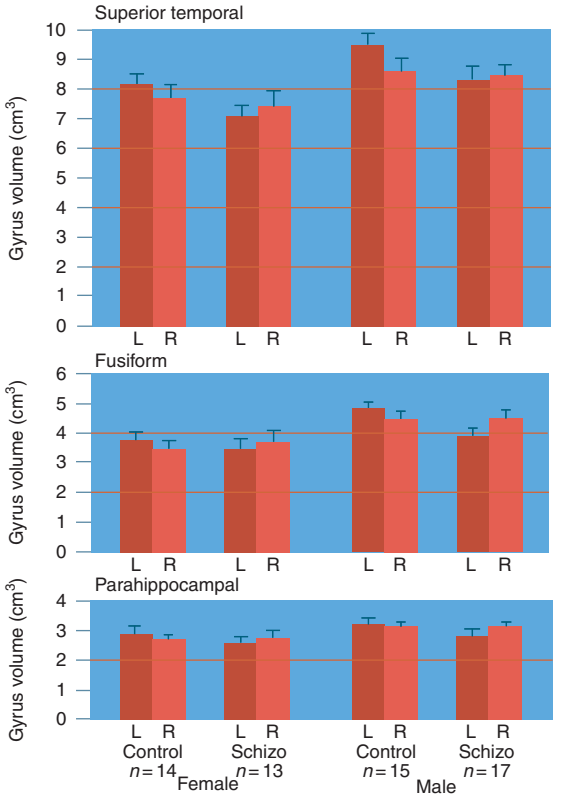


Figure 2 Volumes of cortical gyri (superior temporal, fusiform, parahippocampal) in patients with schizophrenia and controls assessed by stereology on postmortem brain. There are subtle asymmetries to the left in controls, and to the right in patients in each case. Reproduced from Esiri, M. M. and Crow, T. J. 2002. The neuropathology of psychiatric disorder. In: Greenfield's Neuropathology (*eds.* D. I. Graham and P. L. Lantos), 7th edn., vol. II, pp. 431–470. Arnold.

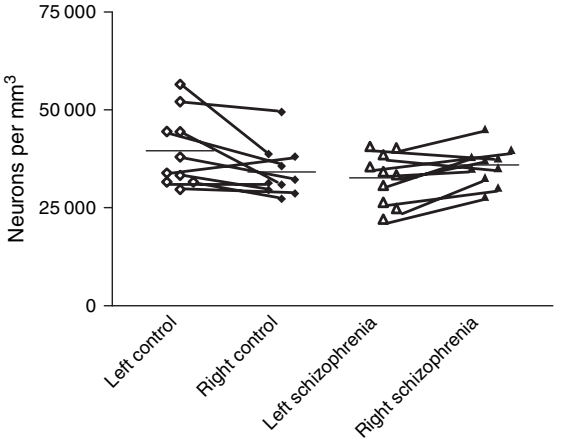


Figure 3 Pyramidal cell density in layer 3 of the left and right hemispheres of schizophrenic and control brains. An asymmetry to the left is present in controls and to the right in patients. Reproduced from Cullen, T. J., Walker, M. A., Eastwood, S. L., Esiri, M. M., Harrison, P. J., and Crow, T. J. 2006. Anomalies of asymmetry of pyramidal cell density and structure in dorsolateral prefrontal cortex in schizophrenia. *Br. J. Psychiatry* 188, 26–31, Elsevier.

this way, age of onset of psychosis may reveal to us the operation of sexual selection in humans in determining the limits of species-specific phenotypic variation (Crow, 1993, 1998a, 2002c) (Figure 3).

In frontal regions, no gross asymmetry and no change in gyral volume was detected in schizophrenia (Highley *et al.*, 2001), but in the density of cells in the cortex (in area 9) there was an asymmetry (greater cell density) to the left in controls and loss or reversal of this asymmetry in patients (Cullen *et al.*, 2006).

4.36.6 The Structure of Language and Its Decomposition in Psychosis

How are the symptoms to be explained? On the basis that the anatomical changes reflect an alteration in connectivity and that the asymmetry of the human brain (the torque) is the foundation of the faculty of language, one can construct a theory of nuclear symptoms – that these are primary disorders of the structure of language, and that they reveal its constituent elements and the way in which the elements are segregated within and relate to each other between the four quadrants of heteromodal association cortex.

4.36.6.1 The Linguistic Sign Is Bihemispheric

de Saussure (1916) maintained that the linguistic sign (the word) was characteristically bipartite,

comprising a signal (the sound pattern or phonological engram) and a signaled (the associated concept or meanings). The association between the sound pattern and its meanings, according to de Saussure, is arbitrary – any sound pattern can be associated with any concept or meaning (the first principle). This is what is distinctive about the human use of words and what makes language so flexible.

There is a two-way relationship between the components, with movement from the sound pattern to the meanings in speech reception, and from the concepts to sound patterns in speech production. One can ask, what is the neural basis of the separation of the two components? If asymmetry is what is characteristic of the human brain, it seems that there must be a relationship between specialization of function of the hemispheres and the feature that de Saussure identifies as the key to language. The most parsimonious hypothesis is that the components are (at least in part) segregated to the two hemispheres.

From Broca's observations, it is clear that what is localized in the hemisphere that is labeled as dominant is the phonological engram. It follows that some part of the signifieds must be assumed to be located in the nondominant hemisphere. For each phonological engram, there must be a corresponding engram – a mirror image – in the nondominant hemisphere, but one that is systematically transformed by the differing terminations of the interhemispheric connections in that hemisphere.

de Saussure's second principle is that speech is linear, 'just a ribbon of sound'. Allied to this is the notion that there must be a speaker and a hearer – speech is necessarily communicative – and the ribbon of sound is what travels between them (Figure 4).

One envisages therefore that speech is encoded, and this is a bihemispheric process, by the speaker from his concepts or thoughts into phonological engrams that are then transformed into the ribbon of sound, and that this is received by the hearer, and decoded into his own meanings or concepts, and that this decodification takes place partly by interhemispheric interactions. Communication depends upon the hearer sharing at least some of the speaker's signifier–signified associations, in other words, that they speak the same language.

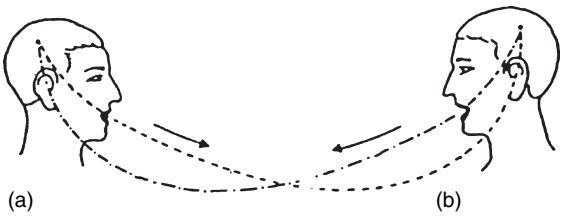


Figure 4 The relationship between the speaker and the hearer according to de Saussure (1916).

4.36.6.2 Deixis and the Significance of the Indexical

The system works well so long as the speaker refers to the world outside himself and the hearer. But a complication arises when he refers to himself. As Hurford (1992) points out, such a referral necessitates further decoding on the part of the hearer – that the 'I' that the speaker refers to, relates not to the 'I' of the hearer but to the 'you' (to him) of the speaker. This class of symbols – the indexicals – referring to the speaker or hearer belongs to the wider class of deictic symbols that include reference to the here of the present place and the now of the present moment in time.

The interest is that deixis – the necessity to define this class of symbols by pointing – has a special status in philosophy and in the structure of language. According to Buehler (1934), this triad of terms defining the present moment and the location and identity of the speaker is the coordinate origin around which language is structured – this place, at the present moment in time, defined by the 'I' of the speaker. Without this, language has no point of reference and loses its capacity to convey meaning.

The concept of the indexical also relates to the nature of psychotic symptoms. While it is true that there is no general misuse of the first person pronoun in individuals with psychosis, those with early-onset developmental disorders such as autism and Asperger's syndrome sometimes have difficulty in acquiring the distinction between the use of 'I' and 'you'. The more general significance arises in relation to Hurford's point that these symbols relate to what is self-generated in speech and what is other-generated – that there is a fundamental dichotomy between speech production and speech perception – and that this dichotomy can be understood in terms of the brain torque (Figure 5).

4.36.6.3 The Human Brain as a Four-Chambered Organ

It is often overlooked that the asymmetry of the human brain is not a simple left–right difference but a deviation across the fronto-occipital axis that transforms the human brain from the standard primate and vertebrate pattern of two-chambers (anterior and posterior corresponding to motor and sensory compartments) into a four-chambered organ in which motor and sensory compartments are distinguished on the left and the right sides. The torque has the effect of differentiating the two

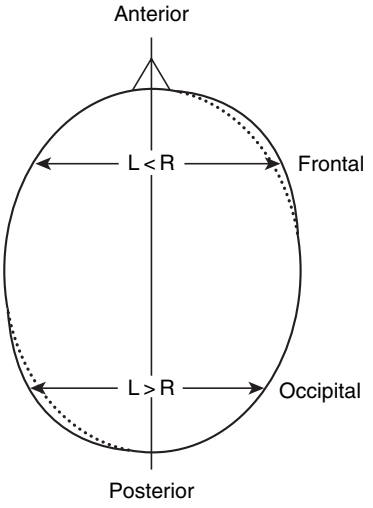


Figure 5 The Yakovlevian torque from right frontal to left occipital.

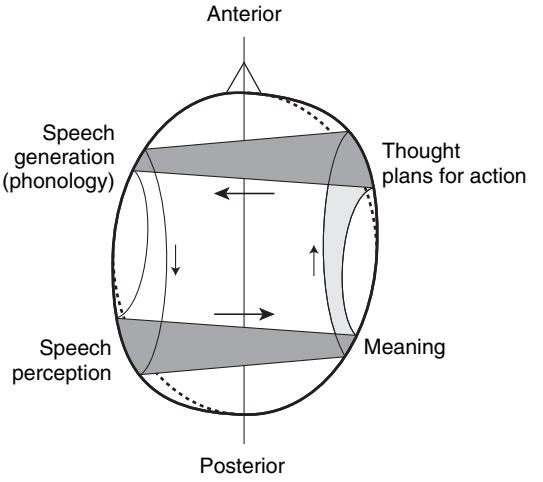


Figure 6 The implications of the torque for the relationship between the four areas of association cortex. Interhemispheric fibers can merge from right to left anteriorly and from left to right posteriorly. The figure also illustrates the segregation of function that has allowed the separation of thoughts from speech production and meaning from speech perception.

sides of the brain by influencing the relative surface area of the cerebral cortex, but it does so in different directions in the anterior (motor) and posterior (sensory) halves. It is a remarkable fact that the volumes of the two hemispheres are closely similar. What has changed relative to other primates is either the distribution of tissue between the two sides along the anteroposterior axis or perhaps even more subtly the sulcogyral folding of the cortical surface. The effect must be assumed to be that the distribution of interhemispheric connections differs on the two sides and it is this that allows the spread of neural activity to be systematically different on one side compared to the other. But the direction of the difference is opposite in the motor and sensory halves of the brain – converging from right to left anteriorly and from left to right posteriorly (Figure 6).

If the torque is what is characteristic of the human brain, these changes, and no others, are critical to the evolution of language. The interconnections between areas of heteromodal cortex have changed and these changes alone must account for what is characteristic of the capacity for language. Two features are suggested – the arbitrariness of the association between the signifier and the signifieds, the phonological engram and its meanings (de Saussure, 1916), and universal grammar, a mechanism that generates the structure of the sentence (Chomsky, 1988). But the striking fact about the evolution of language is that it occurred relatively abruptly, in the transition to modern *H. sapiens*, and all of a piece (Bickerton, 1990, 1995) along with the capacity for symbolic representation (Mellars, 2002). It does not make sense to suppose that there were two sequential innovations, each contributing a revolution to brain function. These two features must reflect different aspects of the same change and that change must be either the introduction of the torque or some modification of a torque that was introduced earlier in hominin evolution.

The key change is the separation of a phonological engram from its associations. The human cortex is not qualitatively different from that of the chimpanzee or any other primate. It must presumably have the same capacity to receive inputs and to transform them into outputs. What is different about the human brain is the interaction between areas of (particularly heteromodal) cortex on the two sides. Thus, if we assume that what is segregated in the dominant hemisphere is the phonological engram – a collection of simple but heavily interconnected motor sequences – this leaves open the possibility that each of these motor patterns has connections with engrams that are systematically different (either more diffuse or more restricted) in the nondominant hemisphere.

But here is the key consequence of the torque – whether the connections are more diffuse or more restricted depends upon whether they are in the motor or sensory halves of the brain. The convergences are in different directions in the anterior and posterior heteromodal association cortices, and this has an obvious implication for the organization of the capacity for language. Whatever transformation takes place from right to left in dorsolateral prefrontal cortex is reversed in sense in the transition from

left to right in occipitoparietotemporal cortex. This can be regarded as the first principle in the neural organization of language.

The second principle is that the sensory phonological engram is distinct from the motor engram. This must obviously be the case and these engrams are presumably located in Wernicke's and Broca's areas, respectively. But it is not so obvious that the form of the engram must be different – the motor engram can be relatively directly associated with motor neurones and with the output, but the sensory engram is one step removed from the acoustic input – word traces need to be filtered out from the totality of incoming sensory information.

Nonetheless, there is a relationship between the two. Words that are heard are recognized as related if not identical to those that are spoken. The form of the relationship presumably is what is established in the course of language acquisition. Conversely, some aspect of the distinction between the two is what is lost in the case of auditory hallucinations; what is clearly intrinsically generated (whether in the process of thought or in motor planning) has activated engrams that are normally accessible only to incoming acoustic stimuli. In this distinction, and in the association of the signifier and the signifieds, lies a solution to the conundrum of psychosis.

The four chambers of the human brain are the framework within which the problem can be solved, in the separation of the motor from the sensory by the central sulcus, and in the segregation of the signifier (the phonological engram) in its two forms in the dominant hemisphere from its primary associations in the nondominant, by the bias of the torque. The key to the solution is to specify the nature of the difference between what is motor and what is sensory and to identify what is intrinsic to the signifier and what is arbitrarily associated with it, on the one hand in its sensory form, and on the other in its motor configuration.

Speech is versatile – what has to be accounted for is the infinity of sentences that can be generated and the diversity of meanings that can be extracted, and the fact that these two processes are integrated with each other and yet separated in the brain.

The simplest approach is to assume that contiguous functions are dealt with in anatomically related areas. Thus, if the phonological engrams of the output are assembled in the association areas of cortex that are focused on Broca's area in the left hemisphere, one must assume that the processes that are related to this assembly, but differ from it in the crucial respect that they confer upon it its arbitrariness and flexibility, are located in the homologous regions in the right hemisphere. In functional terms, these engrams can be loosely referred to as 'thought', the precursor of, or the plans for, speech. It has a relationship to speech in that each element has an associated phonological engram in the left hemisphere, but it is not speech in that without the linear sequence that forms the output on the left, the association between elements is, to a certain degree, random and arbitrary. This presumably is related to the fact that each phonological engram on the left is associated with a number of less well-structured engrams on the right. The relationship from right to left is many-to-one.

In the parietotemporo-occipital junction association areas, the convergence is in the opposite direction. From the phonological engrams that have been extracted from the primary acoustic signal in the auditory association areas on the left, there is a convergence to a smaller area of homologous cortex on the right. Thus, the many-to-one transition in this case is from left to right, and the process of simplification may be identified as the distillation of 'meaning' from the linear sequence of phonology on the left.

According to this concept, the transition from thought to speech takes place from right to left in dorsolateral prefrontal cortex, and from perceived speech to meaning from left to right in parietotemporo-occipital association cortex. But what is the relationship between perceived 'meaning' and the 'thought' that is the plan for speech? Both are concepts or associations (signifieds) removed from the phonological engram, but one is sensory and one is motor. There is a connection between them and that connection is mediated by the uncinate and arcuate bundles in the right hemisphere. The patterns of activity in the right parietotemporo-occipital cortex is thus accessible to the thought processes in right dorsolateral prefrontal cortex, but the activities are distinct, and the distinction differs from that between the activity in the association cortices around Broca's and Wernicke's areas.

4.36.6.4 The Deictic Origin and the Performative Hypothesis

The key here is syntax – the ability to relate the self to the outside world – and to use words to do it. According to Buehler, as noted above, language is built around a coordinate framework with an origin in the self, the present location and the present moment in time defined by the deictic symbols 'I', 'here', and 'now'. Without this, language loses its structure. Language mediates the individual's relationship with the outside world and other individuals. In every linguistic interaction, there is

a speaker and a hearer, and sentences are the substance of the negotiation between them. The distinction between what is self- and what is other-generated and the meanings of these two classes of symbol is critical. This distinction clearly relates to the division between the motor and sensory association cortices in the right hemisphere.

The nuclear symptoms of schizophrenia tell us what happens when the distinction breaks down. Thought, the precursor of speech, loses its characteristic of independence from the outside world – thoughts are inserted into or removed from the individual's mind – while retaining the features of thought, they have lost the relationship to self-generated acts, which is a defining feature of thought as a precursor of speech. The obvious interpretation is that they are influenced by activity in posterior association cortex in a way that differs from the normal exchange between posterior and anterior regions. Conversely, auditory hallucinations such as thoughts spoken aloud or running commentary presumably represent self-generated neural activity (thoughts or plans for action) that activates phonological engrams (perhaps in the superior temporal cortex on the left side) that are normally activated by speech from another individual. In each case, there is a loss of the boundary in symbolic representation (words) between what is self- and what is other-generated. In each case, we can see that the boundary has something to do with what is anterior and what is posterior in association cortex. But we cannot suppose that what is abnormal is simply that some neural activity crosses this boundary. Activity must normally be transmitted between posterior and anterior along, for example, the arcuate and uncinate bundles. The problem is to specify what is normal and what is abnormal. The nature of nuclear symptoms is the clue that we have (Figure 7).

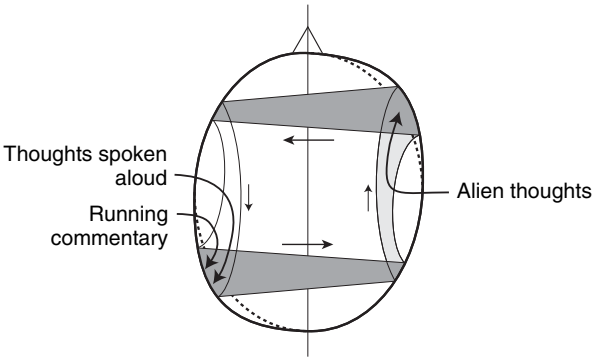


Figure 7 The origin of nuclear symptoms forging the concept of the compartmentation of language. According to this concept, symptoms arise because leakage occurs of neural activity normally segregated within one or more of the chambers.

The possibility must be considered that Buehler's formulation – that language is intrinsically organized around a deictic core – and the distinction between what is self- and what is other-generated that is illustrated by the nature of the nuclear symptoms are both necessary to a solution of the problem of syntax. In his thesis of *How to Do Things with Words*, Austin (1962) made the case that many uses of language are not simply to convey information but to have an effect, that is to say to bring about a state of affairs according to the speaker's intention. Such utterances Austin referred to as performatives to distinguish them from the constatives, the utterances, more usually the subject of linguistic and philosophic analyses, that convey information, generally about the external world. Later he generalized the concept of the performative into the notion that all utterances have an illocutionary force in the sense that they are formulated toward some objective of the speaker.

An interesting parallel to Austin's concept in the performative hypothesis has been formulated in different forms by a small number of theoretical linguists to account for certain features of the use of indexicals (symbols that refer to the speaker or hearer). Ross (1970) defines the theory as that declarative sentences (constatives) "must also be analysed as being implicit performatives, and must be derived from deep structures containing an explicitly represented performative main verb." Performative sentences, he says:

> . . . must have first person subjects and usually have second person direct or indirect objects in deep structure. They must be affirmative and non-negative, they must be in the present tense, and . . . their main verb must belong to the class that includes verbs such as advise, answer, ask, beg, command, declare, implore, inform, pronounce, say, write, in other words the class that designates the transmission of information, instructions, orders etc. The implication of the performative hypothesis is that the declarative sentence has an implicit (unstated) superordinate clause of the form "I say unto you . . ." in the first person and the present tense.

Austin's concept and the performative hypothesis bear a relationship to Buehler's notion of a deictic origin to the coordinate frame of language, and to de Saussure's insistence that language can only be understood in terms of the relationship between the speaker and the hearer. Without the deictic frame, Buehler insists the structure of language dissolves. This may be what happens in the case of thought disorder – the determining focus is lost. What the nuclear symptoms of schizophrenia are telling us is what happens when the distinction between the indexicals 'I' of the speaker and 'you' of the hearer begins to dissolve. If the performative hypothesis is

right, thus conceived they are disorders of the foundations of syntax. They tell us that the phonological engram for the perception of speech is quite separate from the phonological engram for speech production. They tell us that thoughts as the precursor to speech are distinct from the meanings that are extracted from perceived speech in the nondominant hemisphere. They draw attention to the obscure process of the motor and sensory elements of the associations (the signifieds) that takes place in the nondominant hemisphere (Mitchell and Crow, 2005). In each case, the phenomena of psychosis provide evidence on the neural organization of language through what happens when the mechanism goes wrong. We begin to understand this through the structure of the torque.

4.36.7 XY Homology and the Xq21.3/Yp Translocation

The most fundamental prediction of the asymmetry hypothesis is that the genetics of psychosis is the genetics of the speciation of *H. sapiens* (Crow, 2004); in other words, the genetics of asymmetry is conceived as the species-defining characteristic. To approach such predictions, it is necessary that the cerebral dominance gene or right shift factor be identified.

An important clue comes from sex chromosome aneuploidies. Individuals who lack an X chromosome (XO, Turner's syndrome) have nondominant hemisphere (spatial) deficits on cognitive testing. Individuals with an extra X (XXY, Klinefelter's and XXX syndromes) have verbal or dominant hemisphere deficits (Table 1). A possible explanation is that an asymmetry determinant is present on the X chromosome. But then the question arises of why males, who only have one X chromosome do not have spatial deficits such as are seen in Turner's syndrome. The answer must be that the copy of the gene on the X chromosome is complemented by a copy on the Y, i.e., that the gene is in the X–Y homologous class (Crow, 1993). A hormonal explanation will not account for the similarity of the changes in XXY individuals, who are male, and XXX individuals, who are female. The case that the gene is present also on the Y chromosome is strongly reinforced by the verbal deficits/delays that are observed in XYY individuals (Geerts *et al.*, 2003).

The hypothesis is further strengthened by evidence that Turner's and Klinefelter's syndrome individuals have corresponding deviations in anatomical asymmetry (Rezaie *et al.*, 2004) and by the demonstration of a same sex concordance effect – the tendency for handedness and sex to be associated above chance expectation – the hallmark of X–Y linkage (Corballis *et al.*, 1996). A role for an X–Y homologous gene is consistent with the presence of a sex difference – brain growth is faster (Kretschmann *et al.*, 1979) and lateralization is stronger (Crow et al., 1998) in females.

When we come to consider where such a gene might be located, there is an important lead. A major chromosomal rearrangement took place in the course of hominin evolution. Two regions on the human Y chromosome short arm share homology with a single region on the human X chromosome long arm (Xq21.3) (Page *et al.*, 1984; Lambson *et al.*, 1992; Sargent *et al.*, 1996). These homologies were created by the translocation of a 3.5 Mb contiguous block of sequences from a chimpanzee hominin precursor X chromosome to the Y chromosome short arm that was subsequently split by a paracentric inversion (by a recombination, presently undated, of LINE-1 elements (Schwartz *et al.*, 1998; Skaletsky *et al.*, 2003) to give two blocks of homology in Yp11.2 (Figure 8). Genes within this region are therefore present on both the X and Y chromosomes in *H. sapiens* but on the X alone in other great apes and primates, and an explanation for the retention of the sequences on Yp presumably lies in the gene content of this block.

Three genes are known to be expressed within this region; *PABPC5*, a poly (A) -binding protein whose Y gametologue has been lost during hominin evolution; *TGIF2LX* and *Y*, (homeobox-containing genes with testis-specific expression), and *ProtocadherinX* (*PCDH11X*) and *ProtocadherinY* (*PCDH11Y*). *PCDH11X* and *Y* (each comprising seven extracellular cadherin motifs, a short transmembrane region, and an intracellular cytoplasmic tail) that code for cell surface adhesion molecules of the cadherin

Table 1 Neuropsychological impairments associated with sex chromosome aneuploidies

	XX	*XY*	*XO*	*XXY*		
	Normal female	*Normal male*	*Turner's syndrome*	*Klinefelter's syndrome*	*XXX*	*XYY*
No. of sex chromosomes	2	2	1	3	3	3
Verbal ability	Normal	Normal	Normal	Delayed	Delayed	Delayed
Spatial ability	Normal	Normal	Decreased	Normal	Normal	Normal

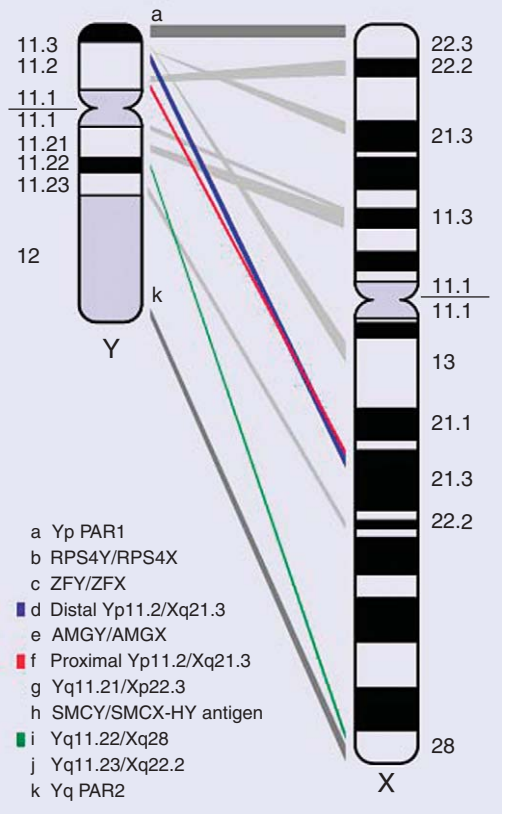


Figure 8 The regions of homology between the X and the Y chromosomes including PAR1 at the telomeres of the short arms and PAR2 at the telomeres of the long arms. Blocks of homology are labeled from a to k on the Y chromosome. Some blocks are identified by their gene content, e.g., RPS4Y/RSP4X, AMGY/AMGX, SMCY/SMCX. Reproduced from Affara, N., Bishop, C., Brown, W., *et al.* 1996. Report on the second international workshop on Y chromosome mapping 1995. *Cytogenet. Cell Genet.* 73, 33–76, Elsevier.

superfamily are of note because both forms of the gene have been retained and are highly expressed both in fetal and adult brain (Yoshida and Sugano 1999; Blanco *et al.*, 2000), including the germinal layer of the cortex (T. H. Priddle, personal communication). The protein products of this gene pair are thus expected to play a role in intercellular communication, perhaps acting as axonal guidance factors and influencing the connectivity of the cerebral cortex. These genes may thus have been subject to selective pressure relating to one or more brain characteristics during hominin evolution.

4.36.8 Implications for Evolutionary Theory

4.36.8.1 The Case for Saltation

To take seriously a structural discontinuity as an explanation for a species difference, it is necessary to consider concepts of evolutionary change other than Darwinian gradualism and the biological or isolation species concept.

The theory of punctuated equilibria (Eldredge and Gould, 1972) was preceded by a long history of challenges to the gradualist version (Bateson, 1894; De Vries, 1901; Goldschmidt, 1940), and some of these have had an explicit genetic basis. Thus, White (1978) and King (1993) have argued strongly for a role for chromosomal change in speciation, but their arguments have not overwhelmed the established view. Against chromosomal change, it is argued (e.g., Coyne and Orr, 1998) that radical rearrangements, for example chromosomal fusions, may apparently have few phenotypic effects, and in some cases alternative chromosomal configurations persist, as it were, as a polymorphism within a species.

The case for saltation in species transitions has been argued at a macroevolutionary level (Stanley, 1998), stating that the amounts of change seen within species and other taxa are simply insufficient to account for the overall pattern of evolutionary change that is seen over time, and at the level of morphology (Mellars, 1998), claiming that the intermediate states in the transition between species that are required by the gradualist theory are absent. But all such general arguments come up against the difficulty that Goldschmidt's (1940) hopeful monster ran into: the greater the magnitude of the saltational change, the less likely it is to have survival value, and the greater the difficulty the hopeful monster will have in identifying a mate. The difficulty is particularly great if the change has the reproductive consequence of reducing fertility in the hybrid state. The possibility that the monster can identify an individual with the same mutation is clearly dependent on reproduction already having taken place, and even then the new mutation is at a severe statistical disadvantage with respect to the existing population.

4.36.8.2 Sexual Selection and the Mate Recognition Principle

However, here Darwin's (1871) juxtaposition of *The Descent of Man* and the theory of sexual selection offers a way out. If sexual selection and speciation were in some way interdependent this might solve the problem of discontinuity and that of mate selection. The case of cerebral lateralization in modern *H. sapiens* illustrates the possibility. All authorities on the genetics of lateralization (Annett, 1985, 2002; McManus, 1985; Corballis, 1997; Crow, 1998a, 1998b; Mckeever, 2000) agree that there is a sex difference: females are more

right-handed than males (although the adult male brain is more asymmetrical than that of the female; Bear *et al.*, 1986; Barrick *et al.*, 2001). The female brain grows faster than that of the male (Kretschmann *et al.*, 1979) and females have greater mean verbal fluency and acquire words earlier (Maccoby and Jacklin, 1975; McGlone, 1980; Halpern, 2000) than males. If language is the species-defining characteristic and lateralization is the process by which it evolved, these facts are related, and they tell us about the nature of the genetic mechanism. Only two explanations of the sex difference in lateralization are conceivable, that it is hormonal in origin (Geschwind and Galaburda, 1985) or that it reflects a sex chromosomal locus (Crow, 1993, 1994), and the facts of sex chromosomal aneuploidies (XXY and XXX individuals who differ in hormonal status have similar hemispheric deviations in development) speak decisively in favor of the latter interpretation. The hypothesis that the asymmetry factor is present on both X and Y chromosomes (Crow, 1993; Corballis *et al.*, 1996) can explain the transmission of handedness within families and apparent dosage effects in the aneuploidies. That there are problems (Corballis, 1997) in accounting for persisting variation in males and females in terms of conventional polymorphisms and heterozygote advantage explanations should not dissuade us from pursuing the line of thought. The genetic principles involved may not be those on which we have hitherto relied.

The paradigm of *H. sapiens* therefore suggests a new version of saltational speciation – that it is not chromosomal changes in general that play a role in speciation but changes on the sex chromosomes, and perhaps particularly changes in regions of X–Y homology that are involved. These regions have a special status because they can account (as in the case of lateralization in humans) for quantitative differences in a characteristic in males and females, and such quantitative differences are a potential substrate for sexual selection. The Y chromosome itself has a unique role, because it is not necessary for survival. There are interindividual differences on the Y (reviewed by Tyler-Smith, 2002), but there are also large interspecific differences. While the X is the most stable chromosome across species, the Y is by far the most variable.

The mammalian Y therefore can be seen as a testbed of evolutionary change. One possibility is that the primary change in speciation takes place on the Y, and when it is located in a region of homology with the X, there is the possibility of correlated but independent change in the two sexes. Such correlated but quantitatively differing ranges of variation have the potential to explain the type of runaway sexual selection envisaged by Fisher (1930), and this may be what occurred with respect to cerebral asymmetry at some point in hominin evolution (Crow, 1998a, 1998b); the introduction of the dimension of symmetry–asymmetry allowed brain growth to equilibrate at a new point of plateau, and this equilibration took place around successive modifications of genes on the Y and then on the X chromosome. There is thus a potential three-way relationship between sexual selection, sex linkage, and speciation, and the pattern suggested by hominin evolution is backed up in the recent literature relating to other species.

A role for sexual selection in modifying a primary change in a sexually dimorphic feature to establish a new species boundary has been argued in relation to Hawaiian Drosophilid species by Kaneshiro (1980) and Carson (1997). Similar arguments apply in the case of the prolific speciation of cichlid fishes in the lakes of East Africa (Dominey, 1984; McKaye, 1991) and may also apply in birds (Price, 1998). Some putative speciation loci, for example, the Odysseus homoeobox (Ting *et al.*, 1998) and the *per* gene (Ritchie and Kyriacou, 1994) that have been identified in *Drosophila* species, are X-linked. In discussing the relationship between the X chromosome and speciation that she finds in Lepidoptera, Prowell (1998) offers three explanations: (1) that X-linked traits evolve more quickly, (2) that traits related to speciation tend to be sex-limited, and that sex-limited traits tend to be on the sex chromosomes, and (3) that female-limited X-linked traits undergo faster rates of evolution when, as in the case of Lepidoptera, the female is heterogametic. These explanations are not mutually exclusive. Prowell asks whether the X chromosome bias is unique to Lepidoptera and concludes that this is unlikely. Haldane's rule is that when, in a species hybrid, one sex is sterile or inviable it is the heterogametic sex. Coyne and Orr (1998) consider various explanations, including faster evolution and recessivity of genes on the X chromosome. While each of these observations and hypotheses is consistent with a relationship between speciation and the sex chromosomes, none of the authors considers the more restrictive formulation suggested by the sequence of events (Sargent *et al.*, 1996, 2001, 2002) on the mammalian Y chromosome, that it is the interaction between the sex chromosomes, particularly the possibility of transfer of material between them, that is critical in speciation.

4.36.8.3 Species-Specific Variation Is Epigenetic

Reinhold considers the case that sexual selection acts selectively on sexual dimorphisms that relate

to sex-linked genes, as suggested by Rice (1984). The sequence of events, including a translocation and a paracentric inversion, suggested by the work summarized by Sargent *et al.* (2002) and by the X–Y hypothesis as relevant to the course of hominin evolution, carries the further implication that epigenetic modification is involved in the process of sexual selection and speciation. In mammals, genes on one X chromosome are subject to the process of X inactivation, but gene sequences that are also represented on the Y chromosome are protected from this influence. Such genes are expressed from both X and Y in males and from both Xs in females, a similar dosage thus being maintained in each sex. The mechanism by which this protection is achieved is unknown (Burgoyne and McLaren, 1985; Crow, 1991). Gene sequences that have been transferred from the X to the Y are in a new situation; whatever the mechanism, a phase of epigenetic equilibration must be assumed (Jegalian and Page, 1998). If X–Y pairing in male meiosis plays a role, the orientation of the sequence on the Y is also relevant. The paracentric inversion on the Y short arm could be critical.

An MRI investigation in monozygotic twins of handedness and asymmetry of the planum temporale (Steinmetz *et al.*, 1995) indicates that there is room for an epigenetic influence on cerebral asymmetry, and this may account for the stochastic element incorporated in genetic theories (Annett, 1985, 2002; McManus, 1985). There is a possibility, therefore, that the genetic mechanisms underlying the development of cerebral asymmetry in humans are a paradigm for a more general interaction between genetic and epigenetic mechanisms in sexual selection and speciation. Perhaps sexual selection and natural selection are mediated by distinct but complementary genetic processes: natural selection ensures the organism's survival through response of any part of the genome to environmental change, whereas sexual selection reflects the sequential response of the female genome (for example, the mammalian XX complement) to change on the Y chromosome, and that this process involves particularly the epigenetic modulation of genes on the X. According to this concept, speciation follows the history of the nonrecombining sex chromosome, in mammals the Y.

4.36.8.4 Speciation Events Occur on the Heterogametic Chromosome

Birdsong has relevance to the genetics of species-specific characteristics (see The Evolution of Vocal Learning Systems in Birds, The Evolution of Vocal Learning Systems in Birds and Humans). The neural capacity is lateralized, season-dependent, and strongly sexually dimorphic, being generally confined to males. The lateralization is a parallel and independent evolutionary development to the cerebral torque of the human brain, such lateralization being absent in intervening vertebrate families such as rodents and primates, and the neural mechanisms are quite distinct. But the individual songs of birds, like the capacity for human language, are species-specific. In that they are a component of mating behavior, they can be regarded as specific mate recognition systems as described by Paterson. Whereas there are elements, for example the season-dependence that is hormone-sensitive, there are also components that are determined by genes and these are located on the Z and W chromosomes (Arnold, 2004). The hormone-sensitive elements are generally those that cross species, whereas the components that are species-specific are mostly genetically determined. Thus, there is the possibility that the species-defining characteristic in song birds, which can reasonably be described as the mate recognition system, is dependent upon genes present on both Z and W chromosomes. Moreover, it appears that the differences between species in this salient characteristic must be determined by genetic differences at the relevant loci on the sex chromosomes.

This theory attributes general significance to X–Y (or W–Z) homologous genes in relation to a mate recognition system in Paterson's sense, and thereby accommodates the role for an X–Y homologous determinant of cerebral asymmetry and language in humans, as suggested above. Perhaps one can propose that it is events on the heterogametic chromosome (the Y in mammals) that have particular significance in relation to speciation, and that it is the interaction between those sequences on the Y that have changed and the pre-existing sequences on the X that is of particular interest. For while the X is remarkably stable in structure across species (the gross structure of the mouse X closely resembling that of the chromosome in humans) the structure of the Y is highly variable even between quite closely related species.

Thus, one can postulate that the primary change in speciation takes place on the Y (more generally the heterogametic chromosome), and the subsequent changes on the X establish the stable sexual dimorphisms that distinguish species.

Ellegren has pointed to a possible molecular correlate of such a sequence. Considered in relation to the rest of the genome and when relative population size in terms of the two sexes is taken into account, the genetic variation on the heterogametic chromosome (the Y in mammals, the W in birds) is

considerably less than would be expected. The reduction in polymorphic loci may reflect a selective sweep at the origin of the species.

The hypothesis emerges that the primary event that leads to a new species is a change (maybe a duplicative translocation as in the hominin lineage, but deletions or insertions are also possible) that occurs on the heterogametic chromosome. The change occurs in one individual (a male in mammals, a female in birds) that represents the rare case in which such a chromosomal change is associated with a reproductive advantage in that such individuals are preferentially selected as mates. Thus, the new chromosome increases in frequency in the population along with its associated phenotypic innovation, but in the first generation is confined to the heterogametic sex. But in the next generation there is the possibility that there will be an impact on the homogametic chromosome because the altered structure of the Y (or the W) has the potential to influence the epigenetic status of the X (or the Z) because regions of the sex chromosomes that are paired are protected from the process of inactivation (meiotic suppression of unpaired DNA) that otherwise leads to the inactivation of one of the two Xs or Zs in the daughters or sons, respectively, of the founder individual and his or her immediate same sex progeny.

Such epigenetic change will be particularly relevant if the primary change occurs in or generates a region of X/Y (or Z/W) homology. Thus, homologies may perhaps be preferentially selected by the speciation process. If the primary event occurs within the nonrecombining region of the heterogametic chromosome (the mammalian Y or the avian W), this allows genes relating to the speciation characteristic 'and its homogametic homologue and related genes' to undergo sequence variation that is independent in the two sexes. The variability in this genetic process could account for the diversity of sexual dimorphisms across species.

These proposals deriving from the case of hominin evolution bear a relationship to concepts of speciation in *Drosophila* developed by R. S. Singh and colleagues as well as to the literature on sexual selection and speciation referred to above. Civetta and Singh (1999) distinguished between sex-related and non-sex-related genes and found a higher rate of Ka/Ks ratios among the former. They suggested that directional sexual selection had shaped the evolution of sex-related genes (defined in a broad sense to include all components of sexuality not confined to courtship and mating) and that these changes were more likely to have occurred in the early stages of speciation. Developing these ideas, Singh and Kulathinal (2005) emphasize the rapid evolution of sex-related traits in a wide variety of taxa, the faster rate of DNA sequence divergence in genes affecting sexual function and fertility, and the evidence for involvement of novel traits and genes in sexual function.

4.36.9 Conclusions

1. Language and the paradox of psychosis:
   - The faculty of language as a defining feature of *H. sapiens* with characteristics absent in the communicative systems of the great apes challenges Darwinian gradualism. It is more readily assimilated to a saltational account of speciation as suggested by T. H. Huxley and developed by R. Goldschmidt.
   - The paradox is that interindividual variation apparently genetic in origin persists at approximately the same frequency in all populations in the face of a fecundity disadvantage.
   - This variation represents a component of variation associated with the capacity for language; it is argued that the phenomena of psychosis are the key to an understanding of the neural organization of language. Thus, psychosis and language have a common evolutionary origin in the speciation event.
2. The cerebral torque and its implications:
   - Broca's hypothesis that cerebral asymmetry is the characteristic that defines *H. sapiens* is supported by recent cross-species comparisons for directional handedness and anatomical asymmetry.
   - The cerebral torque (the bias from right frontal to left occipital across the anteroposterior axis) defines the human brain as a four-chambered organ by comparison with the two chambers (anterior motor and posterior sensory) of the brains of other primates, and dictates a reversal of sign of the convergence of interhemispheric connections (from left to right posteriorly and from right to left anteriorly).
3. The quadripartite structure of language:
   - These transitions are critical to the separation of the sensory and motor phonological engrams in the dominant left hemisphere from some of their associated signifiers (the sensory meanings and the motor thoughts) in the nondominant hemisphere.
   - Critical to the distinction between the speaker and the hearer and to what is motor and what is sensory in the neural representation of speech is the notion (Buehler's) of a deictic origin ('I',

'here', 'now') to the coordinate system of language.
- The nuclear symptoms of schizophrenia (e.g., thoughts spoken aloud, running commentary, thought insertion) are seen as the ectopic presence (leakage) of one or more of the components of language outside the relevant compartment of association cortex.
- The deictic origin is located in Broca's area and defined by its interaction through the uncinate and arcuate bundles with Wernicke's area.

4. The genetic basis of the speciation event:
   - There is a strong case from the phenomena of sex chromosome aneuploidies and a family study of handedness that the cerebral dominance gene is in a region of homology between X and Y chromosomes.
   - A duplicative translocation 6 Mya created a *sapiens*-specific region of homology between Xq21.3 and Yp11, within which there have been subsequent rearrangements including a paracentric inversion.
   - Within the region of homology, the *ProtocadherinXY* gene pair coding for cell surface adhesion proteins has been subject to accelerated evolution.
5. Implications for evolutionary theory:
   - The sequence of events in the speciation of *H. sapiens* was saltational in form. It suggests the general theory of speciation that rearrangements on the heterogametic chromosome play a role by initiating a phase of sexual selection that, on account of sequences shared between the sex chromosomes, involves related characteristics in the two sexes. The interaction establishes a mate recognition system (in Paterson's sense) that defines the new species.

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